BIJLAGE 12

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 modified growth characteristics

Proposed period of release:
01/04/2018 to 31/10/2020

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:	*Poaceae*
b) genus:	*Zea*, section *Zea*
c) species:	*Zea mays*
d) subspecies:	*mays*
e) cultivar/breeding line:	inbred line B104
f) common name:	maize

The modified maize will be a conventional stack of two single events that will be field tested as a hybrid with the non-modified inbred line CML91 as the second parental inbred line.

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 modified growth characteristics resulting from the additional expression of two genes: (1) the GA20oxidase under the control of a pUBIL promoter and (2) the PLA1 gene (also known as the 'KLUH' gene, or CYP78A1 gene) under the control of the GA2oxidase promoter. The GA20oxidase gene is involved in the production of bio-active giberellnes, The PLA1 gene codes for a cytochrome P450 mono-oxygenase enzyme that is involved in the production of factors that control cell proliferation. As a result of the modification the plants grow a bit faster and their growth is prolonged leading to plants that have significantly larger leaves, a higher biomass and a significantly higher cob yield. The modification also results in more branches being formed on the tassel and a shorter anthesis-silking interval.

The plants also contain the *bar* gene which produces the Phosphinotrycine acetyl transferase (PAT) protein as a selection marker gene.

The plants have been made through conventional crossing of two 'single events': one containing the additional GA20oxidase gene, and one containing the additional PLA1 gene. When these single events were combined in the B104 inbred line, this line was crossed with the CML91 inbred line to produce the hybrid that will be tested in the field.

In the field trial three types of genetically modified plants will be present: the stacked event containing both GA20oxidase and PLA1, and the single events containing either the additional GA20oxidase gene, or the additional PLA1 gene.

B3. Type of genetic modification

Insertion of genetic material.

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

The following elements have been inserted into the genome of the recipient maize plants:

Plants containing pUBIL::Ga20ox

Element	**Function**	**Origin**
Left T-DNA-border	T-DNA insert border	*Agrobacterium tumefaciens*
NOS-BAR (= *bar* -T_{NOS})	Phosphinotrycine acetyl transferase followed by the nopaline synthase terminator	*Streptomyces hygroscopicus* and *Agrobacterium tumefaciens*
P_{35S}	Transcription promoter	CaMV
AttB4	Recombination site*	Lysogenic *E.coli*
UBIL	Ubiquinine promoter	Zea mays
AttB1	Recombination site*	Lysogenic *E.coli*
GA20Oxidase-1	Coding sequence for the GA20Oxidase-1 gene	*Arabidopsis thaliana*
AttB2	Recombination site*	Lysogenic *E.coli*
T_{35S}	Transcription terminator of the CaMV 35S gene	CaMV
Right T-DNA-border	T-DNA insert border	*Agrobacterium tumefaciens*

*the AttB1, -2, and -4 recombination sites are synthetically altered versions of a recombination site originally isolated from E.coli.

Plants containing pGA2ox::PLA1

Element	Function	Origin
Left T-DNA-border	T-DNA insert border	*Agrobacterium tumefaciens*
NOS-BAR (= *bar* -T_{NOS})	Phosphinotrycine acetyl transferase followed by the nopaline synthase terminator	*Streptomyces hygroscopicus* and *Agrobacterium tumefaciens*
P_{35S}	Transcription promoter	CaMV
AttB4	Recombination site*	Lysogenic *E.coli*
P_{GA2ox}	Promoter of the GA2oxidase gene	Zea mays
AttB1	Recombination site*	Lysogenic *E.coli*
PLA1	Coding sequence of the PLA1 gene (also known as CYP78A1 or KLUH; a cytochrome P450 mono-oxygenase)	Zea mays
AttB2	Recombination site*	Lysogenic *E.coli*
T_{35S}	Transcription terminator of the cauliflower mosaic virus 35S gene	CaMV
Right T-DNA-border	T-DNA insert border	*Agrobacterium tumefaciens*

*the AttB1, -2, and -4 recombination sites are synthetically altered versions of a recombination site originally isolated from E.coli.

B6. Brief description of the method used for the genetic modification
Immature maize embryos have been co-cultivated with genetically modified *Agrobacterium tumefaciens*. During this co-cultivation the genes of interest (see table above) are transferred to cells of the immature embryo generating transformed cells. The transformed cells have then been selected using a positive screen (based on herbicide tolerance) and induced to regenerate whole plants.

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 confirm the maize's modified growth characteristics under normal field conditions and to measure the effect of the modification on the cob formation and cob filling which is very difficult to measure in greenhouse conditions.

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 (m^2)
The trial plot, including non-modified controls, non-modified fertilizer lines and non-modified buffer row is 735 m^2. There will be 162m^2 of GM plants in this plot.

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
Both single events have been released before. The single event containing the GA20oxidase gene was part of field trial B/BE/11/V4 that took place from 2012 to 2014. And the single event

containing the PLA1 gene was part of the field trial B/BE/14/V2 that took place from 2014 to 2017. The stacked event containing both traits has 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 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. Any concerns about a possible unexpected change in the allergenicity of the maize pollen is also not relevant as the male flowers (the tassels) of the modified maize will be removed before they will be able to shed any pollen. By doing this it is also prevented that any pollen would spread to the environment. 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 removing 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. There will be monitoring on volunteers in the year following the field trial and any volunteer maize plants will be removed and inactivated. The chances that maize generates volunteer plants in Belgium are extremely low, but not zero. In the six years of field trials that we performed with modified maize plants we have never observed a volunteer. 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

-

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.