Adviesraad voor Bioveiligheid
Conseil consultatif de Biosécurité

Advice of the Belgian Biosafety Advisory Council on notification B/BE/25/V1 from VIB for deliberate release in the environment of genetically modified plants for research and development

11 March 2025
Ref. SC/1510/BAC/2025_0345

The notification B/BE/25/V1 has been submitted by the VIB to the Belgian Competent Authority (CA) in January 2025 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 yield.* 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 exhibit the phenotype characteristics observed in the greenhouse: larger leaves, an increased plant height, an increased stem diameter, and an increased cob size.

The notification has been officially acknowledged by the CA on 10 January 2025 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. Three 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 to state that the deliberate release of the edited maize 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 25 February 2025, based on questions prepared by the Biosafety Advisory Council, the CA requested the notifier to provide additional information. Answers to the questions were received by the Secretariat on 26 February 2025.

For 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 regarding 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 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 three growing seasons (from April 2025 until November 2027). The surface of the area for cultivation will not exceed 600 m^2.

An isolation distance of at least 200 m to commercially grown conventional maize will be maintained, to prevent potential outcrossing through pollination. The amount of pollen produced will be small, due to the small scale of the trial. In addition, the inbred maize line B104 has very little overlap in flowering time with conventional maize that is usually commercially grown in Belgium. Removal of male inflorescences would eliminate any risk of outcrossing. However, as the latter practice may influence maize growth and because of the earlier mentioned risk mitigation measures, male inflorescences will not be removed.

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[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

All cobs will be carefully harvested manually and transported in closed bags to the lab. Material will be inactivated if no longer needed for research. Stems and leaves, except for a few which will be harvested for further analysis in the lab, will be shredded and composted on the field. Roots and the lowest part of the stem will be left on the field for composting. After the trial, the field will be left fallow and ploughed at the latest during next spring.

3. Information related to the genetic modification

Multiplex CRISPR (more than one guide RNA is introduced at once) was used to create frameshift mutations in either or both of two key regulatory genes, *ZmGRF10* and *ZmTCP42*, which influence cell proliferation and plant architecture, rendering these genes non-functional.

The CRISPR-Cas9 components were introduced into maize embryos via *Agrobacterium tumefaciens*-mediated transformation. A selectable marker (*hygR*) enabled the identification of transformed plants, while an antibiotic resistance gene (*sm/spR*) was present in the vector backbone. Following selection, transformed plants were backcrossed with the B104 inbred line and subsequently selfed to generate stable homozygous mutants. PCR analysis confirmed the absence of T-DNA and vector sequences in the final lines, ensuring no transgenic DNA was retained.

Through conventional breeding, plants carrying mutations in both *ZmGRF10* and *ZmTCP42* were obtained, alongside single mutants. These lines exhibit increased biomass, larger leaves, and thicker stems in greenhouse conditions, and will be evaluated in the field.

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 increased biomass is not known to confer a selective advantage to survivability. Moreover, the measures taken (see section 2 above) rule out the development and survival of the modified maize in the year(s) after the field trial.

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 frameshift mutations), negative effects on the environment and humans are not expected.

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.

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

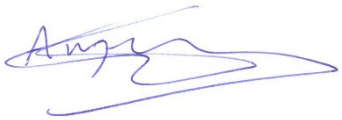
The management measures proposed are considered as sufficient to prevent potential adverse effects to the environment, animal and human health during and after the field trial. Careful manual harvesting of the cobs and storing them in closed bags will prevent seed dispersal. The seeds and the few collected plants will be destroyed after analysis.

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

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/25/V1 (ref: BAC_2025_0286)

Adviesraad voor Bioveiligheid
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Compilation of comments of the experts in charge of evaluating notification B/BE/25/V1

Ref. SC/1510/BAC/25_0286

Coordinator: Wouter Vanhove
Experts: Henri Batoko (UCL), Michel Edmond Ghanem (CIRAD), Nina Papazova (Sciensano), Jan Van Doorsselaere (VIVES)
SBB: Fanny Coppens

INTRODUCTION

Dossier **B/BE/25/V1** 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 10 January 2025 and concerns a field trial to evaluate maize with improved yield.

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: 3 experts

Comment:

I have evaluated this section and have no questions. The public, transformable maize inbred B104 has been widely used for genome editing in recent years. This is well documented in the request.

C. INFORMATION RELATED TO THE GENETIC MODIFICATION

Have evaluated this section and had no comments/questions: 3 experts

Comment:

I have evaluated this section and have no questions. The plants have been modified using CRISPR-Cas technology. This is well documented in the request.

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: 3 experts

Comment:

I have evaluated this section and have no questions. The genetically modified maize plants have an improved yield resulting from the functional knock down of two transcription factor genes (GRF10 and TCP42) that negatively affect cell division and growth. The altered plants have larger leaves, an increased plant height, an increased stem diameter, and an increased cob size.

