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**DATUM** 19 oktober 2018  
**KENMERK** CGM/181019-01  
**ONDERWERP** Advies import en verwerking van gg-maïs DP4114xMON810xMIR604xNK603

Geachte mevrouw Van Veldhoven,

Naar aanleiding van een vergunningaanvraag voor import en verwerking van genetisch gemodificeerde maïs DP4114xMON810xMIR604xNK603 (EFSA/GMO/NL/2018/150), ingediend door Pioneer Hi-Bred International, deelt de COGEM u het volgende mee.

**Samenvatting:**

De COGEM is gevraagd te adviseren over de mogelijke milieurisico's van import en verwerking van de genetisch gemodificeerde (gg-) maïs DP4114xMON810xMIR604xNK603 en alle subcombinaties daarvan. In deze gg-maïs komen de genen *pat* en *cp4 epsps* tot expressie, waardoor de plant tolerant is voor bepaalde herbiciden. Ook komen de genen *cry1F*, *cry34Ab1*, *cry35Ab1*, *cry1Ab* en *mcry3A* tot expressie, waardoor de plant resistent is tegen bepaalde plaaginsecten die behoren tot de vlinder- en keverachtigen. De kruisingslijn bevat ook een *pmi* gen. Dit gen zorgt ervoor dat bij het ontwikkelen van gg-lijnen, na transformatie, gg-plantencellen gemakkelijk geselecteerd kunnen worden.

Verwildering van maïsplanten is in Nederland nooit waargenomen. Maïsplanten uit gemorst zaad (opslagplanten) worden hier nauwelijks aangetroffen. Bovendien zijn er in Nederland geen wilde verwanten van maïs aanwezig, waardoor de ingebrachte sequenties zich niet naar andere soorten kunnen verspreiden.

De moleculaire karakterisering van DP4114xMON810xMIR604xNK603 is voldoende. Er zijn geen redenen om aan te nemen dat expressie van de ingebrachte genen ervoor zorgt dat deze gg-maïs zou kunnen verwilderen. Gezien het bovenstaande acht de COGEM de milieurisico's van de import en verwerking van de gg-maïs DP4114xMON810xMIR604xNK603, en subcombinaties hiervan, verwaarloosbaar klein. Omdat een voedselveiligheidsbeoordeling door andere instanties wordt uitgevoerd, heeft de COGEM bij deze vergunningaanvraag de risico's van incidentele consumptie niet beoordeeld.

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

Hoogachtend,



Prof. dr. ing. Sybe Schaap  
Voorzitter COGEM

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                  Mr. J.K.B.H. Kwisthout, Ministerie van IenW  
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## **Import and processing of genetically modified maize DP4114xMON810xMIR604xNK603 and sub-combinations**

### **COGEM advice CGM/181019-01**

- The present application (EFSA/GMO/NL/2018/150) concerns the authorisation for import and processing for use in feed and food of genetically modified (GM) maize DP4114xMON810xMIR604xNK603 and its sub-combinations;
- Maize DP4114xMON810xMIR604xNK603 was produced by conventional crossbreeding of the four GM parental maize lines;
- Previously, COGEM advised positively on the import and processing of all four parental lines;
  
- The molecular characterisation of DP4114xMON810xMIR604xNK603 has been updated and is adequate;
- The updated bioinformatics analyses do not provide indications for environmental risks;
  
- The GM maize expresses the genes *cry1Ab*, *cry1F*, *cry34Ab1*, *cry35Ab1*, *mcry3A*, *cp4 epsps*, *pat* and *mir604pmi*;
- It is resistant to certain lepidopteran and coleopteran insects, tolerant to glyphosate and glufosinate-ammonium containing herbicides, and able to use mannose as a carbon source;
  
- In the Netherlands, feral maize populations have never been observed and the appearance of volunteers is rare;
- In the Netherlands, wild relatives of maize are absent and hybridisation of maize with other species is therefore not possible;
  
- There are no indications that the introduced traits allow DP4114xMON810xMIR604xNK603 to survive in the Netherlands;
- There are no indications that DP4114xMON810xMIR604xNK603 could establish feral populations in the Netherlands;
  
- COGEM is of the opinion that import and processing of maize DP4114xMON810xMIR604xNK603 and its sub-combinations pose a negligible risk to the environment in the Netherlands;
  
- COGEM abstains from giving advice on the potential risks of incidental consumption since a food/feed assessment is carried out by other organisations.

