Adviesraad voor Bioveiligheid
Conseil consultatif de Biosécurité

Advice of the Belgian Biosafety Advisory Council on notification B/BE/23/V1 from INARI Agriculture N.V. for deliberate release in the environment of genetically modified plants for research and development

14 April 2023
Ref. SC/1510/BAC/2023_0338

The notification B/BE/23/V1 has been submitted by INARI Agriculture N.V. to the Belgian Competent Authority (CA) in January 2023 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: *R&D field trial to evaluate the phenotype and yield of maize lines gene edited for reduced height.* The purpose of the release is to analyse the phenotype and yield potential of the edited maize plants under field conditions.

The notification has been officially acknowledged by the CA on 20 January 2023 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. Four 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.

On 17 March 2023, based on a list of 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 27 March 2023.

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. One question of the public tackling biosafety issues of the edited maize was received.

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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). 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 (EFSA, 2016[3]). 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 will only occur after a warm winter period (with no temperatures lower than 0°C for more than 6 to 8 hours) and will be characterised by a low probability of cross-pollination (Grüber *et al.*, 2008[4]; Palaudelmàs *et al.*, 2009[5]). Given the Belgium weather conditions, volunteers are not likely to occur.

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

The field trial will be conducted during one growing season (from April 2023 until November 2023). The surface of the area for cultivation will not exceed 1824 m^2.

To prevent the dispersal of pollen of the edited maize, border rows of conventional maize will be planted around the trial as pollen catch plants and a distance of at least 200 m from any neighbouring maize field, will be foreseen. In case such a distance cannot be guaranteed, the male flowers will be bagged or removed before flowering.

[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.

To prevent dispersal of seed, seeds will be transported in bags to the trial site; a netting will be applied in the early trial stage to prevent foraging by birds; and after harvest with a small plot combine, cobs and any seeds remaining after cleaning of the combine, will be transported in closed bags to the lab.

All collected plant material will be inactivated if no longer needed for research. Stems and leaves will be shredded on the field and will remain on the location for composting. Roots and the lowest part of the stem will be left in the ground.

The year following the field trial, monitoring for volunteers will be done. The field trial will be left fallow until the next season and ploughed, after which grass/clover will be sown as the following crop. Depending on the number of any volunteer maize plants appearing, they will be manually removed and inactivated or treated by a herbicide.

3. Information related to the genetic modification

A maize line into which a native maize genetic element was delivered in a specific promoter sequence via the CRISPR-Cas technology is subject of this field experiment. Insertion of this genetic element is expected to result in the upregulation of a transcriptional factor gene impacting internode elongation and hence plant height (shorter stature).

The maize line was obtained using a Cas editor line into which the native genetic element, a specific designed guide RNA and a marker plasmid encoding a visual and a selectable marker cassette were introduced via biolistic transformation. The vector backbone of the marker plasmid and the Cas editor line contain antibiotic resistance marker genes.

Transformed plants were selected and subsequently backcrossed with the wild type plant to obtain plants solely containing the edit (and no sequence of the Cas editor line or marker plasmid). The plants to be included in the field were tested for the lack of the *cas* gene and the selectable marker. Upon request of additional experimental data by the Council, the absence of any antibiotic resistance marker genes was demonstrated. With these additional data, the information related to the genetic modification was considered sufficient.

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

No increased persistence in the field or invasiveness into natural habitats of the edited maize line compared to non-edited maize is expected. It is not entirely excluded that the intended changed characteristic (decreased plant height) would result in a selective advantage to survivability. However, the measures taken (careful collection of cobs and seeds during harvest) and monitoring of the field for volunteers in the subsequent year after the trial) will prevent the development and survival of edited maize in the year(s) after the field trial.

Vertical gene transfer to cultivated maize in the surroundings through pollen will be negligible due to the implementation of border buffer rows and at least a 200 m isolation distance from any cultivated maize in the neighbourhood (Devos et al., 2005[6]).

Horizontal gene transfer between plants and micro-organisms is considered as a rare event under natural conditions (Keese, 2008[7]). In case gene transfer from the edited maize to micro-organisms would take place and gene expression would occur, negative effects on the environment and humans

[6] 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. Environ. Biosafety Res. 4: 71-87.
[7] Keese, P. 2008. Risks from GMOs due to horizontal gene transfer. Environ. Biosafety Res. 7: 123-149.

are not expected. The inserted genetic element influencing the regulation of plant growth, will not confer a selective advantage to bacteria.

