Adviesraad voor Bioveiligheid
Conseil consultatif de Biosécurité

Advice of the Belgian Biosafety Advisory Council on notification B/BE/23/V4 from INARI Agriculture N.V. for deliberate release in the environment of genetically modified plants for research and development

16 February 2024
Ref. SC/1510/BAC/2024_0202

The notification B/BE/23/V4 has been submitted by INARI Agriculture N.V. to the Belgian Competent Authority (CA) in December 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 optimised plant architecture.* 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 18 December 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, but only two replied.
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. No requests for additional information were formulated.

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 edited 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). 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 Belgian 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 2024 until December 2024). The surface of the area for cultivation will not exceed 1710 m^2.

To prevent the dispersal of pollen of the edited maize, a distance of at least 200 m from any neighbouring maize field will be foreseen and a 3 m border buffer of 4 rows of conventional maize will be planted around the trial.

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.

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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 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

Two maize lines into which a native maize genetic element was delivered in a specific promoter sequence via the CRISPR-Cas technology, and a cross of these two maize lines, are subject of this field experiment. In the first maize line, the insertion of this genetic element was shown to result in enhanced expression of a transcription factor gene impacting internode elongation and hence plant height (shorter stature) under field conditions (see B/BE/23/V1). In the second maize line, the insertion of the same genetic element is expected to result in the upregulation of a transcriptional activator impacting leaf size and hence leaf biomass (increased biomass) under field conditions.

The maize line with reduced height 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 Cas editor line and the vector backbone of the marker plasmid contain antibiotic resistance marker genes.

The maize line with increased leaf biomass was obtained via RNP biolistic transformation of maize embryos into which the native genetic element, a specific designed guide RNA, a marker plasmid encoding a visual and a selectable marker cassette, and a Cas nuclease protein were introduced. The vector backbone of the marker plasmid contains antibiotic resistance marker genes.

Maize plants with reduced height and maize plants with increased leaf biomass, carrying only the envisaged modification (and no marker or *cas* genes), were selected and subsequently crossed with each other to obtain heterozygous plants containing both edits (reduced height and increased leaf biomass), and further selfed to obtain homozygous plants with both edits, one edit (reduced height or increased leaf biomass) or no edit (null segregants). These homozygous lines (i.e. inbred lines), as well as crosses of these homozygous lines with non-GM male lines (i.e. hybrid lines) will be evaluated in the field trial. The plants selected to be included in the field were re-tested for the lack of the *cas* gene (from the Cas editor line) and the selectable markers. 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 lines compared to non-edited maize is expected. It is not entirely excluded that the intended changed characteristic (decreased plant height and/or increased leaf biomass) 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 an isolation distance of at least 200 m from any cultivated maize in the

neighbourhood, border buffer rows with conventional maize plants serving as pollen catch plants and the use of maize plants that flower later than the maize planted by local farmers (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 are not expected. The inserted genetic element influencing the regulation of plant growth, will not confer a selective advantage to bacteria.

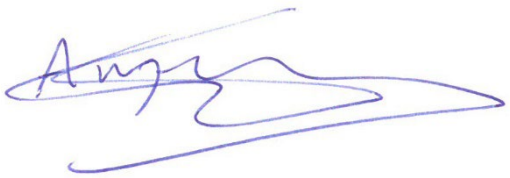
Further, it is not expected that the edited maize would have significant effects on organisms (invertebrates, vertebrates and soil micro-organisms) as no trait that could affect the behaviour of organisms via contact or feeding has been integrated. Given the restricted scale of the field trial, any potential effect to organisms and biogeochemical processes - if these would occur - will be of a local and temporal nature. Given the envisaged trait(s) (shorter maize stature and/or larger leaves), a possible altered allergenicity potential of the transgenic pollen (allergy from maize pollen may occur in case of occupational exposure of humans to high amounts of pollen grains, see *e.g.* Oldenburg *et al*., 2011[8]) is not envisaged. No plant material will enter the food/feed chain.

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 implementation of an isolation distance of 200 m, the use of a pollen barrier (i.e. buffer rows) and the use of a late-flowering maize variety 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/V4 (ref: BAC_2024_0135)

[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.
[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/V4

Ref. SC/1510/BAC/24_0135

Coordinator: Geert Angenon
Experts: Jacques Dommes (ULiège), Nina Papazova (Sciensano) & Jan Van Doorsselaere (VIVES)
SBB coordinator: Adinda De Schrijver

INTRODUCTION

Dossier **B/BE/23/V4** concerns a notification of the 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 18 December 2023 and concerns a field trial to evaluate the phenotype and yield of maize lines gene edited for optimised plant architecture

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 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.

