Deliberate Release Notification

Part 3 – Environmental Risk Assessment

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"R&D Field trial to evaluate the phenotype and yield of maize lines gene edited for reduced height."

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OBJECTIVE OF THE ENVIRONMENTAL RISK ASSESSMENT

In the context of performing a field trial in Belgium with gene edited maize lines, this Environmental Risk Assessment (ERA) has been conducted in accordance with Annex IIA of European Directive 2001/18/EC and Commission Decision 2002/623/EC to identify and evaluate potential adverse effects of the gene edited maize lines, either direct or indirect, immediate or delayed, on human health and the environment which the conduct of a field trial with this material may exert. It is conducted with a view of identifying if there is a need for risk management and if so, to ensure that the most appropriate methods are used to mitigate this risk.

This ERA is performed according to the methodology laid out in Annex IIC of Directive 2001/18/EC, supplemented by the guidance notes in Commission Decision 2002/623/EC.

LIST OF ABBREVIATIONS

EU	European Union
GMHP	Genetically Modified Higher Plant
ILVO	Instituut voor Landbouw-, Visserij- en Voedingsonderzoek
RBD	Randomized block design
RNA	Ribonucleic acid
VIB	Vlaams Instituut voor Biotechnologie

I. CHARACTERISTICS OF THE MODIFIED MATERIAL AND THE RELEASE

Detailed information on the characteristics of the material and the intended release are provided in the technical file of this application. In this section, the main elements relevant for the ERA are summarized.

A. The recipient organism

Maize (*Zea mays*) is a well-known domesticated plant species, with a long history of safe use and which is annually cultivated on global scale. It is a major commodity crop with many applications in food and feed. Furthermore, it has been a primary target for innovative solutions based on genetic engineering and therefore subject of many risk assessments, including by the Belgian and European authorities.

Maize is routinely grown on commercial scale in the region where the trial will be conducted. Furthermore, ILVO, the institute that will take care of the operational aspects of the trial, is equipped for and has a long standing experience in conducting breeding and agronomic trials of maize, conventional as well as genetically modified/ gene edited lines.

Maize is a highly domesticated plant, in many aspects fully relying on the intervention of humans for survival and dispersal:

- Vegetative parts, once severed from the plant, lack the capacity for regrowth or secondary shooting. As an annual plant, individual plants wither at the end of the growing season and die. No survival structures are formed.
- Seeds remain attached to the cobs, preventing seed dispersal before harvest.
- Seeds are not naturally dispersed over long distance. They show no dormancy and are sensitive to low temperatures.
- Maize is not known to cause volunteer issues in farmer's fields or outside (field edges, transportation routes, ..). Maize is not a competitive plant in unmanaged environments.
- There are no compatible wild-relatives in Belgium.
- Although pollen can be carried by wind over long distance, successful pollination remains limited to the immediate vicinity of the plants.

B. The genetic modification(s)

Inari has used a combination of advanced techniques to develop the experimental lines that will be tested in the proposed field trials. Starting with a Cas editor line produced by the research institute VIB, biolistic transformation was used to introduce a native maize genetic element in particular sites of a native maize transcription factor gene. While different components were necessary (including a marker carrying plasmid), during subsequent selection only those lines were retained that showed the intended insertion of the native maize genetic element and lacked any other inserted sequence.

The insertion of the native maize genetic element has been targeted by gene editing based on specifically designed guide RNAs. Off-target effects has been raised as a possible concern for using gene editing. Assessing potential off-targets with the Geneious Prime tools showed that no matches could be identified. Further confirmation will be planned for lines that are carried forward in product development.

Two different guide RNAs were used to insert the native genetic element at two different locations of the transcription factor gene in order to modify the altered expression at two different levels.

- Guide 1: insertion of the native genetic element at one site;
- Guide 2: insertion of the native genetic element at another site.

As such, the edits with the two different guide RNAs resulted in different alleles of the transcription factor gene.

The native genetic element is identified in the maize genome that can alter expression of native genes, this sequence also occurs in the genomes of other crops like soy, rice, and barley. The insertion is not transcribed or expressed as such, and therefore no new proteins are produced.

Insertion of the native genetic element in a native maize transcriptional factor gene is expected to result in the altered expression, which in turn results in suppression of internode elongation. Overall, it can be concluded that no new proteins/enzymes are produced, but instead that the levels of an existing transcription factor are influenced.

C. The modified organism

As pointed out, the only remaining modification is the gene editing resulting in the precise insertion of a native maize genetic element in the native maize transcription factor gene.

