Adviesraad voor Bioveiligheid
Conseil consultatif de Biosécurité

Advice of the Belgian Biosafety Advisory Council on notification B/BE/24/V3 from VIB for deliberate release in the environment of genetically modified plants for research and development

12 March 2024
Ref. SC/1510/BAC/2024_0346

The notification B/BE/24/V3 has been submitted by the VIB to the Belgian Competent Authority (CA) in January 2024 for a request of deliberate release in the environment of genetically modified higher plants for research and development according to Chapter II of the Royal Decree of 21 February 2005.

The title of the notification is: *Scientific field evaluation of maize with improved digestibility.* The purpose of the release is to measure the performance of the modified maize plants under normal field conditions and learn whether they also in these conditions have an altered composition of their cell wall. The field trial also has the goal to determine whether the modification would lead to undesired effects such as a higher vulnerability for breaking of their stem during strong winds.

The notification has been officially acknowledged by the CA on 12 January 2024 and forwarded to the Biosafety Advisory Council for advice.
Within the framework of the evaluation procedure, the Biosafety Advisory Council, under the supervision of a coordinator and with the assistance of its Secretariat, contacted experts to evaluate the dossier. Two experts from the common list of experts drawn up by the Biosafety Advisory Council and the Biosafety and Biotechnology Unit (SBB), answered positively to this request.
The experts assessed whether the information provided in the notification was sufficient and accurate in order to state that the deliberate release of the edited maize lines would not raise any problems for the environment, animal or human health in the context of the intended use. See Annex I for an overview of all comments received.

On 23 February 2024, based on a question prepared by the Biosafety Advisory Council, the CA requested the notifier to provide additional information. An answer to the question was received by the Secretariat on 4 March 2024.

For the purpose of the scientific evaluation, the following legislation has been considered:
- Royal Decree of 21 February 2005 (Belgian Official Journal of 24.02.2005, p. 7129) modified by the Royal Decree of 19 February 2020 (Belgian Official Journal of 02.03.2020, p. 12666).

In parallel to the scientific evaluation, the CA made the dossier available on its website for a one-month public consultation as required in the abovementioned Royal Decree. No questions of the public tackling biosafety issues of the GM maize were received.

Summary of the scientific evaluation

1. Information related to the recipient or parental plants

Zea mays is an allogamous plant that propagates through seed produced predominantly by cross-pollination. Maize pollen can be collected by honeybees and other insects, however these pollinating insects play a minor role in the cross-pollination of maize plants which relies mainly on wind for the dispersal of its pollen (OECD, 2003[1]). Data on pollen dispersal in maize demonstrated that the levels of cross-fertilisation drop rapidly over the initial meters around the pollen source and that most of the released pollen is deposited within about 30 m of the source (Devos *et al.*, 2005[2]). At distances farther than 30 - 50 m from the source, pollen dispersal is very low but not zero. However, vertical wind movements can lift up pollen and distribute it over distances up to kilometers under suitable climatic conditions. In Belgium (and in Europe) there are no sexually cross-compatible indigenous wild relatives with which maize can hybridise and form progeny (OECD, 2003; EFSA, 2016[3]). Teosinte, regarded as an invasive weed in Europe since its first occurrence in France (1990) and Spain (2009), has so far not been reported in Belgium. The only recipient plants that can be cross-fertilised by maize in Belgium are therefore other cultivated maize varieties.

Seed dispersal of individual kernels of domesticated plants are mainly the result of field operations of harvesting the crop and transporting the grain from the harvested fields to storage facilities. Spilled maize seeds can overwinter, germinate and appear in the field as volunteers. However, maize is incapable of sustained reproduction outside the domestic cultivation area as it has lost its ability to survive in the wild due to its long process of domestication (OECD, 2003). Volunteers can only occur after a warm winter period (with no temperatures lower than 0°C for more than 6 to 8 hours) and are characterised by a low probability of cross-pollination (Grüber *et al.*, 2008[4]; Palaudelmàs *et al.*, 2009[5]). In the prevailing Belgian climate, volunteers are unlikely to occur.

2. Information on the design and management conditions in the field trial

The field trial will be conducted during two growing seasons (from April 2024 until October 2025). The surface of the area for cultivation will not exceed 900 m^2.