## 1. Introduction

The present application (EFSA/GMO/NL/2018/150) filed by Pioneer Hi-Bred International concerns import and processing of DP4114xMON810xMIR604xNK603 maize and genetically modified (GM) maize consisting of its sub-combinations. The GM maize was produced by conventional crossbreeding of the four genetically modified (GM) parental maize lines. It expresses the *pat* and *cp4 epsps* genes conferring tolerance to glyphosate and glufosinate-ammonium containing herbicides, and the *cry1Ab*, *cry34Ab1*, *cry35Ab1*, *cry1F* and *mcry3A* genes resulting in resistance to certain lepidopteran and coleopteran insects. In addition, it expresses the *pmi* gene. As a result transformed plant cells are able to use mannose as a carbon source.

Parental lines MON810, MIR604, NK603, and stacked event MON810xNK603 have been authorised for import and processing for use in food and feed in the European Union.<sup>1,2,3,4,5</sup> Parental line DP4114 is not yet authorised, however, the line has been assessed for import and processing for use in food and feed by the European Food Safety Authority (EFSA). EFSA concluded that in the scope of the application maize DP4114 is as safe as its conventional counterpart with respect to potential effects on human and animal health and the environment.<sup>6</sup>

## 2. Previous COGEM advices

Previously, COGEM positively advised on import and processing of the parental lines DP4114, MON810, MIR604, NK603, and on the stacked event NK603xMON810.<sup>7,8,9,10,11</sup> The environmental risks of import and processing of these events were considered negligible. Additionally, COGEM advised positively on cultivation of MON810, MIR604, NK603, and NK603xMON810, and considered the environmental risks of cultivation of these events negligible.<sup>12,13,14,15,16,17</sup>

## 3. Environmental risk assessment

Potential environmental risks of DP4114xMON810xMIR604xNK603 maize and of GM maize consisting of its sub-combinations are assessed as part of the environmental risk assessment carried out by COGEM.

### 3.1 Characteristics of maize

Maize (*Zea mays*) is a member of the grass family *Poaceae*. It is a highly domesticated crop originating from Central America, but nowadays cultivated globally. Maize is wind pollinated,<sup>18,19</sup> and has both male and female flowers that are spatially separated. The female flowers are not attractive to insect pollinators, because they do not produce nectar. Insect pollination of maize is probably highly limited but cannot be excluded.<sup>20</sup>

Recently, the wild relative of maize teosinte was reported as a weed in maize fields in Spain<sup>21,22,23</sup> and France.<sup>24,25</sup> In the Netherlands, no wild relatives of maize are present and hybridisation with other species cannot occur.

Maize requires warm conditions in order to grow and does not tolerate prolonged cold and frost.<sup>20,26</sup> In cultivation areas with warm climatic conditions, the appearance of volunteers can occur

the year following maize cultivation due to spilled cobs or kernels. However, these volunteers are usually killed by common mechanical pre-planting soil preparation practices.<sup>20</sup>

Maize is very sensitive to weed competition.<sup>27</sup> During the long process of domestication, maize has lost the ability to persist in the wild.<sup>19</sup> A soil seed bank, small seeds, and an extended period of flowering and seed production are characteristics often observed in persistent weeds.<sup>28</sup> Maize lacks all these characteristics. After ripening, the seeds (the kernels) adhere to the cob and do not shatter naturally.<sup>20,29</sup> Consequently, seed dispersal is severely hampered.

