

Renewal application cultivation of maize T25

COGEM advice CGM/080806-02

Summary

*The present application by Bayer CropScience AG (file EFSA/GMO/NL/2007/46) concerns the renewal of the authorization for cultivation of the genetically modified maize line T25. This line contains the *pat* gene, resulting in tolerance to herbicides containing glufosinate ammonium.*

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 trait will increase the potential of maize to establish feral populations. The gene introduced in T25 cannot spread to closely related species since wild relatives of maize are not present in Europe. Furthermore, the general surveillance plan is adequate to detect unexpected effects due to cultivation of the maize line. Additionally, maize line T25 is cultivated during ten years in several countries and no adverse effects have been reported.

In view of the above, COGEM is of the opinion that cultivation poses negligible risks to the environment. Regarding the risks for food and feed, the outcome of the assessment by other, primary responsible, organizations (EFSA, RIKILT, RIVM) was not known at the moment of completion of this advice.

1. Introduction

The present application by Bayer CropScience AG, file EFSA/GMO/NL/2007/46, concerns the renewal of authorization for cultivation of maize line T25. This line expresses the *pat* gene resulting in tolerance to herbicides containing glufosinate ammonium.

In 1998, T25 maize was authorized for placing on the market into the European Union for all uses with the exception of food. Furthermore, cultivation of this maize line was granted in, among other countries the United States (1995), Canada (1996), Japan (1997) and Argentina (1998) (1). COGEM advised positively on the cultivation of hybrid maize line T25 x MON810 (2).

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 (4). In Europe, hybridization with other species cannot occur as wild relatives of maize are not present in Europe (3). The appearance of volunteers is rare under Dutch weather 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.

3. Environmental risk assessment

To obtain permission for cultivation of a gm-maize line, an environmental risk assessment has to be carried out by the applicant. The objective of the risk assessment is to identify and evaluate potential adverse effects (direct and indirect, immediate or delayed) of the maize line on human health and the environment. Among others, it includes the evaluation of effects of the gm-maize line on populations of target and non-target organisms and of the likelihood of the maize line becoming more persistent than the recipient plants. The molecular characterization and analysis is also of importance.

3.1 Molecular characterization

Origin and function of the introduced genetic elements

Maize line T25 was generated by introducing plasmid DNA (pUC/Ac) by polyethylene-glycol mediated protoplasts transformation. Vector pUC/Ac consists of the following elements:

- T-35S terminator, derived from *Cauliflower mosaic virus (CaMV)*
- Synthetic *pat* gene, derived from *Streptomyces viridochromogenes*. The *pat* gene encodes phosphinothricin acetyl transferase which confers resistance to glufosinate ammonium containing herbicides
- P-35S promoter, derived from CaMV
- β -lactamase gene (backbone), derived from *Escherichia coli*. Confers resistance to ampicillin
- ColE1 origin of replication (backbone), derived from *E. coli*

Properties of the introduced genes conferring herbicide tolerance

Maize line T25 is tolerant to herbicides containing 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 (5). 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). Maize line T25 expresses the *pat* gene which encodes phosphinothricin-N-acetyl transferase (PAT). This 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 T25 is tolerant to L-phosphinothricin and thus to herbicides containing glufosinate ammonium.

Molecular analysis

In the opinion of the COGEM the molecular analysis of hybrid maize line T25 is adequate. The applicant showed by Southern blot hybridization analysis that a single copy of the insert was integrated in the nuclear genome of maize line T25. Southern blotting and segregating analysis demonstrated that the insert is genetically stable in different generations. The sequences of the 5' and 3' regions that flank the insert were analyzed up to respectively 308 bp and 150 bp and compared to the sequence of a conventional maize line. Results indicate that the flanking regions are native to the maize genome. Although the intention was to insert only the P35S-pat-T35S cassette, sequence analysis of the insert demonstrated that the backbone of plasmid pUC/Ac was partly integrated into the maize genome. Approximately 75% of the *β-lactamase* gene (encoding ampicillin resistance) is present. Consequently, the gene is not stably transcribed nor translated into an active protein. Furthermore, at the 3' end of the insert a duplication of a fragment similar to part of the P35S promoter is present in the maize genome. In addition, comparison of the sequence of genomic DNA from non-transgenic plants and DNA from maize line T25 identified a deletion of 20 base pairs at the presumed insertion locus. Since these alterations do not affect the introduced trait, COGEM is of the opinion that the alterations do not affect the outcome of the risk analysis negatively.