D.2. Information on the sequences, which have been introduced or modified

Have evaluated this section and had no comments/questions: 3 experts

Comment:

I have evaluated this section and have no questions. The plants have been modified using CRISPR-Cas technology. First a T-DNA construct containing the CAS9 gene was introduced into maize plants. Then, plants were derived that were homozygous for the CAS9 construct and these were used for a second transformation through which a T-DNA construct containing 12 guide RNA genes was introduced into the plants.

D.3. Information on the expression

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

Comment:
It is stated in the summary notification sheet (Bijlage 11, section B2) that "the genetically modified maize plants have an improved yield resulting from the functional knockdown of two transcription factor genes (GRF10 and TCP42) that negatively affect cell division and growth." However, the molecular characterization provided indicates that the GMO maize were obtained by CRISPR-Cas editing, resulting in indels in the respective coding sequences (CDS). It remains unclear whether any expression data support the claim of "functional knockdown" as opposed to a complete knockout.

SBB comment: The currently applicable Biosafety Advisory Council guidelines for Molecular Characterisation of GM Plants for a Standard Part B Consent do not require determination of the level of expression at protein level. At field trial stage, it is considered sufficient to give information on the expected expression, rather than the real expression.

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

Have evaluated this section and had no comments/questions: 3 experts

Comment:

I have evaluated this section and have no questions. Increases in leave size, stem diameter, plant height, and cob size.

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

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

Comment:

- Bijlage 2 page 12 figure 8: annotations of transgenic lines Ed1, script4 in the figure as WT? Please correct. **(*)**
- It is necessary to provide data on the DH3 plants and demonstrate homozygosity and absence of T-DNA. **(**)**

SBB and coordinator comment:

(*) This comment was not forwarded to the notifier as the dossier has already been made publicly available.

(**) Homozygosity is not an element of the risk assessment. As the absence of T-DNA is demonstrated for DH2 plants and DH3 plants are the result of self-crossing DH2, it is not necessary to demonstrate absence of T-DNA in DH3 plants.

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: 3 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: 3 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: 3 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: 3 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: 3 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: 3 experts

***Comment**:*
De PCR mix is niet volledig – de eindconcentraties van primers en enzym moeten gegeven worden, alsook de startconcentratie van het enzym.

SBB comment:
(Voor de vereisten van het detectieprotocol, zie het document https://www.biosafety.be/sites/default/files/partb_protocole_gmo_detection.pdf, beschikbaar via https://www.biosafety.be/content/environmental-release-gmos-experimental-purpose-tools-risk-assessment-and-risk-management)

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: 3 experts

Comment:

I have evaluated this section and have no questions. The field trial will take place on grounds belonging to the ILVO research institute in the municipality of Wetteren. The trial plot, including non-modified controls, non-modified fertilizer lines and non-modified buffer rows is 504 m2.

F. INFORMATION RELATING TO THE RELEASE

Have evaluated this section and had no comments/questions: 3 experts

G. INFORMATION RELATED TO CONTROL, MONITORING, POSTRELEASE AND WASTE TREATMENT

G.1. Any measures taken

Have evaluated this section and had no comments/questions: 3 experts

Comment:

Waarom worden in deze proef de mannelijke pluimen niet vóór maturiteit verwijderd, zoals bij andere proeven al gebeurde?

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

Have evaluated this section and had no comments/questions: 3 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: 3 experts

G.4 Information on monitoring plans and techniques

Have evaluated this section and had no comments/questions: 3 experts

Comment:

I have evaluated this section and have no questions. As written in the request, the risk of spread of the modified properties to the environment is mitigated by a combination of factors. Potential outcrossing to commercially grown conventional maize will be mitigated by: (1) an isolation distance of at least 200 meter, (2) the small scale of the field trial resulting in the production of only a small amount of modified pollen, and (3) the use of an inbred maize line that has a very high maturity index compared to the maize varieties that are grown in North-west Europe, meaning that there will be little overlap in the flowering time of the maize plants in the field trial with maize plants in commercially grown conventional maize fields.

The risk of spreading of genetically modified seeds will be strongly reduced by very rigorously manually harvesting all cobs, including the tiniest ones. Experience with such field trials in the past

years has shown that the way the cobs are harvested effectively prevents any volunteers being formed.

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: 3 experts

Comment:

I have evaluated this section and have no questions. The field trial plot is surrounded by a 1.80 m high wire fence to prevent accidental trespassing and accidental removal or spread of GM material.

ANNEX 1. INFORMATION RELATED TO THE RISKS FOR THE ENVIRONMENT

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

Comment:

The requesters claim that the environmental impact from the release is expected to be zero. The modified characteristics are not expected to lead to changes in composition, greater weediness or the ability of the maize to establish in non-agricultural habitats. The modified characteristics are also not expected to change the interaction of the maize with herbivores, or change its susceptibility to disease. This needs to be further documented.

SBB and coordinator comment: The information provided in the dossier is in line with what was provided in earlier similar applications, and was found to be sufficient.