COMMISSIE
COGEM
GENETISCHE
MODIFICATIE

Aan de minister van
Volkshuisvesting, Ruimtelijke
Ordening en Milieubeheer
Mevrouw dr. J.M. Cramer
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DATUM 4 mei 2009
KENMERK CGM/090504-06
ONDERWERP Advies "Import, distribution and retail of GM carnation IFD-26407-2"

Geachte mevrouw Cramer,

Naar aanleiding van een adviesvraag over het dossier C/NL/09/02.co1 betreffende de aanvraag voor een vergunning voor import, distributie en verkoop van snijbloemen van de genetisch gemodificeerde anjervariëteit IFD-26407-2 van Florigene Ltd., deelt de COGEM u het volgende mee.

Samenvatting
De COGEM is gevraagd te adviseren over de vergunning voor import, distributie en verkoop van snijbloemen van de genetisch gemodificeerde (gg-) anjervariëteit IFD-26407-2 in Europa.
Anjers kunnen de blauwe kleurstof die zorgt voor een paarsblauwe bloemkleur van nature niet produceren. Door toevoeging van twee genen kan de gg-anjer IFD-26407-2 dit pigment wel maken waardoor deze anjer paars gekleurde bloemen heeft.
Anjer is niet in staat tot verwildering. De ingebrachte genen hebben geen invloed op het verwilderingspotentieel. Spontane kruisbestuiving met verwante soorten is niet mogelijk. Verspreiding van genen van de gg-anjer naar verwante soorten in de natuur is derhalve uitgesloten.
Bloemblaadjes van anjers worden in zeldzame gevallen gebruikt in gerechten of als garnering. De gg-anjer mag onder de aangevraagde vergunning niet worden gebruikt voor voedseldoeleinden. De COGEM is van mening dat er geen reden is om aan te nemen dat een uitzonderlijk geval van incidentele consumptie risico's met zich mee zou brengen.
Gezien het bovenstaande acht de COGEM de risico's voor mens en milieu bij import van snijbloemen van de genetisch gemodificeerde paarse anjer IFD-26407-2 verwaarloosbaar klein.

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

Hoogachtend,

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Voorzitter COGEM

c.c. Drs. H.P. de Wijs
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Import, distribution and retail of GM carnation IFD-26407-2

COGEM advice CGM/090504-06

Summary

The present application by Florigene Ltd. of file C/NL/09/02.co1, concerns the authorization for the import, distribution and retail of genetically modified (GM) carnation variety IFD-26407-2.

Carnation does not have weedy characteristics and although carnation is grown for centuries it has never been found in the wild. The GM carnation variety IFD-26407-2 has a modified flower color and is tolerant to sulfonylurea herbicides. These traits are not associated with a potential for weediness.

Carnation is not able to fertilize wild relatives spontaneously and, therefore, the risk of transfer of the introduced traits to related species is negligible. Formation of seed on cut flowers is highly improbable.

In rare cases petals of carnation are used in dishes or as garnishing. The application does not concern an authorization for use as food. COGEM is of the opinion that there are no reasons to assume that a rare case of incidental consumption will pose a risk to human health.

In view of the aforementioned, COGEM is of the opinion that the risks for the environment and human health resulting from import of cut flowers of GM carnation variety IFD-26407-2 are negligible.

Introduction

The present application concerns the genetically modified (GM) carnation (*Dianthus caryophyllus*) variety IFD-26407-2. IFD-26407-2 expresses the genes *Cytb5*, *f3'5'h* and *suRB* resulting in a modified flower color and tolerance to sulfonylurea herbicides.

Previous COGEM advices

COGEM issued positive advices on several other modified carnation varieties with an altered flower color.[1,2,3,4,5] Some of these carnation varieties were authorized in Europe. The GM carnation variety 'Moonshadow' was authorized for production, import, distribution and retail in 1998 and grown in Europe from December 1998 until July 1999. Recently, COGEM issued a positive advice concerning the renewal of the authorization for import, distribution and retail of this GM carnation.[5] In 1997 'Moondust' was authorized for production, import, distribution and retail, this variety was grown in Europe between 1998 and 2000. 'Moonlite' was authorized for import, distribution and retail in 2007. The authorization for import of another GM carnation variety 'Moonaqua' is currently under consideration in the European Union.[3,6]

Carnation, aspects of the crop

Carnation belongs to the species *Dianthus caryophyllus* of the widely cultivated genus *Dianthus*. The non-horticultural single-flower form of *D. caryophyllus* (the 'clove pink') is native to southern Europe where it grows on walls, in rock crevices and on dry stony slopes around the Mediterranean coastal regions.[7] *D. caryophyllus* has occasionally been found naturalized in the United Kingdom.