In view of the above, the molecular analysis does not indicate that cultivation of this line would pose a risk to the environment.

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. This application is submitted under Regulation (EC) 1829/2003, therefore a food/feed assessment is carried out by EFSA. Other organizations who advice the competent authorities can perform an additional assessment on food safety although this is not obligatory. In the Netherlands a food and/or feed assessment for Regulation (EC) 1829/2003 applications is carried out by RIKILT and RIVM. Regarding the risks for food and feed, the outcome of the assessment by other organizations (EFSA, RIKILT, RIVM) was not known at the moment of completion of this advice.

3.2 Potential changes in the interaction of T25 with the environment

Based upon the environmental risk analysis of this application, COGEM is of the opinion that the risk assessment is adequate and that no adverse environmental effects are to be expected. Maize is very sensitive to weed competition and cannot persist as a weed (3,4). In the Netherlands, volunteers are rarely found and establishment of maize plants in the wild has never been observed. 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. There is no reason to assume that the maize line has a selective advantage resulting from the modification. Furthermore, T25 is not likely to exert negative effects on non-target organisms as a result of the modification.

3.3 Cultivation of T25

Since 1998, maize line T25 is authorized for cultivation in the Member States of the European Union and in other countries. According to the applicant, T25 is used for breeding activities for the development of commercial maize varieties. These T25 derived maize hybrids are only cultivated in the United States and Canada. During the period 1998-2006, 19 million acres of T25 hybrids were cultivated.

In these ten years, T25 no adverse effects on human health and the environment have been reported.

4. General surveillance plan

To obtain authorization to cultivate gm maize, a monitoring plan considering the environmental impact of cultivation is required. A monitoring plan consists of two parts, a case-specific plan and a general surveillance plan. The case-specific plan should be set up to verify the hypotheses made in the risk assessment. The applicant states that no potential hazards are identified from the environmental risk assessment. Therefore, a case-specific monitoring plan is not necessary according to directive 2001/18/EC. COGEM agrees with the statement that no potential hazards are expected from cultivation of T25 maize.

General surveillance was introduced to be able to observe unexpected effects of genetically modified crops on the environment. This refers to potentially adverse effects that were not anticipated while assessing the genetically modified organism. The applicant has formulated a general surveillance plan for T25 maize in order to detect any unanticipated adverse effects on human health and the environment.

COGEM is of the opinion that the general surveillance plan is acceptable, although there are some uncertainties with respect to the proposed actions to be undertaken. Many stakeholders are invited to participate in the general surveillance. Farmers' questionnaires and existing monitoring systems are considered a useful tool to collect data on the performance and impact of the genetically modified plant. Following the initial placing on the market, general surveillance reports on direct and immediate effects, and on potential indirect or delayed effects, are submitted on an annual basis. Final reports will be made at the end of the consent.

COGEM notices that the plan is based on voluntary participation of stakeholders. It is unclear whether they will agree to cooperate. Additionally, stakeholders participating in the general surveillance are mainly traders and operators involved in handling and processing of maize products. Consequently, less attention is paid to

surveillance of adverse effects of cultivation of T25 at farm level. COGEM suggests that the applicant uses a more detailed plan to monitor effects at farm level.

5. Advice

COGEM has been asked to advice on the cultivation of maize line T25. 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 *pat* gene in T25 increases the potential of maize to establish feral populations. In addition, introgression of the introduced gene into closely related species cannot occur, as wild relatives of maize are not present in Europe. The molecular characterization is adequate and does not indicate that cultivation of this line would pose a risk to the environment. Also, the general surveillance plan is adequate to detect unexpected effects due to cultivation of the maize line.

In addition, hybrids of T25 are commercially grown in, e.g. the United States and Canada. No adverse effects have been reported.

In view of the above, the overall opinion of COGEM is that cultivation of T25 poses a negligible risk to the environment in the Netherlands.

References

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