Import and processing of cotton T304-40

COGEM advice CGM/120105-01

Summary

The present application by Bayer CropScience (file EFSA/GMO/NL/2011/97) concerns the import and processing for use in feed and food of cotton T304-40. Cultivation is not part of this application.

Cotton line T304-40 was produced by Agrobacterium tumefaciens *mediated transformation of conventional cotton. T304-40 expresses the* cry1Ab *gene and* bar *gene. As a result, T304-40 cotton is resistant to certain lepidopteran pests and tolerant to glufosinate ammonium based herbicides. T304-40 has not been previously assessed by COGEM.*

In Northwest Europe, no wild relatives of cotton are present. Modern cotton cultivars do not possess any of the attributes commonly associated with problematic weeds. COGEM does not know of reasons to assume that the introduced traits will increase the potential of cotton to establish feral populations. Moreover, cotton cannot survive the climatologic conditions in Northwest Europe. Therefore, COGEM is of the opinion that incidental spillage of T304-40 seeds will not pose a risk to the environment in Northwest Europe.

The applicant showed by Southern blot analyses that one nearly complete copy of the insert (with the bar *and* cry1Ab *gene) flanked by an inverted incomplete copy (without the* bar *gene) is integrated at a single locus. The applicant further showed that the backbone of the plasmid used for transformation is absent in T304-40. Bioinformatic analysis of the insert itself and the junctions of the insert and the cotton genomic DNA identified a total of 291 open reading frames (ORFs). These sequences were analyzed for similarity to known toxins or allergens. No similarities were found. The molecular characterization and provided General Surveillance plan meet the criteria of COGEM .*

In view of the above, COGEM is of the opinion that the risks for humans and the environment associated with import and processing of cotton line T304-40 are negligible. A food/feed safety assessment is carried out by other organizations. Therefore, COGEM abstains from advice on the potential risks of incidental consumption.

Introduction

The present notification (EFSA/GMO/NL/2011/97) by Bayer CropScience AG concerns import and processing of the genetically modified cotton line T304-40. This cotton line was produced by *A. tumefaciens* mediated transformation of conventional variety Coker 315 and expresses the *cry1Ab* gene from *Bacillus thuringiensis* and the *bar* gene from *Streptomyces hygroscopicus*. As a result, T304-40 cotton is resistant to certain lepidopteran pests and tolerant to glufosinate ammonium based herbicides.

Previous COGEM advice

T304-40 has not been previously assessed by COGEM. However, in the past COGEM has repeatedly advised positively on crops expressing the *cry1Ab* gene. Furthermore, in March of 2011, COGEM advised positively on the import and processing of cotton line GHB614xLLCotton25 expressing the *bar* gene.[1]

Aspects of the crop

Cotton is a member of the genus *Gossypium* and belongs to the *Malvaceae* family. The majority of cultivated cotton (90%) is *Gossypium hirsutum,* but *Gossympium barbadense*, *Gossympium arboreum* and *Gossympium herbaceum* are cultivated as well.[2,3,4] In the South of Europe *G. hirsutum* cotton is grown in Greece, Spain and Bulgaria.[5]

Cotton plants reproduce sexually.[4] Cotton is predominantly a self-pollinating species, but crosspollination may occur. The pollen of cotton is large, heavy and somewhat sticky.[3,4] The viability of *G. hirsutum* pollen decreases rapidly after eight hours.[4] Outcrossing rates for cotton are strongly influenced by the prevalence of insects[4] and dissemination of pollen by wind is (almost) absent.[3] Amongst others bumblebees (*Bombus*), honeybees (*Apis*) and other bee species (*Anthophora, Melissodes* and *Halictus*) are pollinators of cotton flowers.[3] Wild relatives of cotton (*G. hirsutum*) do not occur in Northwest Europe. Therefore, hybridization with wild relatives cannot occur in Northwest Europe.[3]

The climate in Northwest Europe is not suited for cotton growth. Cotton is highly sensitive to temperature and susceptible to frost. Temperature is the main factor to determine the geographic range in which cotton can be grown. *G. hirsutum* seeds do not germinate until the temperature reaches 15ºC[3] and plant development ceases when temperatures are below 12ºC.[4] Activity is delayed when the temperature rises above 38ºC.[3] For normal development, cotton needs an average of 150 days with temperatures between these values.[3] The optimum temperature for germination is 34ºC, for growth of seedlings 24-29ºC and for later continuous growth 34ºC. When the crop is grown at lower temperatures, the production of vegetative branches increases and the cropping period is extended. Because cotton is susceptible to frost, the whole growth period (which can range from 160 to 220 days) has to be free of frost.[2] In places where cotton is grown as a rain-fed crop the average rainfall is 800-1200 mm.[2] In areas where the rainfall is less than 500 mm a year, irrigation should be applied.[3] In the seedling stage cotton does not tolerate shady circumstances, and in later plant stages reduced light intensity affects flowering and fruiting.[2]