The nomenclature is somewhat confusing. Nowadays the common name of *D. caryophyllus* is carnation. However, some carnations are known as 'pinks' and the term carnation is sometimes used to indicate other *Dianthus* species. Moreover, some cultivated carnations are hybrids with *D. plumarius*.[7] This application concerns a cultivated double-flowered carnation (*D. caryophyllus*) variety.

Carnations have been cultured for hundreds of years and are presently amongst the most extensively grown cut flowers with more than ten billion carnations produced around the world each year. Carnations are sold as cut flowers, cuttings or plants. Cultivated carnation is not propagated by seed but vegetatively by cuttings and tissue culture. Propagation in the horticulture involves the use of so-called mother plants.[7] Cuttings of these mother plants are used for the production of flowers for a period of two years. Carnation does not spread vegetatively spontaneously, and it does not produce vegetative organs like bulbs, stolons or rhizomes.

Carnation is highly domesticated by generations of breeding aimed at improvement of flower size and color variation. Carnation is semi-winter hardy[7], has no weedy characteristics and after decades of cultivation it is not able to establish itself in the wild.

Cross-pollination of wild *D. caryophyllus* depends on lepidopteran insects.[8] The domesticated carnation produces little pollen with reduced viability.[8] In the wild, breeding has increased the number of petals present in carnation cultivars. As a result the reproductive tissues of the flower have become enclosed, restricting access to insect pollinators.[8]

Aspects of wild *Dianthus* species

Wild *Dianthus* species occur worldwide.[9] In Europe, *Dianthus* species are found in mountainous areas like the alpine region, mainly in the Balkan and the Mediterranean area.[8] In the Netherlands, some rare *Dianthus* species occur: *D. deltoides* (steenanjer; maiden pink), *D. armeria* (ruige anjer; Deptford pink), *D. superbus*, (prachtanjer; large pink) and *D. carthusianorum* (Kartuizer anjer; Carthusian pink).[10] The species *D. barbatus* (duizendschoon; sweet William) is commonly grown as a garden plant and has established itself in the wild.[10]

Pollination of *Dianthus* in nature occurs exclusively by lepidopteran insects. The nectaries are at the base of the flowers and only insects with a proboscis longer than 2.5 cm can reach them. The number of insects visiting the carnation flower is further limited due to the fact that carnation cultivars have a long distance between the edge of the petals and the nectary, causing difficulty for insects to extract the nectar. *Dianthus* species are protrandous, which means that the anthers and pollen mature before the pistils. Pollen shedding takes place at the opening of the flower. As the flower ages the anthers fall off and the styles become receptive.[8]

It is theoretically possible for carnation to cross-hybridise with other *Dianthus* species and interspecific crossings haven been made manually by breeders to introduce new traits into carnation.[8,9,11] However, spontaneous hybridisation between cultivated carnation and wild *Dianthus* species has never been reported, despite decades of cultivation in gardens and parks.

Molecular characterisation

Origin and function of the introduced genes

The GM carnation variety IFD-26407-2 was produced by *Agrobacterium tumefaciens* mediated transformation using the disarmed *A. tumefaciens* strain AGL0 and the transformation vector pCGP2355.