During field observations in Austria some volunteers and maize plants were observed in non-agricultural habitats.<sup>30</sup> In the Netherlands, the appearance of volunteers is very rare, although maize plants occasionally have been observed outside agricultural fields.<sup>31</sup> COGEM is not aware of any reports of feral maize populations in the Netherlands or elsewhere in Europe.

**Conclusion:** In the Netherlands, feral maize populations do not occur and hybridisation of maize with other species is not possible.

### 3.2 Description of the introduced genes, traits and regulatory elements

Maize DP4114xMON810xMIR604xNK603 was created by conventional crossbreeding of the parental lines. For a description of the parental lines, see previous COGEM advices.<sup>7,8,9,10,11</sup> A description of the inserted genetic elements is listed in the table below. The list is limited to information on the introduced genes, corresponding traits, and regulatory elements (promoters and terminators).

Introduced genes	Encoded proteins	Traits	Regulatory elements
<i>cry1F</i> (DP4114)	Truncated Cry1F protein originating from <i>Bacillus thuringiensis</i> var. <i>aizawai</i> <sup>32</sup>	Resistance to certain lepidopteran insects	Polyubiquitin ( <i>ubiZM1</i> ) promoter and intron from <i>Zea mays</i> and ORF25 terminator from <i>Agrobacterium tumefaciens</i> strain pTi15955
<i>cry34Ab1</i> (DP4114)	Cry34Ab1 protein originating from <i>B. thuringiensis</i> strain PS149B1 <sup>33,34,35,36,37</sup>	Resistance to certain coleopteran insects	<i>UbiZM1</i> promoter and intron from <i>Z. mays</i> and proteinase inhibitor II ( <i>pinII</i> ) terminator from <i>Solanum tuberosum</i>
<i>cry35Ab1</i> (DP4114)	Cry35Ab1 protein originating from <i>B. thuringiensis</i> strain PS149B1 <sup>33,34,35,36,37</sup>	Resistance to certain coleopteran insects	TA Peroxidase promoter from <i>Triticum aestivum</i> and <i>pinII</i> terminator from <i>S. tuberosum</i>
<i>pat</i> (DP4114)	Plant optimised version of phosphinothricin acetyl transferase (PAT) from <i>Streptomyces</i>	Tolerance to glufosinate-ammonium containing herbicides	35S promoter and 35S terminator from <i>Cauliflower mosaic virus</i> (CaMV)

Introduced genes	Encoded proteins	Traits	Regulatory elements
	<i>viridochromogenes</i> strain Tü 494 <sup>38,39,40</sup>		
<i>mcry3A</i> (MIR604)	Variant of the Cry3A protein derived from <i>B. thuringiensis</i> subsp. <i>tenebrionis</i> <sup>41,42,43</sup>	Resistance against certain coleopteran insects	Metallothionein-like ( <i>mtl</i> ) promoter from <i>Z. mays</i> and nopaline synthase ( <i>nos</i> ) terminator from <i>A. tumefaciens</i>
<i>mir604pmi</i> , also known as <i>manA</i> (MIR604)	Variant of phosphomannose isomerase (MIR604PMI) enzyme derived from <i>Escherichia coli</i> strain K12 <sup>44</sup>	Enables transformed plant cells to use mannose as a carbon source	Polyubiquitin promoter and intron ( <i>ZmUbiInt</i> ) from <i>Z. mays</i> and <i>nos</i> terminator from <i>A. tumefaciens</i>
<i>cry1Ab</i> (MON810)	Cry1Ab protein derived from <i>B. thuringiensis</i> subsp. <i>kurstaki</i> <sup>45,46</sup>	Resistance to certain lepidopteran insects	Enhanced 35S ( <i>e35S</i> ) promoter from CaMV; terminator is absent
<i>cp4 epsps</i> (NK603)	5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) from <i>Agrobacterium</i> sp. strain CP4 <sup>47</sup>	Tolerance to glyphosate containing herbicides	<i>Ract 1</i> promoter from <i>Oryza sativa</i> and <i>nos</i> terminator from <i>A. tumefaciens</i>
<i>cp4 epsps L214P</i> (NK603)	Variant of EPSPS from <i>Agrobacterium</i> sp. strain CP4 <sup>47</sup>	Tolerance to glyphosate containing herbicides	<i>E35S</i> promoter from CaMV and <i>nos</i> terminator from <i>A. tumefaciens</i>
<i>See references for a detailed description of the traits</i>			