The size of plants is largely determined by growth of the stem. Stem elongation is affected by the expression of the transcription factor gene. With gene editing the native maize genetic element in the specific sites resulting in altered expression of the native transcription factor gene, internode elongation and, hence, plant height is expected to be reduced. Limited observations in the greenhouse confirm the phenotype, but field trials under realistic growing conditions are required for further evaluation.

The finally selected modified organism carries no other additional characteristics.

D. The intended release

This first confined field trial is a limited scale comparison of two lines (each carrying respectively the native maize genetic element in a different targeted insertion site). Each line is presented both as inbred lines (resulting from self-pollination) and as an experimental hybrid with a conventional line.

The trial includes controls (segregating null-lines) and other maize lines to provide for border rows.

Inbred trial

1	GIBE0104	Control original germplasm
2	Control for the first allele	Null segregant (without edit)
3	First allele	Heterozygous edited
4	First allele	Homozygous edited
5	Control for the second allele	Null segregant (without edit)
6	Second allele	Heterozygous edited
7	Second allele	Homozygous edited
8	Commercial line	Filler for RBD design

Hybrid trial

1	GIBE0104 x crossing partner	Control
2	Control of the first allele x conventional line	Null segregant (without edit)
3	First allele (homozygous edit) x conventional line	Heterozygous edited hybrid
4	Control of the second allele x conventional line	Null segregant (without edit)
5	Second allele (homozygous edit) x conventional line	Heterozygous edited hybrid

The design is a typical randomized block design, with 4 repetitions. In addition to overall development of the plants, the observations will specifically address the intended phenotype (reduced plant and ear height) and yield.

The overall surface, including border rows, will be less than 2000 M^2.

E. The potential receiving environment

The field trial site is in a rural area where agricultural crops, meadows and field margins with herbs and bushes are found. The trial itself is surrounded by grass/clover land.

F. Interactions

No particular interactions between the experimental lines and the environment are expected.

G. Information from releases of similar organisms and organisms with similar traits and their interaction with similar environments

There were no previous releases of these lines. The intended trial is the first field trial with plants containing the edited transcription factor gene.

On the other hand, there is extensive experience with release of genetically modified as well as gene edited maize, including with the team that will be managing the operational aspects of the field trial.

II. ENVIRONMENTAL RISK ASSESSMENT

In this section, the characteristics of the GMO linked to the genetic modification that may result in adverse effects on human health or the environment are reviewed. They are based on a generic series of potential adverse effects of GMOs and some that are not applicable for the specific release were discarded at the first step of the risk assessment, i.e. the “identification of characteristics which may cause adverse effects”. For others, the potential impact as well as the likelihood is further analysed, leading to an estimation of the risk for human health or the environment.

A. Persistence and invasiveness of the GMHP, including plant-to-plant gene transfer.

1. Problem formulation including hazard identification

This section addresses the potential for maize with an edited transcription factor gene to become more persistent or invasive compared to wildtype maize either by itself or through outcrossing to sexual compatible species. Maize by itself is not invasive or persistent.
Genetic traits can be vertically transferred in species sexually compatible with the GMHP. As pointed out before, there are no sexually compatible species for maize in Belgium. Any transfer is therefore limited to other maize plants.

The edited maize plants do not differ from conventional maize except for the altered expression of the transcription factor gene. Neither the native genetic element, nor the transcription factor gene produce any new function as such. Therefore, the effect of the reduced stature of the plant should be assessed.

2. Hazard characterisation

Maize by itself is not invasive or persistent. It is not expected that the reduced stature of the edited plant will have an influence on the persistence or invasiveness. On the contrary, in a plant stand with wildtype maize plants the smaller gene edited plants will be experiencing shadow effects reducing its growth capacities.
However, mixed populations do naturally not occur since maize is only present as human controlled cultivation; the trait will therefore not make the plants more persistent than the recipient or parental plants in agricultural areas.
Outcrossing of the trait will provide a disadvantage to the progeny of the recipient maize plant.

The effect in the natural environment will be neutral, if not negative for the same reason. In any case, maize is not a potent competitor in unmanaged areas and this is not expected to change in relation to the reduced height.

3. Exposure characterisation

The gene-edited maize will be released in a field trial with limited surface surrounded by non-modified border rows to limit outcrossing. Such border rows have been commonly used as pollen traps as well as providing additional pollen to dilute any pollen release from the experimental material. Due to the lower plant height compared to the border plants outcrossing is even more reduced. Therefore, the exposure is very limited.

