BIJLAGE 11

Summary Notification Information Format

A. General information

A1. Details of notification

Notification Number
B/BE/24/V1

Member State
Belgium

Date of Acknowledgement
25 January 2024

Title of the Project
Field evaluation of poplars with a decreased lignin content

Proposed period of release:
15/05/2024 to 15/03/2028

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 name:	Salicaceae
b) genus:	*Populus,* sectie *Populus*. Subsectie *Albidea*
c) species:	*Populus tremula x Populus alba* (*Populus x canescens)*
d) subspecies:	-
e) cultivar / breeding line:	717-1B4, female clone
f) common name:	Grey poplar

B2. Description of the traits and characteristics which have been introduced or modified, including marker genes and previous modifications
The genetically modified trees have an altered wood composition resulting from the introduction of a mutation in the *tra2a* and *tra2b* genes. This gene is involved in the pentose phosphate

pathway that is upstream of the monolignol pathway. The monolignol pathway is at the basis of the production of the lignin polymer which is one of the three main components of plants cell walls next to cellulose and hemicellulose.
The mutations result in the knock-down of *tra2*, which has the downstream effect that less lignin is formed (about 15% less) and about 8% more cellulose. The wood biomass that is formed therefore has a somewhat different composition which has the benefit that it is more easily transformed into sugars. The saccharification efficiency of the wood has increased up to 31%.
The mutations have been introduced using the CRISPR-Cas9 gene editing system. The components of this system have been introduced into the trees using *Agrobacterium tumefaciens* mediated genetic modification. This means that a T-DNA construct was introduced that contains the *hph*-antibiotics resistance gene, the sequences coding for guide RNAs and the *cas9*-gene. The *hph* gene is present as a selection marker gene allowing easy selection of transformed plants, but has not function in the final plant. The guide RNAs that are present in the T-DNA insert were targeted at the *tra2* genes and the *cas9*-gene. This has resulted in the generation of plants that have mutations in the *tra2a*, *tra2b* and *cas9*-gene. As a result of these mutations, the *tra2A*, *tra2B* and the *cas9*-genes are no longer functional. The *cas9*-gene has only temporarily been functional in the initial one cell stage of the transformation and regeration process, long enough to temporarily produce functional CAS9 enzyme, that generated the mutations. The presence of the T-DNA insert (containing a mutated *cas9*-gene) makes that the plants are still transgenic.

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 region which has been inserted, and which is flanked by the T-DNA borders from the Ti-plasmid of Agrobacterium tumefaciens contains the following elements:

Element	Function	Origin
RB	T-DNA right border	*Agrobacterium tumefaciens*
CaMV 35s promoter	Transcription promoter	CaMV
Mutated *cas9*	A mutated version of the cas9 gene resulting in a non-functional CAS9 enzyme	*Streptococcus pyogenes*
rbcs-E9 Terminator	Transcription terminator	*Pisum sativum*
gRNAs	Sequences resulting in the expressing of different guideRNA molecules that form a component in the CRISPR-Cas gene editing system	Synthetic
hph	hygromycine phosphotransferase gene under control of the *nopaline synthase* (*nos)* promoter and the *nos* terminator. Results in the resistance against hygromycine.	Tn5
T_{NOS}	Transcriptie terminator	*Agrobacterium tumefaciens*
LB	T-DNA left border	*Agrobacterium tumefaciens*

B6. Brief description of the method used for the genetic modification
The method used for the genetic transformation is based on Agrobacterium tumefaciens cocultivation of excised internodes from in vitro grown poplar plantlets (Leplé et al., 1992). After this cocultivation step where the gene transfer takes place, the transformed cells are selected using a positive screen (based on antibiotic resistance) and induced to regenerate a whole plant.

B7. If the recipient or parental plant is a forest tree species, describe ways and extent of dissemination and specific factors affecting dissemination
Grey poplar (*P. x canescens*) can disseminate vegetatively through the production of suckers

from superficial roots. Pollen and seed are disseminated by the wind, possibly over rather long distance. The seed is very small and devoid of albumen: for this reason the seed viability in the wild is rather short (between 2 and 4 weeks). In fact, seed regeneration is not often observed as ecological conditions necessary for seed germination and plantlet development are seldom met: naked soil, no competition at all with any other species, full light, permanent humidity, but not in excess.