### 3.3 Molecular characterisation

Previously, COGEM evaluated the molecular characterisation of each parental line and considered these to be adequate.<sup>7,8,9,10</sup>

The applicant confirmed by Southern blot analysis that the stacked line contained the parental transgenic inserts of DP4114, MON810, MIR604, and NK603.

The applicant compared the DP4114, MON810, MIR604, and NK603 insert and flanking sequences in DP4114xMON810xMIR604xNK603 with the sequences of the corresponding single events. According to the applicant the DP4114, MON810, and NK603 inserts and flanking DNA regions in DP4114xMON810xMIR604xNK603 maize are identical to the DNA sequences determined previously for the respective single events.

For MIR604, sequencing data suggest an adenine insertion in a homopolymer region in the 5' genomic flanking sequence 833 to 842 basepairs from the insert. Homopolymers longer than eight base pairs have been associated with slipped-strand mispairing in PCR amplification and are technically challenging to sequence.<sup>48,49,50</sup> Such homopolymeric regions may result in sequence differences between PCR template and final PCR amplicon.

The applicant updated the bioinformatics analyses of the inherited inserted elements, and the sequences spanning the insertion sites and the 5' and 3' flanking regions using recent databases. DNA sequences spanning the 5' and 3' junctions of the inserts were translated *in silico* from stop to stop codon in all six reading frames. According to the applicant, no essential endogenous genes were disrupted at the insertion sites and the putative products of the open reading frames (ORFs) did not show significant protein sequence similarity with known allergens, toxins or other biologically active proteins.

COGEM notes that for the bioinformatics analyses of MIR604 the applicant does not clarify whether the sequence of the breeding stack was used, or the sequence of the single event. As mentioned above, the sequence of the breeding stack flanking at the 5' end of the insert suggests a nucleotide insertion compared to the single event sequence. Because the homopolymeric region is located considerably upstream of the insert, COGEM is of the opinion that the possible adenine insertion is not relevant for the risk assessment.

Considering the above, COGEM is of the opinion that the molecular characterisation of maize DP4114xMON810xMIR604xNK603 is adequate. The results from the updated molecular characterisation do not provide indications that DP4114xMON810xMIR604xNK603 could pose a risk to the environment.

**Conclusion:** The molecular characterisation of maize DP4114xMON810xMIR604xNK603 is adequate and no indications for potential environmental risks were identified.

### ***3.4 Phenotypic and agronomic characteristics***

Previously, COGEM evaluated the phenotypic and agronomic characteristics of each parental line of DP4114xMON810xMIR604xNK603, and found no deviations influencing the outcome of the environmental risk assessment.

The applicant analysed the phenotypic and agronomic characteristics of DP4114xMON810xMIR604xNK603 and noted that these, except for the introduced traits, are not different to those of its conventional counterpart, and are equivalent to the reference varieties, taking into account natural variation. The results of the phenotypic evaluation do not give reason to assume that the GM maize could pose an environmental risk.

COGEM notes that the GM maize is resistant to certain insect pests, which may increase its vigour if these pests are present. However, there are several characteristics which contribute to the absent invasiveness and persistence of maize. Examples of these characteristics are the structure of the corn cob (seed shed enclosed in husks), the poor dormancy of kernels and the frost-sensitivity of maize.<sup>26,51</sup> As persistence and invasiveness are controlled by so many other characteristics, there is no reason to assume that the introduced traits will allow the GM maize to establish in the Netherlands.

In conclusion, COGEM is of the opinion that there are no reasons to assume that the introduced traits in DP4114xMON810xMIR604xNK603 allow the GM maize to survive or establish in the Dutch environment.