Although pollen can be carried over long distance by wind, the potential for a successful cross-pollination with a compatible plant drops quickly over distance. Hence, with the additional provision of an isolation distance of 200 m to the nearest maize field the likelihood for successful fertilization is extremely low. In case it is observed that this isolation distance cannot be met, an alternative approach to avoid pollen flow to other maize will be established (bagging flowers before anthesis and pollination by hand or detasseling of experimental plants well before anthesis).

Even in the unlikely case of a successful fertilization, the consequence is deemed irrelevant: the seeds on the remote plant that may then carry the gene edited function, will not enter the environment. Rather they will be harvested and treated for downstream use. Farmers do not use farm saved seed for maize, so no reintroduction will occur.

4. Risk characterisation

Taken the hazard characterisation together with the exposure characterisation the risk is negligible.

5. Risk management strategies

No risk management strategies are warranted.

6. Overall risk evaluation and conclusions

Results from the assessment support the conclusion that the ability of the gene edited maize to persist in agricultural fields or invade non-agricultural habitats are lower to those of conventional maize. Also, the probability of gene transfer through pollen dispersal will be limited and the transferred trait represents rather a disadvantage. The negligible hazard and the very low levels of environmental exposure lead to the conclusion that the edited maize lines do not pose a risk to the environment.

B. Gene transfer from plants to microorganisms.

1. Problem formulation including hazard identification

Gene transfer from the edited maize to microorganisms may bring advantages or disadvantages to the microorganism receiving the genetic information.
The edited maize plants do not differ from conventional maize except for the altered expression of the transcription factor gene. No new genetic elements, not already present in maize, have been inserted. Therefore, for both the genetic material originating from edited or non-edited maize plants, no difference is expected in the effect of transfer of genetic material to microorganisms.

Therefore, this topic is not applicable and not further elaborated.

C. Interactions of the GMHP with target organisms.

1. **Problem formulation including hazard identification**
   The edited maize lines do not target organisms, such as predators, parasitoids and pathogens. Therefore, this topic is not applicable and not further elaborated.

D. Interactions of the GMHP with non-target organisms.

1. **Problem formulation including hazard identification**
   Introduced traits may have an effect on all kind of organisms interacting with the gene-edited maize plants. Organisms involve beneficial organisms as well as pests and disease-causing organisms.

   The gene-edited maize plants do not differ from conventional maize except for the altered expression of the transcription factor gene. Neither the native genetic element, nor the transcription factor gene produce any new function as such. Therefore, any non-target organism interacting with the gene edited lines would already have been exposed to the functions.

   The resulting phenotype, i.e. the reduced stature of the plants, is also not expected to have any immediate and/or delayed environmental effects.

   Therefore, this topic is not applicable and not further elaborated.

E. Effects of the specific cultivation, management and harvesting techniques.

1. **Problem formulation including hazard identification**
   Changes in the gene-edited maize plants may influence the way the plants are cultivated, managed and harvested.
   The reduced stature of the plants may result in a more efficient fertilizer use and disease treatment by allowing more targeted applications later in the season, when really needed.
   Such changes are expected to improve the overall agronomic management and contribute to more sustainable agriculture.

   In the first field trial focussing on confirming the phenotype and plant development, the standard agronomic practices will be used. No change in management will be investigated or implemented yet.

   Therefore, this topic is not applicable and not further elaborated.

F. Effects on biogeochemical processes.

1. **Problem formulation including hazard identification**
   Maize is not known to play any specific role in biogeochemical processes.
   The edited maize plants do not differ from conventional maize except for the altered expression of the transcription factor gene. Neither the native genetic element, nor the transcription factor gene produce any new function as such. Also the resulting phenotype has no correlation with any biogeochemical processes.

   Therefore, this topic is not applicable and not further elaborated.

G. Effects on human and animal health.

Human health

1. Problem formulation including hazard identification:
Handling gene-edited maize plant potentially have effects on persons working with, coming into contact with or in the vicinity of these plants.

The edited maize plants do not differ from conventional maize except for the altered expression of the transcription factor gene. Neither the native genetic element, nor the transcription factor gene produce any new function as such.

Maize pollen is known to cause allergies in frequently exposed persons. Yet, neither the native genetic element, nor the gene encoding the transcription factor are linked with any known allergen.

The resulting phenotype, i.e. the reduced stature of the plants, is also not expected to have any immediate and/or delayed effects on human health.

Therefore, this topic is not applicable and not further elaborated.

Animal health

1 Problem formulation including hazard identification
The trait introduced in the maize plants may have an effect on animals fed with the gene-edited maize plants depending on their composition and/or presence or antinutritional factors.