Prior to complete formation, tassels from the modified maize will be removed by hand to prevent the dispersal of pollen. Once the last leaf has been formed, monitoring of emerging tassels will take place every two days until all tassels have been removed and will continue until September 15. Removed tassels will be transported in closed bags to the lab and inactivated.

During harvest, maize cobs will be collected manually and transported in closed bags to the lab. Material will be destroyed if no longer needed for research. Stems and leaves, except for a few which will be

[1] OECD, 2003. Consensus Document on the biology of *Zea mays* subsp. *Mays* (maize). Series on Harmonisation of Regulatory Oversight in Biotechnology (ENV/JM/MONO(2003)11), No. 27:1-49. http://www.olis.oecd.org/olis/2003doc.nsf/LinkTo/NT0000426E/$FILE/JT00147699.PDF

[2] Devos *et al.*, 2005. The co-existence between transgenic and non-transgenic maize in the European Union: a focus on pollen flow and cross-fertilization. Environmental Biosafety Research 4, 71-87.

[3] EFSA (European Food Safety Authority), 2016. Relevance of new scientific evidence on the occurrence of teosinte in maize fields in Spain and France for previous environmental risk assessment conclusions and risk management recommendations on the cultivation of maize events MON810, Bt11, 1507 and GA21. EFSA supporting publication 2016:EN-1094. 13 pp.

[4] Grüber *et al.*, 2008. Post-harvest gene escape and approaches for minimizing it. CAB International 2008 (http://www.cababstractsplus.org/cabreviews)

[5] Palaudelmàs *et al.*, 2009. Effect of volunteers on maize gene flow. Transgenic Res. 18, 583-594

harvested for further analysis in the lab, will be shredded on the field. Roots and the lowest part of the stem will be left in the ground.

After the field trial, the field will be left fallow and ploughed at the latest during next spring.

3. Information related to the genetic modification

In first instance plants with a single homozygous mutation in one gene involved in the biosynthesis of lignin were made, creating a pool of plants, each with a mutation in a different gene involved in the biosynthesis of lignin. This reduced lignin content is expected to contribute to better digestibility of maize thereby improving the feed conversions rate.

These single mutant maize lines were obtained using a vector containing Cas9 and guide RNA (gRNA) genes on a T-DNA construct. The T-DNA construct used for transformation also contains a *hygR* gene that served as a marker for the selection of transformants after *Agrobacterium tumefaciens*-mediated transformation. The *hygR* gene results in resistance to the antibiotic hygromycin. Also the vector backbone contains an antibiotic resistance marker gene, namely the *sm/spR* gene, rendering resistance to streptomycin and spectinomycin.

Transformed plants selected based on hygromycin were subsequently backcrossed with the inbred line B104 and finally selfed to obtain homozygous plants solely containing the mutation (and no T-DNA or vector backbone DNA). The homozygous plants were tested for the lack of vector sequences, including the antibiotic resistance marker genes, via quantitative real-time PCR using several construct-specific primers. These tests indicated the absence of vector DNA.

Subsequently, using conventional crossing, plants were generated that combine homozygous mutations in three different genes. Two single mutants and ten triple mutant maize lines obtained by conventional breeding are subject of this field experiment.

4. Potential risks for the environment, animal or human health associated with the release of the GM maize

No increased persistence in the field or invasiveness into natural habitats of the modified maize lines compared to non-GM maize is expected, as the reduced lignin content is not known to confer a selective advantage to survivability. Moreover, the measures taken (removal of tassels and manual collection of cobs) rule out the development and survival of the modified maize in the year(s) after the field trial.

Vertical gene transfer to cultivated maize in the surroundings through pollen can virtually be ruled out due to tassel removal.

Horizontal gene transfer between plants and micro-organisms is considered as a rare event under natural conditions (Keese, 2008[6]). In case gene transfer from the modified maize to micro-organisms would take place and gene expression would occur (although unlikely as the modified maize gene is non-functional due to deletions), negative effects on the environment and humans are not expected. The mutated genes, expressing proteins involved in lignin biosynthesis, are unlikely to confer a selective advantage to bacteria.