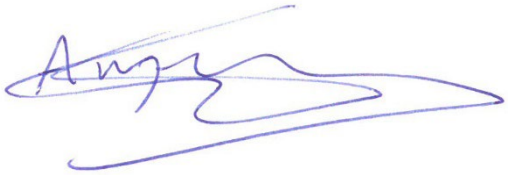
Further, it is not expected that the edited maize would have significant effects on organisms (invertebrates, vertebrates and soil micro-organisms) and humans, as no trait that could affect the behaviour or development of organisms via contact or feeding has been integrated. Given the restricted scale of the field trial, any potential effect to organisms and biogeochemical processes - if these would occur - will be of a local and temporal nature. Given the envisaged trait (shorter maize stature), 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[8]) is not envisaged.

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 use of a pollen barrier (i.e. buffer rows) and the implementation of an isolation distance of 200 m will prevent gene flow by pollen spread. Careful harvesting of the cobs and any remaining seeds and storage and transport of the cobs/seeds in closed bags will prevent seed dispersal. The collected seeds will be destroyed after analysis. In the year following the field trial, the site will be monitored for the appearance of any volunteers.

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 trials with edited maize 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/23/V1 (ref: BAC_2023_0261)

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

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Compilation of comments of the experts in charge of evaluating notification B/BE/23/V1

Ref. SC/1510/BAC/23_0261

Coordinator: Geert Angenon
Experts: Jacques Dommes (ULiège), Patrick du Jardin (ULiège), Michel Ghanem (CIRAD), Nina Papazova (Sciensano) & Jan Van Doorsselaere (VIVES)
SBB: Adinda De Schrijver

INTRODUCTION

Dossier **B/BE/23/V1** concerns a notification of INARI Agriculture N.V., 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 3 February 2023 and concerns a field trial to evaluate the phenotype and yield of maize lines gene edited for reduced height.

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: 4 experts

C. INFORMATION RELATED TO THE GENETIC MODIFICATION

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

Comment 1:

In table 1, the method for calculating a "specificity score" and assessing possible off-targets refers to Zhang et al. 2013, but this reference is missing in the reference list and was not provided in the information package made available to the reviewer. It is thus impossible to properly evaluate the search for off-targets performed in this application. The reference should be asked to the applicant.

Note coordinator & SBB: The reference is indeed missing, but the software used to assess potential off-targets [Geneious Prime, Biomatters Ltd] and criteria for determination of off-targets are mentioned in the application. We further want to note that the search for/verification of off-target changes has not been considered a safety issue for other Part B field trial notifications.

Comment 2:

The material has been generated using CRISPR-Cas technology, a site directed nuclease (SDN) system, allowing the precise insertion of a 36bp maize DNA sequence at a target location. The detailed information is provided correctly.

D. INFORMATION RELATED TO THE GENETICALLY MODIFIED PLANT

D.1. General description of the traits and characteristics, which have been introduced or modified

Have evaluated this section and had no comments/questions: 4 experts

D.2. Information on the sequences actually inserted/deleted

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

Comment 1:

Please provide a figure showing an alignment of the sequencing results of the ZmDEC1 promoter from WT and edited lines.

Have the developed lines T2, T3 been tested for hygromycin resistance (via *in vitro* or PCR analysis) and PAT resistance (via spraying or *in vitro* analysis)?

Please show the results of PAT qPCR demonstrating absence of this marker.

Biolistics is known as a “dirty” transformation method; has it been tested if parts of the backbone of the plasmid with the PAT (pIN1184) are incorporated?

Note coordinator & SBB: Sequencing data to prove the presence of the intended modification are not considered as a requirement for the evaluation of Part B trials with GM plants (see guidelines of the Council). Having said this, some indication of the copy number (plus proof) should be provided for transgenic plants.

Comments will be formulated as such: Data, not necessarily sequence data, should be provided on the presence of a single copy of the enhancer element and the absence of the marker plasmid and the Cas9 editor construct, and specifically the absence of antibiotic resistance genes. If the absence of the editor line and marker plasmid is not demonstrated, the presence of these sequences should in principle be taken up in the risk assessment.

Comment 2:

In this application, null segregants without edit were used as comparators for studying the phenotype of the edited plants. This is certainly the best control from a scientific point of view, but the EU legislation prohibits the use of such negative segregant as sole comparator for the comparative analysis between GM and non-GM plants in Part C dossiers. I consider that there is no safety issue for such Part B dossiers.

D.3. Information on the expression of the donor material

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

Comment 1:

The applicants provide Figure 8 as a proof of expression. However, they mention that this figure compiles all publicly available data which is inserted in the maize database. Applicants are required to provide data about the expression of the insert during the lifecycle of the plant, as the current Figure 8 is not convincing since we cannot distinguish the native expression from the modified plants.

Note coordinator & SBB: The 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 this first field trial stage (where several lines are tested), it is sufficient to give information on the expected expression (provided in Fig.8), rather than the real expression.

