COMMISSIE
COGEM
GENETISCHE
MODIFICATIE

Aan de staatssecretaris van
Infrastructuur en Milieu
dhr J.J. Atsma
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DATUM	30 maart 2011
KENMERK	CGM/110330-01
ONDERWERP	Teelt, import en verwerking van de genetisch gemodificeerde aardappel AV43-6-G7

Geachte heer Atsma,

Naar aanleiding van een adviesvraag (EFSA/GMO/NL/2009/69) betreffende een vergunningaanvraag voor teelt van de genetisch gemodificeerde aardappel AV43-6-G7 van AVEBE, deelt de COGEM u het volgende mee:

Samenvatting

De COGEM is gevraagd te adviseren over de mogelijke milieurisico's van teelt, import en verwerking van de genetisch gemodificeerde aardappel AV43-6-G7. Deze aardappel bevat een modificatie waardoor er nauwelijks amylose en hoofdzakelijk amylopectine wordt gevormd.

Voor zijn verspreiding en overleving maakt de aardappel gebruik van zaden en knollen. De aardappel kan in Nederland niet uitkruisen naar wilde verwanten. De aardappel kan wel uitkruisen naar andere aardappelrassen, maar slechts in beperkte mate en alleen over korte afstanden. Opslag uit zaad heeft in de normale landbouwkundige praktijk een lage concurrentiekracht ten opzichte van andere gewassen. De gevormde plantjes zijn klein en zwak en over het algemeen niet in staat zich in een opvolgend gewas te handhaven en knollen te vormen. Aardappelknollen zijn vorstgevoelig en eventuele opslag uit knollen wordt evenals zaailingen in het kader van de verplichte bestrijding van *Phytophthora infestans* vernietigd.

Er zijn geen redenen om aan te nemen dat expressie van het geïnserteerde gen het verwilderingspotentieel van de aardappel vergroot. De COGEM is van mening dat moleculaire karakterisatie van de aardappellijn aan alle eisen voldoet.

Gezien het bovenstaande heeft de COGEM geen bezwaar tegen teelt van aardappel AV43-6-G7 en acht zij de risico's voor mens en milieu verwaarloosbaar klein.

De door de COGEM gehanteerde overwegingen en het hieruit voortvloeiende advies treft u hierbij aan als bijlage.

Hoogachtend,

Prof. dr. ir. Bastiaan C.J. Zoeteman
Voorzitter COGEM

c.c. Drs. H.P. de Wijs
Dr. I. van der Leij

Met het oog op eventuele belangenverstrengeling is het COGEM buitenlid dr. P.M. Bruinenberg niet betrokken geweest bij de besluitvorming over dit advies.

Cultivation, import and processing of genetically modified potato AV43-6-G7

COGEM advice CGM/110330-01

This application concerns cultivation, import and processing of the genetically modified potato AV43-6-G7. AV43-6-G7 is an amylose-free potato obtained by down regulation of the Potato Granule Bound Starch Synthase I (GBSSI) gene. The genetic modification did not introduce any new traits or components such as new metabolites or proteins.

Previously, COGEM issued several positive advices concerning field trials with AV43-6-G7 potato in the Netherlands. Furthermore, COGEM advised positively on the cultivation, import and processing of a similar genetically modified amylose-free potato in 2004.

In the Netherlands, the presence of potato (S. tuberosum) *volunteers is possible, but* S. tuberosum *does not establish itself. There are no reasons to assume that the inserted trait will allow AV43-6-G7 potato to establish feral populations. In addition, routine agricultural practices like ploughing, harrowing, herbicide application and the compulsory measures to control potato blight, eliminate emerging volunteer plants in the field. In the Netherlands, outcrossing between* S. tuberosum *and wild relatives is not possible, since efficient incompatibility barriers prevent hybridization between* S. tuberosum *and these wild relatives.*

COGEM is of the opinion that the molecular characterization of AV43-6-G7 potato is adequate. In addition, COGEM considers the General Surveillance plan sufficient for cultivation, import and processing of AV43-6-G7 potato.

Based on the aspects discussed above, COGEM is of the opinion that cultivation of AV43-6-G7 potato poses a negligible risk to the environment and has no objections against an authorization for cultivation of AV43-6-G7 potato. COGEM points out that 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 scope of the present notification (EFSA/GMO/NL/2009/69) by AVEBE concerns the cultivation, import and processing of the genetically modified amylopectin starch potato AV43-6-G7. AV43-6-G7 potato contains cDNA sequences derived from the *Granule Bound Starch Synthase I* (GBSSI) gene isolated from potato in a sense and antisense orientation. The sequences form an inverted repeat of the 5' part of the *GBSSI* cDNA. The RNA transcribed from this inverted repeat forms a hairpin which is subsequently cleaved into small RNA fragments. These RNA fragments will not be translated into a protein, but will lead to RNA interference which results in the degradation of the endogenous *GBSSI* mRNA. Due to this degradation process no GBSSI protein is formed and the production of amylose in AV43-6-G7 potato is inhibited.

