

Summary Notification Information Format

A. General information

A1. Details of notification

Notification Number

B/BE/25/Vx

Member State

Belgium

Date of Acknowledgement

xx January 2025

Title of the Project

Scientific field evaluation of maize with improved yield

Proposed period of release:

15/04/2025 to 30/11/2027

A2. Notifier

Name of the Institute

VIB

A3. Is the same GMPt release planned elsewhere in the Community?

No.

A4. Has the same GMPt been notified elsewhere by the same notifier?

No

B. Information on the genetically modified plant

B1. Identity of the recipient or parental plant

- | | |
|----------------------------|---------------------------------|
| a) family: | <i>Poaceae</i> |
| b) genus: | <i>Zea</i> , section <i>Zea</i> |
| c) species: | <i>Zea mays</i> |
| d) subspecies: | <i>mays</i> |
| e) cultivar/breeding line: | inbred line B104 |
| f) common name: | maize |

B2. Description of the traits and characteristics which have been introduced or modified, including marker genes and previous modifications

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.

B3. Type of genetic modification

Targeted multiplex editing of genes using CRISPR-Cas.

B4. In case of insertion of genetic material, give the source and intended function of each constituent fragment of the region to be inserted

There is no donor DNA inserted into the genetic material. The genome editing has resulted in the addition of one DNA basepair in both transcription factor genes thereby generating an out-of-frame mutation in both of these genes.

B6. Brief description of the method used for the genetic modification

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. This results in plants in which mutations will be introduced in the genes that are targeted by the guide RNAs. This process generates plants with different numbers of mutated target genes. In some plants two target genes will be mutated, in others three, or four or even more. A plant with mutations in the two transcription factor genes (GRF10 and TCP42) is at the basis of the plant material in this field trial. This plant was crossed with the haploid inducer line RWS-GFP, and the embryos resulting from this cross were grown on a medium containing colchicine, which results in a doubling of their genome. These double haploid plants were selfed and from the resulting next generation, plants were chosen that neither contained T-DNA nor vector backbone sequences. In other words, the plants in the field trial only contain the CRISPR-Cas generated mutations, but no foreign sequences.

B7. If the recipient or parental plant is a forest tree species, describe ways and extent of dissemination and specific factors affecting dissemination

Not applicable.

C. Experimental Release**C1. Purpose of the release**

The purpose of the release is to determine the performance of the modified maize plants under normal field conditions and learn whether they have an increased biomass in these conditions resulting from increases in leave size, stem diameter, plant height, and cob size.

C2. Geographical location of the site

The field trial will take place on grounds belonging to the ILVO research institute in the municipality of Wetteren.

C3. Size of the site (m²)

The trial plot, including non-modified controls, non-modified fertilizer lines and non-modified buffer rows is 504 m².

C4. Relevant data regarding previous releases carried out with the same GM-plant, if any, specifically related to the potential environmental and human health impacts from the release

The plants have not been field tested before.

D. Summary of the potential environmental impact from the release of the GMPts

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.

E. Brief description of any measures taken for the management of risks

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 reduced to zero by very rigorously manually harvesting all cobs, including the tiniest ones. Experience with such field trials in the past 13 years has shown that the way the cobs are harvested effectively prevents any volunteers being formed. 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.

F. Summary of foreseen field trial studies focused to gain new data on environmental and human health impact from the release

There are no specific studies foreseen to gain new data on the environmental and human health impact from the release other than the study of the phenotype and growth characteristics of the maize.

G. Final report

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H. European Commission administrative information

To be filled in by the Commission

I. Consent given by the Competent Authority:

To be filled in by the Commission.