**Conclusion:** There are no indications that the introduced traits allow DP4114xMON810xMIR604xNK603 to survive in the Netherlands. DP4114xMON810xMIR604xNK603 does not have an increased potential for the establishment of feral populations in the Netherlands.

#### **4. Food/feed assessment**

This application is submitted under Regulation (EC) 1829/2003, therefore a food/feed assessment is carried out by EFSA and national organisations involved in the assessment of food safety. In the Netherlands, RIKILT carries out a food and/or feed assessment for Regulation (EC) 1829/2003 applications. The outcome of the assessment by other organisations (EFSA, RIKILT) was not known when this advice was completed.

#### **5. Post-market environmental monitoring (PMEM)**

The applicant supplied a general surveillance plan as part of the PMEM. COGEM has published several recommendations for further improvement of the general surveillance (GS) plan,<sup>52,53</sup> but considers the current GS plan adequate for the import and processing of maize DP4114xMON810xMIR604xNK603.

#### **6. Overall conclusion**

There are no indications that expression of the introduced traits will alter the fitness of maize DP4114xMON810xMIR604xNK603 under natural conditions. COGEM is of the opinion that import and processing of DP4114xMON810xMIR604xNK603 maize and its sub-combinations pose a negligible risk to the environment in the Netherlands. COGEM abstains from giving advice on the potential risks of incidental consumption since other organisations carry out a food/feed assessment.

#### **References**

1. European Commission (2013). Commission Implementing Decision of 6 November 2013 authorising the placing on the market of pollen produced from maize MON 810 (MON-ØØ81Ø-6) pursuant to Regulation (EC) No 1829/2003 of the European Parliament and of the Council (2013/649/EU). Official Journal of the European Union. L 302/44
2. European Commission (2017). Commission Implementing Decision (EU) 2017/1207 of 4 July 2017 renewing the authorisation for the placing on the market of genetically modified maize MON 810 (MON-ØØ81Ø-6) products pursuant to Regulation (EC) No 1829/2003 of the European Parliament and of the Council (C(2017) 4453). Official Journal of the European Union. L 173/18



3. European Commission (2011). Commission Decision of 30 November 2009 authorising the placing on the market of products containing, consisting of, or produced from genetically modified maize MIR604 (SYN-IR604-5) pursuant to Regulation (EC) No 1829/2003 of the European Parliament and of the Council (2009/866/EC). Official Journal of the European Union. L 314/102
4. European Commission (2015). Commission Implementing Decision (EU) 2015/684 of 24 April 2015 authorising the placing on the market of genetically modified maize NK603 (MON-ØØ6Ø3-6) and renewing the existing maize NK603 (MON-ØØ6Ø3-6) products, pursuant to Regulation (EC) No 1829/2003 of the European Parliament and of the Council. (C(2015) 2753). Official Journal of the European Union. L 112/6
5. European Commission (2007). Commission Decision of 24 October 2007 authorising the placing on the market of products containing, consisting of, or produced from genetically modified maize NK603xMON810 (MON-ØØ6Ø3-6xMON-ØØ81Ø-6) pursuant to Regulation (EC) No 1829/2003 of the European Parliament and of the Council (2007/701/EC). Official Journal of the European Union. L 285/37
6. European Food Safety Authority (2018). Assessment of genetically modified maize 4114 for food and feed uses, under Regulation (EC) No 1829/2003 (application EFSA-GMO-NL-2014-123). Scientific Opinion. EFSA Journal 16: 5280
7. COGEM (2015). Import and processing of genetically modified maize 4114. COGEM advice CGM/150528-01.
8. COGEM (1996). Advies C/FR/95/12-07. COGEM advies CGM/960807-01 [In Dutch]
9. COGEM (2005). Import and processing of maize variety MIR604. COGEM advice CGM/051122-02
10. COGEM (2003). Markttoelating 'NK603 maize tolerant for glyphosate'. COGEM advies CGM/030319-08 [In Dutch]
11. COGEM (2017). Renewal of the authorisation for import and processing of genetically modified NK603xMON810 maize. COGEM advice CGM/170628-01
12. COGEM (2016). Application for cultivation of genetically modified maize MON810: assessment of COGEM advice in view of EFSA opinions. COGEM advice CGM/160929-01
13. COGEM (2011). Cultivation of insect resistant maize MIR604. COGEM advice CGM/110404-01
14. COGEM (2006). Cultivation of herbicide tolerant maize line NK603. COGEM advice CGM/060704-01
15. COGEM (2007). Cultivation of genetically modified maize line NK603 x MON810. COGEM advice CGM/070308-02
16. COGEM (2008). Reactie van de COGEM naar aanleiding van het Franse rapport getiteld 'Project d'avis sur la dissémination du MON810 sur le territoire français'. COGEM advies CGM/080131-04 [In Dutch]
17. COGEM (2008). Renewal application cultivation of genetically modified maize MON810. COGEM advice CGM/080414-01
18. Hin CJA (2001). Landbouwkundige risico's van uitkruising van GGO-gewassen. Centrum voor Landbouw en Milieu (CLM) [In Dutch]
19. Treu R & Emberlin J (2000). Pollen dispersal in the crops Maize (*Zea mays*), Oil Seed Rape (*Brassica napus* ssp. *oleifera*), Potatoes (*Solanum tuberosum*), Sugar Beet (*Beta vulgaris* ssp. *vulgaris*) and Wheat (*Triticum aestivum*). Evidence from Publications. Soil Association