Previous COGEM advice

In 2004, 2007 and 2008 COGEM issued positive advices on small and large-scale field trials with AV43-6-G7 potato in the Netherlands.[1,2,3] Furthermore, in 2004, COGEM advised positively on cultivation, import and processing of a similar genetically modified amylose free potato.[4] COGEM was of the opinion that the risks for humans and the environment associated with cultivation, import and processing of this potato and the field trials with AV43-6-G7 are negligible.

Aspects of the crop

Potato *Solanum tuberosum* belongs to the family *Solanaceae* which also includes tomato (*Lycopersicon esculentum*), eggplant (*Solanum melogena*), tobacco (*Nicotiana tabacum*) and pepper (*Capsicum annuum*). *S. tuberosum* is divided into two subspecies: *tuberosum* and *andigena*. The subspecies *tuberosum* is cultivated in Europe.[5]

Potato (*S. tuberosum*) is not able to form feral populations. In the Netherlands wild relatives of the potato are present, including black nightshade (*Solanum nigrum*), bittersweet (*S. dulcamara*), hairy nightshade (*S. physalifolium*) and cutleaf nightshade (*S. triflorum*).[5,6] Outcrossing between *S. tuberosum* and these wild relatives is not possible since efficient incompatibility barriers prevent hybridization.[7] In the Netherlands *S. tuberosum* is an important agricultural crop species of which the different varieties can be divided into potatoes for starch production and potatoes for human consumption.

Potato can disseminate and survive through pollen, seed and tubers. Tubers are sensitive to frost and are usually unable to survive Dutch winters. Only during mild winters or in the presence of an isolating cover of snow, tubers might be able to survive and generate seedlings or volunteers in the next year.[7]

Potato pollen is relatively heavy and is spread via wind and insects. Potato can reproduce sexually by cross- or self-pollination. Under field conditions 80 to 100% of the seeds are formed due to selfing.[8] Outcrossing to other cultivated potato species is possible but reduces markedly with increased distance. Several studies have shown that outcrossing barely occurs after ten meters.[9,10,11]

Studies have shown that viable seeds can stay behind after harvest. A small part of these seeds is able to survive the winter and germinate the next year. However, potato seedlings are fragile and are in competitive disadvantage with other plants.[12] In addition, routine agricultural practices like ploughing, harrowing and herbicide application eliminate emerging volunteer plants from seed. Therefore, they usually do not survive. Furthermore, remaining seedlings in the field will be eliminated due to compulsory measures to control potato blight.

Molecular characterization

The genetically modified potato AV43-6-G7 was produced by *A. tumefaciens*-mediated transformation of internodal stem segments of potato cultivar Karnico with binary vector

pKGBA50mf-IR1.1. This vector consists of a T-DNA region and the vector backbone. The T-DNA region contains three cDNA sequences derived from the Granule Bound Starch Synthase I (*GBSSI*) gene isolated from potato. The GBSSI enzyme is involved in the synthesis of amylose. The first *GBSSI* sequence in the T-DNA region is the 851 bp *GBSSI* promoter sequence. This sequence gives strong expression in tubers, pollen and root tips. The second sequence is the 1.1 kb 5' part of the *GBSSI* gene cloned in sense orientation downstream of the *GBSSI* promoter. The third sequence is the complete *GBSSI* cDNA, cloned in antisense orientation downstream of the 1.1 kb sense part of the cDNA. This third sequence consists of the 1.3 kb 3' part of the cDNA (which functions as a spacer sequence) and the 1.1 kb 5' part of the cDNA. The result of this cloning is an inverted repeat of the 5' part of the *GBSSI* cDNA. The RNA originating from this inverted repeat forms a hairpin which is subsequently cleaved into small RNA fragments. These RNA fragments will not be translated into a protein, but will lead to RNA interference which results in the degradation of the endogenous *GBSSI* mRNA. Due to this degradation process no GBSSI protein is formed and the production of amylose is inhibited. A complete overview of the introduced sequences is given below:

- pTiT37 fragment with right border sequence*;*
- M13mp19 fragment from the M13 phage genome;
- M13mp19 fragment, part of the Lac operon (*lacI*);
- Genomic *GBSSI* fragment (*PGBSSI*) functional as a promoter in plants;
- Polylinker sequence;
- *GBSSI* cDNA fragment; 5' 1.1 kb part, sense orientation in relation to the promoter sequence;
- Polylinker sequence;
- *GBSSI* cDNA fragment; 3' 1.3 kb part, antisense orientation in relation to the promoter sequence;
- *GBSSI* cDNA fragment; 5' 1.1 kb part, antisense orientation in relation to the promoter sequence;
- Polylinker sequence;
- pTiT37 fragment containing the nopaline synthase gene terminator (T-NOS), functional as a polyadenylation sequence in plants;
- Polylinker sequence;
- M13mp19 fragment part of the Lac operon (*lacZ*);
- M13mp19 fragment contains the M13 ori;
- M13mp19 fragment part of the Lac operon (*LacI*);
- pTiT37 fragment including left border sequence.