Further, it is not expected that the modified maize would have significant effects on organisms (invertebrates, vertebrates and soil micro-organisms) and humans, as no trait that could affect the behaviour or development of organisms via contact or feeding has been integrated. Given the restricted scale of the field trial, any potential effect to organisms and biogeochemical processes - if these would occur - will be of a local and temporal nature. As the release of pollen in the environment is prevented,

[6] Keese, P. 2008. Risks from GMOs due to horizontal gene transfer. Environ. Biosafety Res. 7: 123-149.

a possible altered allergenicity potential of the transgenic pollen (allergy from maize pollen may occur in case of occupational exposure to high amounts of pollen grains, see *e.g.* Oldenburg *et al*., 2011[7]) does not form a concern for human health.

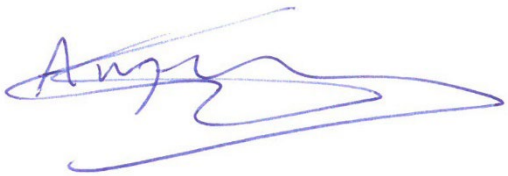
5. Information related to the control, monitoring, post-release and waste treatment

The management measures proposed are considered as sufficient to prevent potential adverse effects to the environment, animal and human health during and after the field trial. The monitoring and removal of any appearing tassel in the transformed lines will prevent gene flow by pollen spread. Careful manual harvesting of the cobs and storing them in closed bags will prevent seed dispersal. The seeds and the few collected plants will be destroyed after analysis.

Years of experience have shown that no volunteer plants appear in the year following maize field trials. The field will be left fallow and ploughed at the latest during next spring.

Conclusion

Provided that the trials are conducted as described in the dossier, the Biosafety Advisory Council concludes that it is very unlikely that this proposed small scale field trial with modified maize lines will harm human health, animals or the environment.



Prof. Dr. ir. Geert Angenon
President of the Biosafety Advisory Council

Annex I: Compilation of comments of experts in charge of assessing the dossier B/BE/24/V3 (ref: BAC_2024_0236)

[7] Oldenburg 2011. Maize pollen is an important allergen in occupationally exposed workers. Journal of Occupational Medicine and Toxicology 6: 32.

Adviesraad voor Bioveiligheid
Conseil consultatif de Biosécurité

Compilation of comments of the experts in charge of evaluating notification B/BE/24/V3

Ref. SC/1510/BAC/24_0236

Coordinator: Wouter Vanhove
Experts: Marc De Loose (ILVO), Nina Papazova (Sciensano) & Jan Van Doorsselaere (VIVES)
SBB coordinator: Adinda De Schrijver

INTRODUCTION

Dossier **B/BE/24/V3** concerns a notification of the VIB, for deliberate release in the environment of genetically modified higher plants (GMHP) according to Chapter II of the Royal Decree of 21 February 2005.

The notification has been officially acknowledged on 12 January 2024 and concerns a field trial to evaluate maize with improved digestibility.

Experts were invited to evaluate the GMHP considered in the notification as regards their potential impacts on the environment, including human and animal health, and information relating to pre- and post-release treatment of the site.

The comments of the experts are roughly structured as in
- Annex II (principles for the risk assessment) of the consolidated version of the Royal Decree of 21 February 2005
- Annex III (information required in notifications) of the Royal Decree of 21 February 2005

EVALUATION FORM

The comments below served as basis for a list of questions that the competent authority forwarded to the notifier with a request to provide additional information. The comments highlighted in grey correspond to the questions/comments selected and sent to the notifier.

B. INFORMATION RELATED TO THE RECIPIENT OR (WHERE APPROPRIATE) PARENTAL PLANTS

Have evaluated this section and had no comments/questions: 2 experts

C. INFORMATION RELATED TO THE GENETIC MODIFICATION

Have evaluated this section and had no comments/questions: 2 experts

D. INFORMATION RELATED TO THE GENETICALLY MODIFIED PLANT

D.1. Information related to the traits and characteristics, which have been introduced or modified

Have evaluated this section and had no comments/questions: 2 experts

D.2. Information on the molecular characteristics of the final GMO

Have evaluated this section and had no comments/questions: 2 experts

D.3. Information on the expression (of the insert)

Have evaluated this section and had no comments/questions: 2 experts

D.4. Information on how the GM plant differs from the recipient plant

Have evaluated this section and had no comments/questions: 2 experts

D.5. Genetic stability of the insert and phenotypic stability of the GMHP

Have evaluated this section and had no comments/questions: 2 experts

D.6. Any change to the ability of the GMHP to transfer genetic material to other organisms