The insertion cassette of the transformation vector pCGP2355 contained the following sequences:

- LB left border region, derived from the Ti plasmid of *A. tumefaciens*
- 35S constitutive promoter, derived from *Cauliflower mosaic virus* (CaMV)
- Cab 5'UTR, 5'untranslated region of the chlorophyll a/b binding protein, derived from *Petunia* x *hybrida* cDNA
- *suRB* gene and its terminator, derived from *Nicotiana tabacum* and encoding acetolactate synthase (ALS)
- CHS promoter, petal specific promoter, derived from the chalcone synthase (CHS) gene from *Antirrhinum majus* (snapdragon)
- Cytochrome b5 enzyme from *Petunia x hybrida* to increase F3'5'H activity
- D8 terminator, derived from a putative phospholipid transfer protein homologue ('D8') from *Petunia* x *hybrida*
- ANS promoter, flavonoid promoter from anthocynidin synthase from *Dianthus caryophyllus*
- *f3'5'h* cDNA, derived from *Petunia x hybrida* and encoding the flavonoid 3'5'hydroxylase protein
- *ANS terminator,* Flanonoid pathway terminator from anthocyanidin synthase from *Dianthus caryophyllus*
- RB, right border region, derived from *A. tumefaciens*

Properties of the introduced genes resulting in a modified flower color

Carnations cannot produce the blue pigment delphinidin because part of the anthocyanin biosynthetic pathway is absent. Therefore, it is impossible to produce blue carnations by traditional breeding methods.

Introduction of the *f3'5'h* gene in IFD26407-2 enables the production of the blue pigment delphinidin. The *f3'5'h* gene encodes the flavonoid 3'5' hydroxylase (F3'5'H) enzyme which converts dihydrokaempferol (DHK) to dihydromyricetin (DHM).[12] Both products can be used as substrates by the dihydroflavonol 4-reductase (DFR) enzyme of carnation. The orange/red pigment pelargonidin is produced if DHK is converted and the blue pigment delphinidin is produced if DHM is converted.

The *Cytb5* gene that was introduced in IFD26407-2 encodes cytochrome b5 which increases the activity of F3'5'H.[13]An increased activity of F3'5'H results in a higher production of DHM and therefore in a higher amount of delphinidin.

Properties of the introduced gene conferring herbicide tolerance

The *suRB* gene has been introduced in the GM carnation variety IFD-26407-2 to allow the selection of genetically modified plants in the transformation process. The *suRB* gene encodes a mutant acetolactate synthase (ALS) protein which confers tolerance to ALS inhibiting (sulfonylurea) herbicides.

ALS inhibiting herbicides bind to the ALS enzyme which is required for the production of branched chain amino acids (valine, leucine and isoleucine).[14] This results in the production of reduced quantities of branched chain amino acids and a shortage of these amino acids. This shortage leads to rapid inhibition of cell division and subsequently to plant death.[14] The *suRB* gene encodes an ALS protein that is insensitive to sulfonylurea herbicides thus conferring tolerance to these herbicides.

Molecular analysis

The applicant demonstrated by Southern blot hybridization that vector backbone sequences (including the tetracycline resistance gene) are not present in IFD-26407-2. The absence of the tetracycline resistance gene was also confirmed by PCR analysis.

In addition, hybridizations with eleven different probes show that IFD-26407-2 contains one insert with one copy of the different elements. The insert and 150bp of its flanking regions were sequenced. The junctions between the insert and its flanking regions were analyzed for the presence of putative open reading frames (ORFs). Nine ORFs were translated *in silico* and analyzed for similarity with known allergens or toxins. No similarities with known toxins were found. The similarity search with the allergen database resulted in two amino acid sequences of six amino acids which were identical to amino acid sequences of known allergens. Hydrophilicity plots revealed that both deduced amino acid groups are most likely located at the internal region of the putative proteins and not on external regions. The applicant concluded that the sequences identified did not correspond to the hydrophilic and thus antigenic regions of the allergenic proteins.

COGEM points out that using a similarity search with a sequence of six amino acids frequently results in a high number of false-positive matches. Recently, COGEM reconsidered the elements of the molecular characterization which are needed for commercial releases of genetically modified crops.[15] The molecular characterization of IFD-26407-2 fulfils the requirements of COGEM for the molecular characterization. In this report, COGEM stated that the flanking regions should be analyzed for the presence of putative open reading frames from stop to stop codon with a minimum length of eight amino acids. This minimum is based on the average epitope length for allergens[16]. The applicant used a minimum length of six amino acids for the analysis of the GM carnation IFD-26407-2.