A. GENERAL INFORMATION

4. INFORMATION RELATING TO THE RELEASE

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

5. INFORMATION RELATING TO THE SITE OF RELEASE

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

B. SCIENTIFIC INFORMATION

1. INFORMATION RELATING TO THE RECIPIENT PLANT OR, WHERE APPROPRIATE, TO THE PARENTAL PLANTS

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

2. MOLECULAR CHARACTERISATION

(a) Information relating to the genetic modification

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

(b) Information relating to the genetically modified higher plant (GMHP)

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

(c) Conclusions of the molecular characterisation

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

3. INFORMATION ON SPECIFIC AREAS OF RISK

(a) Any change to persistence or invasiveness of the GMHP, and its ability to transfer genetic material to sexually compatible relatives, and the adverse environmental effects thereof

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

(b) Any change to the ability of the GMHP to transfer genetic material to microorganisms and the adverse environmental effects thereof

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

(c) Mechanism of interaction between the GMHP and target organisms and the adverse environmental effects thereof

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

(d) Potential changes in the interactions of the GMHP with non-target organisms resulting from the genetic modification and the adverse environmental effects thereof

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

(e) Potential changes in agricultural practices and management of the GMHP resulting from the genetic modification and the adverse environmental effects thereof

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

(f) Potential interactions with the abiotic environment and the adverse environmental effects thereof

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

(g) 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: 1 expert

Comment:
Is it known which endogenous maize genes are upregulated due to overexpression of a gene coding for a transcription factor and a transcriptional activator? If yes, has it been checked if these proteins are toxic or allergenic?

SBB: Which endogenous genes are upregulated, is indeed not described in the dossier. Maize has however a history of safe use as food or feed and is not considered to have allergenic properties (OECD, 2002)[1]. Given the nature of the trait, it is not expected that the production of antinutrients will be affected. Moreover, plant material derived from the field trial will not be fed to animals or used for human consumption, and measures are taken to prevent feeding of birds (netting) and mammals (fence) on the modified maize plants.

(h) Conclusions on the specific areas of risk

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

4. INFORMATION ON CONTROL, MONITORING, POST-RELEASE AND WASTE TREATMENT PLANS

(a) Any measures taken

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

(b) Description of methods for post-release treatment of the site

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

(c) Description of post-release treatment methods for GM plant material, including wastes

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

[1] OECD 2002. Consensus Document on Compositional Considerations for New Varieties of Maize (Zea Mays): Key Food and Feed Nutrients, Anti-nutrients and Secondary Plant Metabolites. OECD Environmental Health and Safety Publications. Series on the Safety of Novel Foods and Feeds. nr.6, 42 pp

(d) Description of monitoring plans and techniques

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

(e) Description of any emergency plans

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

(f) Description of methods and procedures to protect the site

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

5. DESCRIPTION OF DETECTION AND IDENTIFICATION TECHNIQUES FOR THE GMHP

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

Comment:
p29: 5 Description of detection and identification techniques for the GMHP
It is mentioned that this method does not allow to detect that this mutation is caused by gene editing.
This phrase is strange: all the lines have arisen due to gene editing with crispr/cas. It is extremely unlikely that the edit is arisen due to random mutagenesis. This phrase should be deleted.

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.

6. INFORMATION ABOUT PREVIOUS RELEASES OF THE GMHP, IF APPLICABLE

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 plant to plant gene transfer

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

2. Plant to micro-organisms gene transfer

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

3. Interactions of the GMHP with target organisms

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

4. Interactions of the GMHP with non-target organisms

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

5. Impacts 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

OTHER INFORMATION

Comment 1:
p12 and p19: The story line of the third mutation is not relevant and should be deleted.

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.

Note coordinator: The notifier correctly provided detailed description of the methods used.

Comment 2:
Given the fact that biolistics can result in mutations of the DNA at different random sites in the genome, did Inari investigate whether this is the case (e.g. presence of mutation at other locations and presence of fragments of plasmids used for the bombardment)?

Note SBB: This information has not been provided. We want to note that the search for/verification of off-target changes is and has not been considered a safety issue for Part B field trial notifications (due to the restricted environmental release and use).