20. Andersson M & Carmen de Vicente M (2010). Gene flow between crops and their wild relatives. The John Hopkins University Press, Baltimore, Maryland, The United States of America
21. Pardo G *et al.* (2014). El Teosinte (*Zea mays*, spp.). Cent. Sanid. y Certificación Veg. DGA [in Spanish]
22. Pardo G *et al.* (2015). Presencia de teosinte (*Zea* spp.) como mala hierba en los regadíos del valle del Ebro. XV Congr. la Soc. Española Malherbología La Malherbología y la Transf. tecnológica Sevilla, 417-424 [In Spanish]
23. Pardo G *et al.* (2016). El teosinte: descripción, situación actual en el valle del Ebro y resultados de los primeros ensayos. Vida Rural.408: 42-47 [In Spanish]
24. Agri79 (2013). Téosite - La téosite exige une vigilance toute particulière.  
<http://agri79.reussir.fr/public/index.php?a=article&codeArticle=JFNK3KKU> (visited: October 8th 2018) [In French]
25. Trtikova M *et al.* (2017). Teosinte in Europe - Searching for the origin of a novel weed. Sci. Rep. 7: 1560. doi: 10.1038/s41598-017-01478-w
26. Miedema P (1982). The effect of low temperature on *Zea mays*. Advances in Agronomy 35: 93-128
27. CAB International (2007). Crop Protection Compendium. *Zea mays* (maize). CD-ROM edition, Wallingford
28. Kos SP *et al.* (2012). Can transgenic crops go wild? A literature study on using plant traits for weediness pre-screening. COGEM research report CGM 2012-01
29. Organisation for Economic Cooperation and Development (OECD) (2003). Consensus document on the biology of *Zea mays* ssp. *mays* (Maize)
30. Pascher K (2016). Spread of volunteer and feral maize plants in Central Europe: recent data from Austria. Environ. Sci. Eur. 28: 30
31. van de Wiel CCM *et al.* (2011). Crop volunteers and climate change. Effects of future climate change on the occurrence of Maize, Sugar Beet and Potato volunteers in the Netherlands. COGEM research report 2011-11
32. US-EPA (U.S. Environmental Protection Agency; 2010). Biopesticide registration action document, Cry1Ab and Cry1F *Bacillus thuringiensis* (Bt) corn plant-incorporated protectants.  
[https://www3.epa.gov/pesticides/chem\\_search/reg\\_actions/registration/decision\\_PC-006481\\_1-Sep-10.pdf](https://www3.epa.gov/pesticides/chem_search/reg_actions/registration/decision_PC-006481_1-Sep-10.pdf) (visited: October 8th 2018)
33. Herman RA *et al.* (2002). Binary insecticidal crystal protein from *Bacillus thuringiensis*, strain PS149B1: effects of individual protein components and mixtures in laboratory bioassays. J. Econ. Entomol. 95: 635-639
34. Ellis RT *et al.* (2002). Novel *Bacillus thuringiensis* binary insecticidal crystal proteins active on Western corn rootworm, *Diabrotica virgifera virgifera* LeConte. Appl. Environ. Microbiol. 68: 1137-1145
35. Moellenbeck DJ *et al.* (2001). Insecticidal proteins from *Bacillus thuringiensis* protect corn from corn rootworms. Nat. Biotechnol. 19: 668-672
36. Kelker MS *et al.* (2014). Structural and biophysical characterization of *Bacillus thuringiensis* insecticidal proteins Cry34Ab1 and Cry35Ab1. PLoS ONE 9 doi: 10.1371/journal.pone.0112555