Several publications have demonstrated that matches of six amino acids with any allergen occurs frequently by chance, thus resulting in false positives, which limit the suitability for predicting allergenicity[16,17,18]. In the opinion of COGEM a group of six amino acids is not considered to provide significant value for the bioinformatics evaluation of novel proteins in a safety assessment. Summarizing, COGEM is of the opinion that it is highly unlikely that the modification of IFD-26407-2 will lead to the production of proteins with a significant similarity to known toxins or allergens.

Advice

This application concerns the import of cut flowers of the GM carnation variety IFD-26407-2. A General Surveillance plan to observe and register adverse effects of the import of IFD-26407-2 was provided. COGEM considers the General Surveillance plan provided sufficient for import, distribution and retail of the GM carnation variety IFD-26407-2.

Carnation is not able to spread vegetatively and cut flowers are not able to form roots. This excludes the possibility that the imported material will give rise to plants and establish itself in the wild. Nevertheless, carnation can be propagated by stem cuttings, a method used both by professionals in the flower industry and amateur gardeners. Therefore, it cannot be completely ruled out that buyers will propagate the material to plant in their gardens. However, carnation has no weedy characteristics.[8] Although carnation is cultivated for decades, it has never been found growing in the wild. The introduced traits (modified flower color and herbicide tolerance) do not introduce a potential for weediness.

Formation of seed on cut flowers is highly improbable. Carnation is pollinated exclusively by butterflies or moths. Outcrossing during production or transport is unlikely as flowers are cut before opening and transported refrigerated. Theoretically, it is possible that cut flowers in the vase are pollinated by butterflies. Carnation plants require five to six weeks for seed development while the vase life of carnation flowers is only three to four weeks. Therefore, it is improbable that cut flowers will produce seed.

Carnation can only theoretically hybridize with wild relatives. Although, theoretically, it is possible that cut flowers in a vase are visited by butterflies, it is unlikely that they will distribute viable pollen. Carnation produces only a few anthers and little pollen with a reduced viability. Pollen shedding only takes place at the opening of the flower. The applicant compared IFD-26407-2 to its parental line and reported that IFD-26407-2 has on average longer styles, less viable anthers and more and shorter filaments. In view of the general characteristics of carnation and the data provided on IFD-26407-2, the possibility of hybridisation with wild relatives is considered unlikely. Most importantly, there has never been any evidence of spontaneous hybridisation between carnation and wild *Dianthus* species, despite the fact that carnation is cultivated worldwide for decades.

Moreover, the environmental risks linked to hybridization of this GM carnation variety with wild relatives are comparable with those of conventional carnation. The genetic modification involves genes which play a role in the anthocyanin pathway. The resulting blue pigmentation does not alter the ecological characteristics of carnation. Neither the *f3'5'h* gene, the *cytochrome b5* gene nor the herbicide tolerance gene *suRB* offers selectable advantages in nature. Accordingly, gene flow to wild relatives will not pose an environmental risk.

Therefore, COGEM concludes that the risk of transfer of genetic traits from the transgenic carnation variety to species in unmanaged environments is insignificant.

In rare cases petals of carnation are used in dishes and as garnishing.[19,20,21] This notification refers to the import and distribution of cut flowers and not to food purposes. Therefore, retailers will not be allowed to sell the petals of the GM carnation for food purposes. However, it cannot be entirely excluded that individuals will use petals of bought flowers in dishes or to garnish their plates. In general, people are advised against using flowers from flower shops or commercial

growers for food purposes because these might contain residues from pesticides or other chemicals.

The GM carnation variety IFD-26407-2 expresses the *Cytb5, f3'5'h* and *als* (*suRB*) genes. The *Cytb5* and *f'3'5'h* genes have been introduced to produce delphinidin. Delphinidin is also produced in fruits like blueberries. In addition, the applicant provides evidence that the ALS protein that is encoded by the *als (suRB)* gene is not allergenic or toxic. In view of the above, COGEM is of the opinion that there are no reasons to assume that a rare case of incidental consumption will pose a risk.

Summarizing, the application involves import of cut flowers. The GM carnation has no weedy characteristics and is not able to establish itself in the wild, the risk of transfer of the introduced genes to wild relatives is negligible, and the GM-variety does not pose a threat to the health of consumers. In view of the above mentioned, COGEM is of the opinion that the proposed import of cut flowers of carnation variety IFD-26407-2 poses a negligible risk to human health or the environment.

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