Properties of the gene introduced in AV43-6-G7

The two major components of starch are amylose and amylopectin. Amylose consists of long linear chains of α-1,4 linked glucose residues with relatively few α-1,6 linked branches whereas

amylopectin is a highly branched molecule of shorter α-1,4 linked glucose molecules and more frequent α-1,6 branches.[13]

The genetically modified potato AV43-6-G7 contains an inverted repeat of the *GBSSI* cDNA. GBSSI is one of the key enzymes catalyzing the formation of amylose. As a consequence of the modification, the GBSSI protein is not formed and the production of amylose is inhibited. Therefore, AV43-6-G7 produces predominantly amylopectine. Pure amylopectin is used in, amongst other things, the paper and chemical industries.

Molecular analysis

The applicant showed by Southern blot analyses that one truncated copy of the T-DNA containing the GBSSI expression cassette was integrated at a single integration locus in the genome of AV43-6-G7. Furthermore, the applicant demonstrated by Southern blot analysis and PCR that the backbone of pKGBA50mf-IR1.1 was absent in AV43-6-G7. Therefore, the kanamycin resistance gene *nptIII* located on the backbone of the vector was not integrated in the AV43-6-G7 genome.

Sequence analyses spanning the 5' and 3' junctions of the insertion site and the genomic DNA revealed a 237 bp deletion at the right border side and a 1056 bp deletion at the left border side. These deletions do not affect the functioning of the transgene, since it was shown by Western blot analyses that neither mature GBSSI protein nor truncated GBSSI protein was observed in AV43-6-G7. This shows that silencing of the endogenous GBSSI gene is efficient.

Bioinformatic analysis of the entire sequence of the integrated T-DNA and the right and left border regions revealed twenty-eight open reading frames (ORFs). Homology searches were performed using the NCBI protein database (BLASTn, BLASTx, BLASTp) and the Allergen Online Database (v9.0). No matches with toxins or known allergens were found.

DNA sequences spanning the 5' and 3' junctions of the insertion site and the genomic DNA were analyzed from stop codon to stop codon. An additional eleven ORFs were identified. These have been examined for homology to toxic proteins in BLASTp and presence of linear allergenic epitopes in the FARPP allergen database. The results of these analyses revealed no sequence similarities between any known toxins or allergens and the eleven putative polypeptides.

COGEM is of the opinion that the molecular characterization of AV43-6-G7 potato has been adequately performed and meets the criteria laid down by COGEM.[14]

Field trials

AV43-6-G7 potato has been tested in field trials at eight locations in the North-Eastern part of the Netherlands during the period 2004-2007. According to the applicant, growth characteristics such as emergence, development, flowering and maturity were not different for AV43-6-G7 potato compared to its parent Karnico. Also, there was no statistically significant difference in resistance against a number of diseases including late blight and wart disease.

S. tuberosum is cold sensitive for temperatures below zero degrees Celsius.[8] Previous experiments have shown that a lower amylose content has no effect on frost sensitivity of *S. tuberosum*.[4,15] Based on these results, COGEM is of the opinion that this conclusion extends to all genetically modified potatoes with a lower amylose content, including AV43-6-G7 potato.[16]

General surveillance

General surveillance has been introduced to be able to observe unexpected adverse effects of genetically modified crops on the environment. The setting or population in which these effects might occur is either not, or hardly predictable. The central tool for general surveillance in case of cultivation of AV43-6-G7 potato is the so-called *'Optimeel registration system'* which includes a questionnaire. According to the applicant, Optimeel comprises since 1998 detailed monitoring and reporting of starch potato cultivation and represents the baseline for starch potato cultivation. Comparison with such a baseline allows monitoring, recording and detecting of unexpected deviations as a result of growing any new cultivar and more in particular AV43-6-G7 potato. Use of this form will be mandatory for all AV43-6-G7 farmers, both in seed tuber multiplication and actual starch production. According to the applicant, special attention will be drawn to characteristics which are not already covered by Optimeel. For this purpose Optimeel will be extended with additional items, including the observation of unexpected effects in relation to animals feeding on the crop and the observation of deviant potato plants. COGEM is of the opinion that the general surveillance plan is sufficient for cultivation of AV43-6-G7 potato.

Advice

COGEM has been asked to advice on the cultivation of AV43-6-G7 potato. This potato expresses an inverted repeat of the *GBSSI* cDNA which leads to silencing of the endogenous GBSSI gene. This modification prevents the formation of amylose. As a result the potato produces predominantly amylopectin. In the past COGEM issued several positive advices concerning field trials with AV43-6-G7 potato.

Potato (*S. tuberosum*) does not establish outside cultivated areas. There are no reasons to assume that the inserted trait has an effect on the persistence or invasiveness of AV43-6-G7 potato. Therefore COGEM is of the opinion that the introduced sequences do not lead to an increased persistence and invasiveness. In the Netherlands, outcrossing between AV43-6-G7 and its wild relatives including black nightshade, bittersweet, hairy nightshade and cutleaf nightshade is not possible. In addition the molecular analysis of AV43-6-G7 potato is adequate.

Based on the aspects discussed, COGEM is of the opinion that cultivation AV43-6-G7 potato poses a negligible risk to human health and the environment.

A food/feed safety assessment is carried out by other organizations. Therefore, COGEM abstained from advice on the potential risks of incidental consumption.

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