Cottonseed may be dispersed by wind or water but may also be spread during transport or when feeding cattle.[4] In addition, cottonseed may be transported by birds or rodents. Germination is less likely to occur in undisturbed sites and roadsides than in disturbed sites.[4] Seeds from cotton cultivars do not possess dormancy[3,4] and will germinate in autumn if conditions are favourable. In addition, seeds will usually not survive in humid soil.[4] In regions with mild and dry winters, cottonseeds may overwinter and germinate in spring. Seedlings are sensitive to competition from weeds.[3]

Modern cotton cultivars do not possess any of the attributes commonly associated with problematic weeds, such as dormancy, persistence in soil banks, germination under adverse environmental conditions, rapid vegetative growth, a short life cycle, very high seed output, high seed dispersal and long-distance dispersal of seeds. Cotton volunteers occur in cotton growing areas and may occur when cottonseed is used as livestock feed. The occurrence of volunteer cotton is limited by the availability of adequate soil moisture or the occurrence of frost.[4] There are reports that *G. hirsutum* and *G. herbaceum* cotton are naturalised in some Southern European countries, e.g. Greece and Spain.[6,7,8,9]

Molecular characterisation

Cotton T304-40 was developed by *A. tumefaciens* mediated transformation of tissue from cotton variety Coker 315 using vector pTDL008. Glufosinate ammonium was used as the

selective agent. The following elements were introduced in T304-40:

- Left border repeat; 24 bp *Cis*-acting element for T-DNA transfer from *A. tumefaciens.*
- 3'me1; Terminating signal of the *cry1Ab* gene from *Flaveria bidentis.*
- *cry1Ab*; Insect resistance *cry1Ab* gene from *B. thuringiensis.*
- 5'e1; Leader sequence for high level constitutive expression, especially in cotton leave and squares.
- Ps7s7; Duplicated promoter from *Subterranean clover stunt virus* for high level constitutive expression, especially in cotton leave and squares.
- P35S3; Promoter from *Cauliflower mosaic virus* for high level constitutive expression.
- *bar*; Glufosinate ammonium tolerance gene from *S. hygroscopicus.*
- 3'nos; Terminating signal of the *bar* gene from *A. tumefaciens.*
- Right border repeat, 24 bp *Cis*-acting element for T-DNA transfer from *A. tumefaciens.*

Expressed proteins

The *cry1Ab* gene encodes a δ-endotoxin which is effective in controlling certain lepidopteran larvae, including cotton bollworm larvae (*Helcoverpa zea*) and tobacco budworm larvae (*Heliothis virescens*), which are common pests of cotton.

Upon ingestion by susceptible larvae, the Cry1Ab protein is solubilized in the midgut and activated by midgut proteases to release a toxin fragment. This toxin fragment binds to specific receptors on the epithelial surface of the midgut and causes pores to open. This leads to disruption of the movement of solutes across the gut epithelium and causes the influx of water leading to cell swelling and lysis. The gut contents are released into the body cavity allowing bacteria to breed ultimately leading to septicaemia and death.[10,11]

T304-40 is also 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.[12] The application of glufosinate ammonium leads to reduced glutamine and increased ammonia levels in non-transgenic plants.[12] Photosynthesis is inhibited and eventually the plant dies.[13] T304-40 expresses the *bar* 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.[12] As a result T304-40 is tolerant to L-phosphinothricin and thus to herbicides containing glufosinate ammonium.

Molecular analysis

The applicant demonstrated by Southern blot, PCR and sequence analysis that T304-40 cotton contains a partial 3'me1 terminator sequence followed by two partial copies of the *cry1Ab* gene cassette in a tail-to-tail orientation. One of these cassettes contains part of the Ps7s7 promoter while the other one contains only part of the 3'me1 terminator and a partial copy of the bar gene cassette in which only part of the 3'nos terminator is present. The applicant further showed that the DNA is integrated at a single locus and that T304-40 does not contain backbone sequences of plasmid pTDL008.

The applicant determined the sequence of the integration site. A comparison with the sequence after the T-DNA insertion revealed a 32 bp deletion in T304-40. The 5' and the 3' flanking sequences of the integrated DNA are of cotton origin. No unknown sequences were found.

The junctions between the T-DNA insert and the flanking plant genomic DNA as well as the junctions between the various integrated partial T-DNA segments were sequenced and screened for potential newly created open reading frames (ORFs). The ORFs were defined as a putative protein encoding sequence between two stop codons. In totall, 291 ORFs were identified. The amino acid sequences of these ORFs were compared with known allergens and toxins in the AllergenOnline database and Bayer toxin database, respectively. These analyses showed no biologically significant sequence similarities with known toxins and allergens.