D.4. Information about the differences between the genetically modified plant and the recipient plant

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

Comment 1:

On page 7 of the technical dossier, the authors refer to the function of the corresponding DEC1 gene in rice and indicate that this gene controls sensitivity to the plant hormone gibberellic acid (‘GA’). The same is expected in maize. GA is known to control seed dormancy (break) in higher plants. Considering that the edited maize plants are expected to have a decreased sensitivity to GA, effects on seed dormancy, like a prolonged dormancy, may be hypothesized. In such a scenario, reproduction, dissemination and persistence of seeds might be affected.

On page 16 of the dossier, the applicant considers that no effects on the 'mode and rate of reproduction', on 'Dissemination' and on 'Survivability' are expected. They do not base their assumption on a discussion of the relevant literature, but on greenhouse observations. The applicant claims that 'no changes have been observed', but the traits (assessment endpoints) are not described.

For the sake of completeness and accuracy of the risk assessment, further information should be asked to the applicant on the following:

1) Literature review of the impact of DEC1 on GA sensitivity and associated seed traits (in rice and other plants if available);
2) Description of the traits (assessment endpoints) used in the phenotypic evaluation of the greenhouse-grown plants, supporting their conclusions.

Comment 2:

The number of plants used in Figures 9 and 10 is extremely low if we suppose that each dot represents a plant. Applicants are required to give additional precisions about the number of plants evaluated and the condition under which the plants were grown in the greenhouse as this may influence the phenotype.

Note coordinator & SBB: To assess the potential risks, we start from the presumption that the desired modification will be achieved in the field trial. We therefore do not see the need to ask for more information on the greenhouse experiment to come to a risk/safety conclusion on the field trial.

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: 4 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: 4 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: 4 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: 4 experts

D.11. Potential interactions with the abiotic environment

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

Comment 1:

The applicants claim that the editing of the transcription factor ZmDEC1 gene results in the shortening of the maize plants and conclude that "*it has no influence on interactions with the abiotic environment*". This is a bit of hasty argument that is not supported by data as shortening the plant might have an effect on how plants behave under various conditions such as drought. This needs to be clarified.

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

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

Comment 1:

I did not access the 'annex' mentioned on page 17 of the technical dossier and could not check the details of the detection method.

Comment 2:

Specifics of the gel electrophoresis, including % of agarose gel, time, voltage must be provided.

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

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

E. INFORMATION RELATING TO THE SITE OF RELEASE

Have evaluated this section and had no comments/questions: 4 experts

F. INFORMATION RELATING TO THE RELEASE

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

Comment 1: *Jan Van Doorsselaere*

I do not see a reason not to detassel the homozygous/heterozygous edited plants since null segregants and null segregant hybrdis and commercial maize cultivars (from the buffer zone) are present for cross-pollination and seed set.
If detasseling is not done, pollen dispersal will occur to the border zone and potentially to maize crops in the environment. According to legislation pollen from NBT (such as CRISPR/CAS) are considered as a GMO.

What will happen with the plants from the 4 row border zone after the experiment?

What will be planted around the GMO field trial? Maize?

Note coordinator & SBB: Under E.2 (p.19) it is clarified that the trial will be surrounded by grass/clover.

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: 3 experts

Comment 1:

As stated before (see F), detasseling should be done on the homozygous and heterozygous edited plants.

Also, what will be measured after the trial? Height of the plants, cob, ear parameters…?

Pollination by hand: of what?

Note coordinator & SBB: What will be measured by the notifier is not a biosafety question and we therefore do not see the need to ask this question to come to a risk/safety conclusion. Measures proposed are clear from the application (no hand pollination if isolation distance is as expected, otherwise hand pollination). The Biosafety Advisory Council will decide on the measures to be taken in order to guarantee that the field trial is conducted safely.

G.2. Description of methods for post-release treatment of site

Have evaluated this section and had no comments/questions: 4 experts

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

Have evaluated this section and had no comments/questions: 4 experts

G.4 Description of monitoring plans and techniques

Have evaluated this section and had no comments/questions: 4 experts

G.5. Description of any emergency plans

Have evaluated this section and had no comments/questions: 4 experts

G.6. Description of methods and procedures to protect the site

Have evaluated this section and had no comments/questions: 4 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: 3 experts

Comment 1:

See comments about seed dormancy in section D.4 above.

2. Gene transfer from GM plants to micro-organisms

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

3. Interactions of the GM plant with target organisms

Have evaluated this section and had no comments/questions: 4 experts

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

Have evaluated this section and had no comments/questions: 4 experts

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

Have evaluated this section and had no comments/questions: 4 experts

6. Effects on biogeochemical processes

Have evaluated this section and had no comments/questions: 4 experts

7. Effects on human and animal health

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

OTHER INFORMATION

Do you have any other questions/comments concerning this notification that are not covered under the previous items?

Comment 1:

The technical dossier (Part 1) communicated to the expert contains editorial highlights (in blue) and event left-over edits (see each mentioning of a figure in the text). I guess this is a mistake from the applicant, but this should obviously be avoided.