

Summary Notification Information Format

A. General information

A1. Details of notification

Notification Number

.....

Member State

Belgium

Date of Acknowledgement

.....

Title of the Project

Scientific field evaluation of maize with an impaired DNA-repair mechanism and maize with modified growth characteristics

Proposed period of release:

01/04/2018 to 31/10/2018

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 maize plants have been edited using the CRISPR/Cas9 system resulting in the mutation of either the ALT gene or the NGAL2 gene. In both cases one extra DNA base pair has been introduced into the target genes resulting in the knock-out of these genes by means of a frameshift mutation.

The ALT gene plays a role in the repair of DNA damage and in these plants faults in the DNA are believed to accumulate to a higher degree. The plants have thereby become a good biosensor for the type of DNA damage that is caused by different forms of abiotic stress such as drought, heat, or environmental pollution.

The NGAL2 is a transcription factor that has been shown to negatively affect the expression of the PLA1 gene. The PLA1 gene codes for a cytochrome P450 mono-oxygenase enzyme that is involved in the production of factors that control cell proliferation. By knocking out this gene the levels of PLA1 may go up resulting in altered growth characteristics, which are phenotypically shown by resulting in a significant larger leaf size.

B3. Type of genetic modification

Frameshift mutation.

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

Not applicable.

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

A CRISPR/Cas9 gene cassette, necessary to induce the desired mutation, was introduced into maize plant by means of *Agrobacterium tumefaciens* mediated transformation. Transformants containing the gene cassette and the desired mutations were selected, and by means of conventional crossing with wild-type plants the CRISPR/Cas9 gene cassette was removed by selecting T1 plants that only contained the desired mutation, but no longer contained the gene cassette (null-segregants). The plants in the field trial therefore do not contain foreign genetic material. They only contain the desired frameshift mutation.

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 (1) assess the phenotype of the ALT^{KO} plants under realistic environmental conditions and assess whether abiotic stress leads to measurable DNA damage, and (2) assess whether NGAL2^{KO} plants show similar characteristics under realistic field conditions as plants in which the PLA1 gene is being additionally expressed.

C2. Geographical location of the site

The field trial will take place in the town of Zwijnaarde, near Ghent, Belgium.

C3. Size of the site (m²)

The trial plot, including non-modified controls is approximately 120m².

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

Not applicable.

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 mutations are not expected to result in characteristics such as 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 other non-target organisms and also not to change the toxicity and allergenicity of the maize. But we have not tested the latter, as this is not necessary and not required for such a small-scale field trial of which the produced materials are not going to be consumed by humans or animals. The modified seeds that will be formed are well retained in the cobs and these cobs will be very carefully hand harvested, also the tiniest ones, to prevent any spread of seeds.

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 performing the trial on an isolated location more than 1 km away from any cultivated maize fields and by in almost all cases by removing the tassel, or in exceptional cases hulling the tassel, thus preventing the spread of modified pollen to non-modified maize plants in the surroundings. The formed modified seeds are, as already stated above, well retained in the cobs and these cobs will be very carefully hand harvested, thus preventing any spread of seeds to the environment. In case some seeds would be lost during the harvest, they are not expected to result in the establishment of the maize outside the field. The field trial is surrounded by a 1.50 m high wired 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, genotype and growth characteristics of these maize mutants.

G. Final report

-

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.