The edited maize plants do not differ from conventional maize except for the altered expression of the transcription factor gene. Neither the native genetic element, nor the transcription factor gene produce any new function as such. They are not known to code for any toxin or anti-nutritional factor.

2. Hazard characterisation
Only if the composition of nutrients and antinutrients of the gene-edited maize has changed compared to conventional maize varieties, this might have an effect on the nutritional value of the feed. Furthermore, it is highly unlikely that the edited trait, the reduced stature, has any influence on the composition of maize feed.

3. Exposure characterisation
The gene-edited plants in the proposed field trial are not intended to be fed to animals. As all the material, with the exception of samples for further analysis, will be destroyed upon termination of the trial, no materials will enter the feed/food chain.

Any equipment used for the management of the trial, in particular for seeding and harvesting, will be thoroughly cleaned on the site to exclude any carry-over to other fields and dispersal.

Above it was argued that -although unlikely- successful fertilisation of remote maize plants in commercial field may occur via pollen flow. Such harvested material will be oriented to animal feeding. It must be highlighted that although pollen will contribute to the genetic make-up of the seed and hence the next generation, the main quality characteristics of the grain are maternally determined. The genetic sequences relating to the native genetic element and the transcription factor gene are already present in all maize feed. Therefore, even in this unlikely scenario, no impact is expected.

Direct consumption (not as feed) can occur in the field e.g. by birds or mammals. It is good practice to avoid damage to the field trials, so whenever needed additional measures will be taken (e.g. netting of the area after seeding to prevent bird damage, fencing in case rabbits damage would be observed). Nevertheless, as the genetic sequences relating to the native genetic element and the transcription factor gene are already present in all maize feed, no new effects are expected. Finally, given the limited area of the field trial and the fact that it is

surrounded by border plants, the chance that wild animals will feed on the gene-edited plants is low.

4. **Risk characterisation**
   Giving the fact that the edited maize in the field trial is not intended for animal feed, the risk is negligible. Also, for wild animals feeding on the maize plants in the trial, the hazard characterisation taken together with the exposure characterisation makes the risk negligible.

5. **Risk management strategies**
   No risk management strategies are warranted.

6. **Overall risk evaluation and conclusions**
   Results from the assessment support the conclusion that the gene-edited trait will have no effect on feeding animals. The negligible hazard and the very low levels of environmental exposure lead to the conclusion that the edited maize lines do not pose a risk to animal health.

III. CONCLUSIONS ON THE POTENTIAL ENVIRONMENTAL IMPACT FROM THE RELEASE

This ERA was performed according to the methodology laid out in Annex IIC of Directive 2001/18/EC, supplemented by the guidance notes in Commission Decision 2002/623/EC. Information on the points listed in Annex IIID of Directive 2001/18/EC, as transposed into the Royal Decree of 21 February 2005 (and amendments) were provided in the previous section and are summarized in this overall conclusion.

Points listed in Annex IIID of Directive 2001/18/EC	Overall risk evaluation
1. Persistence and invasiveness of the GMHP, including plant-to-plant gene transfer.	The ability of the gene edited maize to persist in agricultural fields or invade non-agricultural habitats are lower to those of conventional maize. The probability of gene transfer trough pollen dispersal will be limited and that the transferred trait represents rather a disadvantage. The negligible hazard and the very low levels of environmental exposure lead to the conclusion that the edited maize lines do not pose a risk to the environment.
2. Gene transfer from plants to microorganisms.	The effect of transfer of genetic material form the edited maize to microorganisms is not different from transfer from non-modified maize. Therefore, the edited maize lines do not pose a risk to the environment.
3. Interactions of the GMHP with target organisms.	This topic is not applicable.
4. Interactions of the GMHP with non-target organisms.	This topic is not applicable.
5. Effects of the specific cultivation, management and harvesting techniques.	This topic is not applicable.
6. Effects on biogeochemical processes.	This topic is not applicable.
7. Effects on human and animal health	Human health: this topic is not applicable.

	Animal health: the gene-edited trait will have no effect on feeding animals. The negligible hazard and the very low levels of environmental exposure lead to the conclusion that the edited maize lines do not pose a risk to animal health

The applicant submits that the overall conclusion of the environmental risk assessment is that the intended field trial with the gene edited maize lines will not entail any adverse effects, either direct or indirect, immediate or delayed, on human health and the environment.

The proposed trial includes already several measures (e.g. handling of seeds, use of specialised equipment, cleaning of equipment, provision of an isolation distance to any other maize, planting of border rows with conventional maize) that will contribute to confinement for the material. No need for additional risk management was identified in the ERA.