37. Narva KE *et al.* (2017). Safety considerations derived from Cry34Ab1/Cry35Ab1 structure and function. *J. Invertebr. Pathol.* 142: 27-33
38. Wohlleben W *et al.* (1988). Nucleotide sequence of the phosphinothricin N-acetyltransferase gene from *Streptomyces viridochromogenes* Tü494 and its expression in *Nicotiana glauca*. *Gene* 70: 25-37
39. Eckes P *et al.* (1989). A synthetic gene confers resistance to the broad spectrum herbicide L-phosphinothricin in plants. *J. Cell. Biochem. Suppl.* 13D, 334, Abstract M516
40. Organisation for Economic Cooperation and Development (OECD) (1999). Consensus document on general information concerning the genes and their enzymes that confer tolerance to phosphinothricin herbicide
41. Murray EE *et al.* (1989). Codon usage in plant genes. *Nucleic Acids Res.* 17: 477-498
42. Sekar V *et al.* (1987). Molecular cloning and characterization of the insecticidal crystal protein gene of *Bacillus thuringiensis* var. *tenebrionis*. *PNAS* 84: 7036-7040
43. Chen E & Stacy C (2007). Modified Cry3A toxins. Syngenta Participations Ag, assignee. U.S. Patent No. 7,276,583. Washington, DC: U.S. Patent Office
44. Negrotto M *et al.* (2000). The use of phosphomannose-isomerase as a selectable marker to recover transgenic maize plants (*Zea mays* L.) via *Agrobacterium* transformation. *Plant Cell Reports* 19: 798-803
45. Fischhoff DA *et al.* (1987). Insect tolerant transgenic tomato plants. *Nat. Biotechnol.* 5: 807-813
46. Höfte H & Whiteley HR (1989). Insecticidal crystal proteins of *Bacillus thuringiensis*. *Microbiol. Rev.* 53: 242-255
47. Funke T *et al.* (2006). Molecular basis for the herbicide resistance of Roundup Ready crops. *PNAS* 103: 13010-13015
48. Shinde D *et al.* (2003). Taq DNA polymerase slippage mutation rates measured by PCR and quasi-likelihood analysis: (CA/GT)<sub>n</sub> and (A/T)<sub>n</sub> microsatellites. *Nucleic Acids Res.* 31: 974-980
49. Fazekas AJ *et al.* (2010). Improving sequencing quality from PCR products containing long mononucleotide repeats. *BioTechniques* 48: 277-285
50. Clarke LA *et al.* (2001). PCR amplification introduces errors into mononucleotide and dinucleotide repeat sequences. *Mol. Pathol.* 54: 351-353
51. OECD (2003). Consensus document on the biology of *Zea mays* subsp. *mays* (Maize)
52. COGEM (2010). General Surveillance. COGEM topic report CGM/100226-01
53. COGEM (2015). Advice on improving the general surveillance of GM crops. COGEM advice CGM/150601-02