

# Summary Notification Information Format

## A. General information

### A1. Details of notification

**Notification Number**

B/BE/24/V2

**Member State**

Belgium

**Date of Acknowledgement**

25 January 2024

**Title of the Project**

Field evaluation of poplars with a modified wood composition

**Proposed period of release:**

15/05/2021 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:                    | <i>Populus</i> , sectie <i>Populus</i> . Subsectie <i>Albidea</i>                  |
| c) species:                  | <i>Populus tremula</i> x <i>Populus alba</i> ( <i>Populus</i> x <i>canescens</i> ) |
| 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 additional expression of the F6'H1 and COSY genes in lignifying cells which produce the enzymes Feroloyl-

CoA 6'-Hydroxylase1 and Coumarin Synthase. The production of these enzymes in lignifying cells results in the production of the secondary metabolite Scopoletin which is then built into the lignin polymer. Lignin is one of the tree components of the cell wall next to cellulose and hemicellulose. The amount of lignin in the cell wall has not changed, but the presence of the scopoletin monomer results in lignin that is less recalcitrant during attempts to break it down. Under alkaline conditions the saccharification efficiency of the altered wood has increased up to 31% as a result of the altered lignin composition. The higher the saccharification efficiency is, the more interesting the wood becomes for use in the circular bio-economy, for instance for the production of bio-ethanol.

The trees also contain the nptII antibiotic resistance gene which has been used in the process of transformation to facilitate the selection of shoots that have taken up the modification.

### 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	Functie	Origin
RB	T-DNA right border	<i>Agrobacterium tumefaciens</i>
attB4	Recombinatie site*	Lysogene <i>E.coli</i>
CesA8-B	Cellulose synthase transcription promoter	<i>Populus trichocarpa</i>
attB1	Recombinatie site*	Lysogene <i>E.coli</i>
COSY	Coumarin synthase	<i>Arabidopsis thaliana</i>
T2A	Self-cleaving peptide	Thosea asigna virus 2A
F6'H1	FERULOYL-COA 6'-HYDROXYLASE1	<i>Arabidopsis thaliana</i>
attB2	Recombinatie site*	Lysogene <i>E.coli</i>
T35S	Transcription terminator	CaMV
nptII	Neomycine phosphotransferase gene under control of the <i>nopaline synthase (nos)</i> promoter and the <i>nos</i> terminator. Results in resistance against kanamycin, neomycin.	Tn5
LB	T-DNA left border	<i>Agrobacterium tumefaciens</i>

\*the AttB1, -2 and -4 are mutated versions of the AttB sequences that were originally taken from lysogenic *E.coli*

### 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 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.

### **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<sup>2</sup>)**

The trial site is in total about 400 m<sup>2</sup>, 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 trunk and branches will not become older than three years, and therefore are 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 modified poplars will be grown in a way that branches will never be older than 3 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