Import and processing of herbicide tolerant genetically modified maize MZHG0JG

COGEM advice CGM/170330-01

- The present application (EFSA-GMO-DE-2016-133) concerns the authorisation for import and processing for use in feed and food of genetically modified (GM) maize MZHG0JG;
- Maize MZHG0JG was produced by *Agrobacterium tumefaciens* mediated transformation of maize line NP2222 with plasmid pSYN18857;
- The GM line expresses the *mepsps* and *pat* genes conferring tolerance to glyphosate and glufosinate-ammonium containing herbicides;
- 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;
- The molecular characterisation of maize MZHG0JG meets the criteria of COGEM;
- There are no indications that the introduced traits alter the fitness of maize;
- There are no reasons to assume that the introduced traits will allow GM maize MZHG0JG to survive in the Dutch environment;
- COGEM is of the opinion that import and processing of maize MZHG0JG poses 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-DE-2016-133), filed by Syngenta, concerns import and processing of genetically modified (GM) maize MZHG0JG, for use in feed and food. Maize MZHG0JG was produced by *Agrobacterium tumefaciens* mediated transformation. The GM maize contains the *mepsps* and *pat* genes conferring tolerance to glyphosate and glufosinate-ammonium containing herbicides.

2. Previous COGEM advices

COGEM did not previously advise on the import and processing of maize MZHG0JG, or maize lines harbouring the combination of modified *epsps* and *pat* genes without additional transgenic traits. However, COGEM positively advised several times on the import and processing of maize lines containing modified *epsps* and *pat* genes in combination with additional transgenic traits, such as insect resistance.[1,2,3,4,5]

3. Environmental risk assessment

3.1 Aspects of the wild-type crop

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 predominantly wind pollinated,[6,7] and has both male and female flowers that are spatially separated. Female flowers are not attractive to insect pollinators, because they do not produce nectar. As insects do not visit the female flowers, insect pollination of maize is limited.[8]

In the Netherlands, no wild relatives of maize are present and hybridisation with other species cannot occur. Domesticated maize requires warm conditions in order to grow and does not tolerate prolonged cold and frost.[8,9] After ripening, the kernels remain on the cob and do not shatter naturally.[8,10] In cultivation areas with warmer 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.[8] Some volunteers and maize plants in non-agricultural habitats were observed during field observations in Austria.[11] In the Netherlands, the appearance of volunteers is very rare to absent.[12]

Maize is very sensitive to weed competition.[13] During the long process of domestication, maize has lost the ability to survive in the wild.[7] Establishment of maize plants in the wild has never been observed in the Netherlands and COGEM is not aware of any reports of feral maize populations 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 and traits

Maize MZHG0JG was produced by *A. tumefaciens* mediated transformation of maize line NP2222 with plasmid pSYN18857. The T-DNA contains a *mepsps* and *pat* expression cassette conferring tolerance to glyphosate and glufosinate-ammonium containing herbicides. The plasmid backbone contains, amongst other things, an *aadA-03* antibiotic resistance gene, which is used as a selection marker.

Introduced genes	**Encoded proteins (enzymes)**	**Traits**
mepsps	Modified 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) originating from *Zea mays*[14]	Tolerance to glyphosate containing herbicides, because of a decreased binding affinity for glyphosate
pat	Variant of phosphinothricin N-acetyltransferase (PAT) originating from *Streptomyces viridochromogenes* strain Tü 494[15,16,17]	Tolerance to glufosinate-ammonium containing herbicides
For a detailed description of the traits see references		

3.3 Molecular characterisation

Southern blot analyses showed that maize MZHG0JG contains one copy of the insert at a single integration locus and that there are no T-DNA fragments elsewhere in the MZHG0JG genome. Southern blot analyses also demonstrated the absence of pSYN18857 backbone sequences.

The applicant determined the sequence of the MZHG0JG insert and adjacent flanking maize genome DNA sequences. Sequence comparison with the transformation plasmid pSYN18857 showed that no rearrangements or base pair changes occurred in the MZHG0JG insert. However, a 22 bp deletion in the right border and a 21 bp deletion in the left border of the inserted T-DNA were identified. A comparison with the parental maize line revealed that 22 bp were deleted from the maize genome. An additional 43 bp were inserted into the integration site: a 4-bp DNA sequence is present at the junction between the insert and the 5′ flanking region and a 39-bp DNA sequence at the 3′ junction. The 39-bp insertion is a duplicated maize sequence inserted in reverse orientation at the 5′ flanking region.