Have evaluated this section and had no comments/questions: 2 experts

D.7. Information on any toxic, allergenic or other harmful effects on human health arising from the genetic modification

Have evaluated this section and had no comments/questions: 2 experts

D.8. Information on the safety of the GMHP to animal health, particularly regarding any toxic, allergenic or other harmful effects from the genetic modification, where the GMHP is intended to be used in animal feedstuffs

Have evaluated this section and had no comments/questions: 2 experts

D.9. Mechanism of interaction between the genetically modified plant and target organisms (if applicable)

Have evaluated this section and had no comments/questions: 2 experts

D.10. Potential changes in the interactions of the GMHP with non-target organisms resulting from the genetic modification

Have evaluated this section and had no comments/questions: 2 experts

D.11. Potential interactions with the abiotic environment

Have evaluated this section and had no comments/questions: 2 experts

D.12. Description of detection and identification techniques for the GM plant

Have evaluated this section and had no comments/questions: 1 expert

Comment 1:
Het ontstaan van bijvoorbeeld 'brown midrib' mutaties in maïs is zeldzaam. Volgens de literatuur zijn er ook nog geen spontane lignine mutanten ontstaan in het veel gebruikte maïsmodel B104. Het is zeer onwaarschijnlijk dat de planten die beschreven zijn in het dossier (met meerdere mutaties in CCR genen) ontstaan zouden zijn door toeval. Twee zinnen in paragraaf D12 moeten worden verwijderd.

Note SBB: We want to note that the dossier is already made publicly available. Proposals for textual changes will therefore not be forwarded to the notifier.

Comment 2:
De volgende informatie ontbreekt in het detectieprotocol:

- Een figuur die de positie van de primers en hun doelsequenties illustreert.
- Een correcte PCR mix (het totaal aan µl klopt niet)
- De electroforese-condities en een gel van de amplicons
- De genomen veiligheidsmaatregelen
- De sequencing-condities
- De interpretatie van de resultaten, inclusief informatie over de referentiesequentie.

Richtsnoeren voor welke informatie aangeleverd dient te worden voor Deel B dossiers, zijn te vinden op: https://www.biosafety.be/sites/default/files/partb_protocole_gmo_detection.pdf

D.13. Information about previous releases of the GM plant, if applicable

Have evaluated this section and had no comments/questions: 2 experts

E. INFORMATION RELATING TO THE SITE OF RELEASE

Have evaluated this section and had no comments/questions: 2 experts

F. INFORMATION RELATING TO THE RELEASE

Have evaluated this section and had no comments/questions: 2 experts

G. INFORMATION RELATED TO CONTROL, MONITORING, POST-RELEASE AND WASTE TREATMENT

G.1. Any measures taken

Have evaluated this section and had no comments/questions: 2 experts

G.2. Information on methods for post-release treatment of site

Have evaluated this section and had no comments/questions: 2 experts

G.3. Information on post-release treatment methods for the GM plant material, including wastes

Have evaluated this section and had no comments/questions: 2 experts

G.4 Information on monitoring plans and techniques

Have evaluated this section and had no comments/questions: 2 experts

G.5. Information on any emergency plans

Have evaluated this section and had no comments/questions: 2 experts

G.6. Information on methods and procedures to protect the site

Have evaluated this section and had no comments/questions: 2 experts

ANNEX 1. INFORMATION RELATED TO THE RISKS FOR THE ENVIRONMENT

1. Persistence and invasiveness of the GM plant, including of gene flow from plant to plant

Have evaluated this section and had no comments/questions: 2 experts

2. Gene transfer from GM plants to micro-organisms

Have evaluated this section and had no comments/questions: 1 expert

3. Interactions of the GM plant with target organisms

Have evaluated this section and had no comments/questions: 2 experts

4. Interactions of the GM plant with non-target organisms

Have evaluated this section and had no comments/questions: 2 experts

5. Effects of the specific cultivation, management and harvest techniques

Have evaluated this section and had no comments/questions: 2 experts

6. Effects on biogeochemical processes

Have evaluated this section and had no comments/questions: 2 experts

7. Effects on human and animal health

Have evaluated this section and had no comments/questions: 2 experts