Import and processing of herbicide tolerant genetically modified maize DP202216 with enhanced grain yield potential

COGEM advice CGM/191129-04

- The present application (EFSA/GMO/NL/2019/159) concerns the authorisation for import and processing for use in feed and food of genetically modified (GM) maize DP202216;

- Maize DP202216 was produced by *Agrobacterium tumefaciens* mediated transformation;
- Maize DP202216 expresses the *zmm28* gene leading to increased and extended production of the endogenous ZMM28 protein resulting in plants with an enhanced grain yield potential. Furthermore it expresses a modified *pat* gene (*mo-pat*) conferring tolerance to glufosinate-ammonium containing herbicides;

- In the Netherlands, feral maize populations have never been observed and the appearance of volunteers is rare;
- In the Netherlands, the wild relative of maize, teosinte is not present in maize fields or in nature and hybridisation of GM maize with other species is therefore not possible;

- The molecular characterisation of maize DP202216 meets the criteria of COGEM;
- There are no indications that the introduced traits alter the fitness of maize DP202216 under natural conditions;
- There are no reasons to assume that the introduced traits will allow GM maize DP202216 to survive in the Dutch environment;

- COGEM is of the opinion that import and processing of maize DP202216 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/NL/2019/159), filed by Pioneer Hi-Bred International, concerns the import and processing of genetically modified (GM) maize DP202216, for use in feed and food. Maize DP202216 was produced by *Agrobacterium tumefaciens* mediated transformation. Maize DP202216 expresses a modified *pat* gene (*mo-pat*) conferring tolerance to glufosinate-ammonium containing herbicides. Furthermore, it expresses the *zmm28* gene leading to increased and extended production of the endogenous ZMM28 protein resulting in plants with an enhanced grain yield potential.[1,2]

2. Previous COGEM advices

COGEM previously issued several advices on the import, processing and cultivation of GM maize lines containing the *pat* gene in combination with additional transgenic traits. COGEM advised positively on the import, processing and cultivation of Bt11 maize containing the *pat* gene (in combination with the *cry1Ab* gene).[e.g. 3,4,5,6] COGEM has not advised previously on the import, processing or cultivation of maize lines expressing the *zmm28* gene. COGEM did advise positively on the import and processing of maize MON87403 containing the gene *ATHB17*. As a result maize MON87403 has an increased ear biomass.[7]

3. Environmental risk assessment

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,[8,9] 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 highly limited but cannot be excluded.[10]

Hybridisation of GM maize with other species than teosinte, the wild relative of maize, cannot occur. Recently, teosinte was detected in Spain[11,12,13] and France.[14,15] In these countries, it is present as a weed in some arable fields. In the Netherlands, teosinte is absent in maize fields and in nature.[16]

Maize requires warm conditions in order to grow.[10,17] 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.[10] Maize does not tolerate prolonged cold and frost.[16]

Maize is very sensitive to weed competition.[18] During the long process of domestication, maize has lost the ability to persist in the wild.[9] A soil seed bank, small seeds, and an extended period of flowering and seed production are characteristics often observed in persistent weeds.[19] Maize lacks all these characteristics. After ripening, the seeds (the kernels) adhere to the cob and do not scatter naturally.[10,20] Consequently, seed dispersal is severely hampered.

During field observations in Austria some volunteers and maize plants were observed in non-agricultural habitats.[21] In the Netherlands, the appearance of volunteers is rare, although maize plants occasionally have been observed outside agricultural fields.[15,22] Any volunteers emerging in will be killed by frost at the onset of winter.[16] COGEM is not aware of any reports of feral maize populations in the Netherlands.[16]

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

3.2 Description of the introduced genes, traits and regulatory elements

DP202216 was developed using *Agrobacterium tumefaciens* mediated transformation with plasmid PHP40099. The inserted transfer DNA (T-DNA) from plasmid PHP40099 contains two gene cassettes. 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 (promotors and terminators).