Bioinformatics analyses indicated that the T-DNA was inserted in a repetitive region of the maize genome. According to the applicant, no endogenous genes were disrupted at the insertion site or in the genomic DNA flanking the insertion site.

The applicant screened the junctions between the T-DNA insert and the flanking plant genomic DNA as well as the entire insert for potential newly created open reading frames (ORFs) from stop to stop codon. The applicant states that no biologically meaningful protein sequence similarities with allergens or toxic proteins were detected in these bioinformatics analyses.

The molecular characterisation was conducted according to the criteria previously laid down by COGEM.[18]

Conclusion: The molecular characterisation of maize MZHG0JG is adequate and no indications for potential environmental risks were identified.

3.4 Phenotypic and agronomic characteristics

The applicant analysed the phenotypic and agronomic characteristics of MZHG0JG. The introduced traits do not give reason to assume that the parental lines have an altered survivability compared to conventional maize. The applicant noted a small but significant difference in plant height between the GM line and the non-GM near-isogenic line, and non-GM reference varieties. However, this is just one of many plant traits that determine fitness. It is highly unlikely that sensitivity to weed competition or survivability in the wild is altered by this difference. Therefore, COGEM is of the opinion that there are no indications to assume that the introduced traits in MZHG0JG allow maize to survive or establish in the Dutch environment.

Conclusion: MZHG0JG 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 new general surveillance plan as part of the PMEM. COGEM has published several recommendations for further improvement of the general surveillance (GS) plan,[19,20] but considers the current GS plan adequate for import and processing of maize MZHG0JG.

6. Overall conclusion

COGEM is of the opinion that import and processing of maize MZHG0JG poses 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. COGEM (2012). Import and processing of genetically modified maize line Bt11xMIR162x1507xGA21. COGEM advice CGM/120816-01
2. COGEM (2012). Import and processing of genetically modified maize line Bt11x59122xMIR604x 1507xGA21. COGEM advice CGM/120816-02
3. COGEM (2009). Import and processing of Bt11xGA21 maize. COGEM advice CGM/091019-02
4. COGEM (2009). Import and processing of Bt11xMIR162xGA21. COGEM advice CGM/090917-04
5. COGEM (2009). Import and processing of Bt11xMIR162xMIR604xGA21. COGEM advice CGM/090917-05
6. Hin CJA (2001). Landbouwkundige risico's van uitkruising van GGO-gewassen. Centrum voor Landbouw en Milieu (CLM)
7. 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
8. 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
9. Miedema P (1982). The effect of low temperature on *Zea mays.* Advances in Agronomy 35: 93-128
10. Organisation for Economic Cooperation and Development (OECD) (2003). Consensus Document on the Biology of *Zea mays* ssp. *mays* (Maize)
11. Pascher K (2016). Spread of volunteer and feral maize plants in Central Europe: recent data from Austria. Environ. Sci. Eur. 28: 30

12. 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
13. CAB International (2007). Crop Protection Compendium. *Zea mays* (maize). CD-ROM edition, Wallingford
14. Lebrun M *et al*. (2003). Mutated 5-enolpyruvylshikimate-3-phosphate synthase, gene coding for said protein and transformed plants containing said gene. United States Patent no. US 6,566,587 B1
15. 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
16. Wohlleben W *et al*. (1988). Nucleotide sequence of the phosphinothricin *N*-acetyltransferase gene form *Streptomyces viridochromogenes* Tü494 and its expression in *Nicotoana tabacum*. Gene 70: 25-37
17. Petit L *et al*. (2006). *Zea mays* transgenic phosphinothricin acetyltransferase gene, partial cds; and beta lactamase and phosphinothricin acetyltransferase genes, complete cds. GenBank: DQ156557.1 www.ncbi.nlm.nih.gov/nuccore/DQ156557.1
18. COGEM (2014). Reconsideration of het molecular characterisation criteria for marketing authorisation of GM crops. COGEM topic report CGM/140929-02
19. COGEM (2010). General Surveillance. COGEM topic report CGM/100226-01
20. COGEM (2015). Advice on improving the general surveillance of GM crops. COGEM advice CGM/150601-02