C. Experimental Release

C1. Purpose of the release
As already specified, the genetically modified poplars are modified in their lignin content. Lignin is very important for both tree growth and development, particularly for water conduction and mechanical support. Different transgenic lines of poplars with a modified lignin content have already been evaluated in previous field trials in the UK, France, and Belgium, for agricultural performances and for evaluation of the technological properties of wood for pulp and paper making. This release has the purposes to test the performance of these new TRA2 mutated poplar lines under real life conditions and to produce wood to evaluate its properties to serve as a good biomass source for extracting sugars and other valuable compounds. Lignin composition, lignin/cellulose ratio and the accessibility to cellulose are critical for the extraction of sugars from ligno-cellulosic feedstock. The poplar trees will be grown as a short rotation intensive culture using sustainable low-input 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 (m2)
The trial plot is in total about 400 m2, which includes a surrounding non-GM buffer.

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 genetically modified plants have not been released 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 since it is expected that the GM poplars will not flower and any suckers from superficial roots will be destroyed. Spontaneous regrowing of trees from fallen branches is extremely unlikely, as it is known that *P.x canescens* and the clone 717-1-B4 does not easily shoot. Only under ideal conditions in the laboratory with the application of shooting powder, *P. x canescens* is able to shoot. This means that there will be no transfer of transgenes to native or cultivated poplars, and no spread of the GM poplars themselves. The poplars will be grown in a way that the branches will not become older than three years, and are therefore not expected to flower. Grey poplar normally starts to flower between 5 – 8 years of age, only in some cases after 4 years. But anyhow, if monitoring would reveal any flowering, these flowers will be removed. For information: The clone used as a recipient is a female clone, unable to produce male flowers and therefore also unable to produce pollen.

The modification of the trees is not expected to lead to environmentally relevant effects on non-target species. In former trials only some effects were identified on the composition of bacteria

living in the trees. The amount and species diversity of micro-organisms living in the rhizosphere was not affected. From scientific literature it can be deduced that lignin modified trees do not have an effect on the interaction with pathogens, that there is no or very limited effect on leaf-eating insects, and that for the decay of lignin-modified wood other factors like environmental conditions, the chosen poplar species and clone have more significant effects than the lignin modification.

And as outlined above, there is no expected selective advantage of the GM poplar.

The toxicity or allergenicity of the trees has not been tested, but there are no reasons to suspect that an altered wood composition resulting from a lower lignin and higher cellulose content would have an effect on the toxicity or the allergenicity of the plant. Allergenicity of poplar is mostly associated with pollen, but as we are working with female trees that do not produce pollen this is not a concern.

It is also known that trees with comparable alterations in the lignin content already exist in nature (in loblolly pine in the U.S. and in black poplar in Europe). If there would be any alteration of the way the modified trees interact with nature and in particular with non-target organisms, this altered interaction would be comparable with the interactions of those wild-type trees. Also, there are no indications from the loblolly pine and black poplar mutants that the modified wood would have any negative impact on the health of humans or animals.

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

Grey poplar (P. x canescens) is dioecious (every tree is either male or female). The 717-1B4 clone is female. There is therefore no risk of dissemination through pollen. Moreover, as flower development occurs before vegetative bud burst and leaf development, it is very easy to identify and eliminate female catkins, before their full development. But as the branches will not never be older than 3 years (they will be harvested after one, two or three years), the GM poplars are not expected to flower. Suckers are also regularly monitored and destroyed by either removing them or killing them with a contact herbicide. After a storm the site will be inspected for possible fallen branches and these will be removed. The site is designed in such a manner that fallen branches will not disperse by wind from the plot and will remain within the boundaries of a fence surrounding the trial.
At the end of the trial, the rootstock will be mechanically removed and the soil will be worked with a rotary cultivator. The plot will be monitored for at least two years for suckers, which will be destroyed using a suitable contact herbicide. If necessary monitoring will be extended until there has been one year without any suckers.
The field trial plot will be 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 trees.

G. Final report

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H. European Commission administrative information

I. Consent given by the Competent Authority:

Not known