Introduced genes	**Encoded proteins**	**Traits**	**Regulatory elements**
zmm28	MADS-domain transcription factor gene originating from *Zea mays* encoding for ZMM28 protein[2,1,23,24]	Plants with enhanced grain yield potential	Constitutive promoter (*zm-gos2*) and intron from the ubiquitin gene 1 (*ubi*ZM1) from *Z. mays* and proteinase inhibitor II (*pin*II) gene terminator from *Solanum tuberosum*
mo-pat	Maize codon-optimised version of phosphinothricin acetyl transferase (*pat*) gene from *Streptomyces viridochromogenes*[25,26,27]	Tolerance to glufosinate-ammonium containing herbicides	Promoter, 5'UTR and intron from the *ubi*ZM1 gene from *Z. mays* and *pin*II terminator from *S. tuberosum*
See reference for a detailed description of the trait			

3.2.1 Overexpression of zmm28 in DP202216

In conventional maize, the *zmm28* gene is expressed in leaf tissue at the V6 to R5 growth stage and the ZMM28 protein is not expressed at detectable levels in R6 grain. DP202216 maize contains a gene cassette with a constitutive maize promoter (*zm-gos2*), which drives expression of the maize gene (*zmm28*) and initiates expression of the ZMM28 protein at an earlier growth stage in leaves (V2), and expresses the protein for an extended period of time in grain (including the R6 growth stage).[1] According to the applicant, the increased and extended expression of the ZMM28 protein has been shown to enhance grain yield potential in plants across multiple years and diverse growing locations.[2]

3.3 Molecular characterisation

The applicant performed Next Generation Sequencing (NGS) method known as Soutern-by-Sequencing (SbS) to analyse the first generation progeny plants after transformation (T1) of DP202216 maize. SbS analysis showed that for six plants a single intact PHP40099 T-DNA (intact with exception of the border regions, see below) was inserted into the genome of DP202216 maize and that no backbone sequences are present in DP202216. Furthermore, the exact sequence of the insert and the flanking borders was obtained by Sanger sequencing. The total length of genomic sequence determined in DP202216 maize is 10,091 bp, comprising of 1,283 bp of the 5' flanking genomic border sequence, 1,372 bp of the 3' flanking genomic border sequence, and 7,436 bp of the inserted T-DNA (PHP40099 T-DNA length 7,470 bp). The inserted T-DNA in DP202216 maize was found to lack 22-bp at the right border and 12-bp at the left border. Right border and left border termini trimming often occurs in *Agrobacterium*-mediated transformation.[28] The remainder of the DP202216 insert is intact.

Bioinformatic analyses of the DP202216 insertion flanking borders revealed that the insert is most likely located on chromosome 1 of the maize genome, and that 6 bp are deleted at the insertion site from the maize genome.

According to the applicant, bioinformatics analyses indicate that 1,283 bp upstream of the insert no endogenous maize genes are present. Downstream of the insert, there was a single alignment between the 3'genomic border (1,372 bp) and a predicted mRNA in the nucleotide database, but there were only two moderate-quality expressed sequence tag (EST) alignments and no significant protein alignments. Overall, according to the applicant, it is unlikely that endogenous genes are disrupted at the DP202216 insertion site.

The applicant screened the junctions between the T-DNA insert and the flanking maize genomic DNA as well as the entire insert (stop codon-bracketed frames) for potential newly created open reading frames (ORFs). According to the applicant, except for two low level identity alignments with two known allergens that were considered false positives, no significant sequence similarities between the putative products of these ORFs and no known allergenic, toxic or other biologically active proteins were detected in these bioinformatics analyses.

The molecular characterisation was conducted according to the criteria previously laid down by COGEM.[29]

Conclusion: The molecular characterisation of maize DP202216 is adequate and no indications for potential environmental risks were identified.

3.4 Phenotypic and agronomic characteristics

The applicant evaluated germination and detected that germination rates of DP202216 maize under warm, cold, diurnal (simulated emergence under daily temperature fluctuations) growing conditions were comparable to control maize under corresponding conditions.

The applicant analysed the phenotypic and agronomic characteristics of DP202216. The introduced traits do not give reason to assume that DP202216 has an altered survivability compared to non-GM conventional counterparts and commercial reference maize lines under natural conditions.

Therefore, COGEM is of the opinion that there are no indications that the introduced traits allow maize DP202216 to survive or establish in the Dutch environment.

Conclusion: DP202216 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, a food and/or feed assessment for Regulation (EC) 1829/2003 applications is carried out by Wageningen Food Safety Research (WFSR). The outcome of the assessment by other organisations (EFSA, WFSR) 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,[30,31] but considers the current GS plan adequate for the import and processing of maize DP202216.

6. Overall conclusion

COGEM is of the opinion that import and processing of maize DP202216 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.

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