In view of the above, COGEM is of the opinion that the molecular characterisation of T304-40 has been adequately performed and meets the criteria laid down by COGEM.[14]

Environmental risk assessment

Cotton is predominantly a self-pollinating species, but crosspollination may occur. Wild relatives of cotton (*G. hirsutum*) do not occur in Northwest Europe. Therefore, hybridization with wild relatives cannot occur in Northwest Europe.[3]

Cotton plants are very sensitive to temperature. As mentioned before, a reasonably high temperature (an average of 150 days with a temperature between 15 and 38˚C) is required in all stages of development. The optimum temperature for germination is 34ºC, for growth of seedlings 24-29ºC and for later continuous growth 34ºC. In areas where the rainfall is less than 500 mm a year, irrigation should be applied for cotton growth. In the Netherlands, May, June, July, August and September have average monthly temperatures above 12ºC, but below 18ºC.[15] In addition, in May, June, July, August and September the average monthly precipitation does not exceed the 100 mm.[15] Therefore, the Dutch climate is unsuited for cotton growth.

Climate conditions in other parts of the European Union e.g. Greece, Spain, Bulgaria and Portugal are more suitable for growing cotton.[5] Seeds from cotton cultivars do not possess dormancy[3,4] and will germinate in autumn if conditions are favourable. In addition, seeds will usually not survive in humid soil.[4] In regions with mild and dry winters, cottonseeds may overwinter and germinate in spring.[2] The occurrence of volunteer cotton is limited by the availability of adequate soil moisture or the occurrence of frost.[4]

There is no indication that the introduced traits, which confer resistance to certain lepidopteran pests and tolerance to glufosinate ammonium containing herbicides, will increase the ability of cotton to survive in the environment. The applicant carried out an agronomic assessment for T304-40. The COGEM is of the opinion that the agronomic assessment does not give any indication to assume that T304-40 has an increased survivability compared to conventional cotton lines. Furthermore it is unlikely that cotton will grow in the Netherlands.

In view of the above, there are no reasons to assume that T304-40 has an increased potential for the establishment of feral populations in case of incidental spillage because the climate in Northwest Europe is not suited for cotton growth.

Since 2008 COGEM abstains from giving advice on the potential risks of incidental consumption in case a food/feed assessment is already carried out by other organisations.[16]

This application is submitted under Regulation (EC) 1829/2003, therefore a food/feed assessment is carried out by EFSA. Other organisations who advise 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. Regarding the risks for food and feed, the outcome of the assessment by other organisations (EFSA, RIKILT) was not known at the moment of the completion of this advice.

General surveillance plan

General surveillance (GS) has been introduced to be able to observe unexpected adverse effects of genetically modified (GM) crops on the environment. The setting or population in which these effects might occur is either not, or hardly predictable. The GS plan states that unanticipated adverse effects will be monitored by existing monitoring systems which include the authorization holder and operators involved in the handling and use of viable maize line 5307. In 2010, COGEM formulated criteria which GS plans concerning Dutch applications for import and cultivation of GM crops have to comply with.[17] COGEM concluded that the GS plans could be improved by a guarantee that operators will monitor for unanticipated effects. In the present GS plan on cotton line T304-40 the authorization holder states that the operators have agreed to provide information relevant to the monitoring of T304-40 to the authorization holder. More important, it is stated that the authorization holder will be able to give evidence that the operators collect this information. This is in line with the criteria laid down by COGEM.[17]

Advice

The present application concerns import and processing for feed and food purposes of the genetically modified cotton line T304-40. Cultivation is not part of the application. Therefore, the risk assessment focuses on the accidental spillage of cottonseeds.

Cotton plants are very sensitive to temperature. The Northwestern European climate is unsuited for cotton growth. There is no indication that the introduced traits, which confer resistance to certain lepidopteran pests and tolerance to glufosinate ammonium containing herbicides, will increase the ability of cotton to survive in the environment. Therefore, there are no reasons to assume that T304-40 has an increased potential for the establishment of feral populations in case of incidental spillage. The climate in Northwest Europe is not suited for cotton growth. Therefore, in COGEM's view there is no risk that incidental spillage of cottonseeds will lead to the spread of cotton within Northwest Europe. The molecular characterization of T304-40 meets the criteria of COGEM.

In view of the above, COGEM is of the opinion that the risks for humans and the environment associated with import and processing of cotton line T304-40 are negligible. A food/feed safety assessment is carried out by other organizations. Therefore, COGEM abstains from advice on the potential risks of incidental consumption.

Additional remark

The observations for agronomic characteristics were collected from field trials performed in 2007 and 2008 in Spain. In determining the agronomic criteria, the applicant used a randomized complete block design on multiple locations with several replications and treatments per replication. COGEM notes that in order to obtain statistical significant

differences, it would be better to sample fewer locations and take more replicas per location. COGEM also notes that the applicant did not perform a power analysis on the data. However, since the raw data provided by the applicant suggest that there are few differences between cotton T304-40 and its nontransgenic counterpart, there are no reasons to assume that T304-40 has an increased survivability compared to conventional cotton lines.

Since this application only concerns import and processing of cotton and it is unlikely that cotton will grow in the Netherlands, a detailed analysis of agronomic characteristics is of minor concern. However, this is a point of attention for possible future applications for cultivation of this cotton line in Europe.

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