BIJLAGE 8

Summary Notification Information Format

A. General information

A1. Details of notification

Notification Number
B/BE/07/V2

Member State
Belgium

Date of Acknowledgement
30/11/2007

Title of the Project
Field evaluation of poplars with an altered wood composition for the production of bio-ethanol

Proposed period of release:
01/05/2008 to 31/12/2014

A2. Notifier

Name of the Institute(s) or Company(ies)
VIB

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

The same and similar plants have been introduced in France by INRA, see B/FR/07/06/01 and B/FR/99/02/15. One transgenic line WT/52-40 will be introduced into the environment for the first time and will not be released elsewhere.

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*
(c) Species:	*Populus x canescens (Populus alba x Populus tremula)*
(d) Subspecies:	-
(e) Cultivar / breeding line:	717-1B4
(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 poplars exhibit modified lignin (a major constituent of wood) due to the decreased activity of an enzyme of the lignin biosynthetic pathway. Depending on the transgenic line, the altered enzyme is:
- CCR (Cinnamoyl coenzymeA reductase): 2 transgenic lines WT52-3, and WT52-40.
- CCoAOMT (Caffeoyl coenzymeA O-methyl transferase): 2 transgenic lines 101 and 416.
The down-regulation has been obtained either by antisense strategy (101) or by co-suppression (WT52-3, WT/52-40, 416). The enzyme residual activity varies between 3 to 100 % and is not necessarily uniform within the plant. Consequently, the quality or/and quantity of lignin is modified. These modifications and the consequences on some wood properties have been described in several publications (Baucher et al., 1996, van Doorsselaere et al., 1995 ; Meyermans et al., 2000 ; Lapierre et al., 1999 ; Pilate et al., 2002 ; Lapierre et al., 2004).
In addition, all transgenic lines have also integrated a selection gene (hpt) that confers an antibiotic resistance. This antibiotic resistance has been used during in vitro culture steps to select for genetically modified cells.

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 inserted genetic material is the T-DNA from the Ti plasmid of Agrobacterium tumefaciens harbouring the gene of interest (for lignin modification) and the gene for selection (antibiotic resistance). The gene of interest is one among two poplar genes coding for one among two enzymes of the monolignol biosynthetic pathway. Monolignols are the elementary units of the lignin polymer. The coding sequence of any of these 2 genes is inserted in sense or antisense orientation between i) the promoter of the cauliflower mosaic virus (CaMV) in a duplicated version (p70) and ii) a terminator sequence, either from the T7 gene from the T-DNA (pAg7) or from the gene coding for the CaMV 35S RNA (pA35S). The antisense insertion aims to turn off the expression of the corresponding endogenous gene: The mRNA of the antisense gene interferes with the corresponding endogenous mRNA that results in a strong reduction in the production of the endogenous protein. A sense insertion leads in a few transgenic lines (this is the case for the sense transgenic lines included in this application) to a similar effect, i.e. a reduction in the activity of the target enzyme, through another mechanism named co-suppression.
The two poplar genes listed below derive from cDNA sequences isolated from a xylem cDNA library from the Populus trichocarpa “Trichobel“ clone (for CCoAOMT and CCR cDNA).

i) CCR (Cinnamoyl coenzymeA reductase): the full-length cDNA coding for CCR (accession AJ224986 ; Leplé et al., 1998) inserted in sense orientation. The corresponding chimeric gene (p70-S-CCR-pA35S) once introduced in the pBIBHygro binary vector generates the pBIBHygro/S-CCR pBIBHygro transformation vector.

ii) CCoAOMT (Caffeoyl coenzymeA O-methyl transferase): the full-length cDNA coding for CCoAOMT (accession AJ224894 ; Meyermans et al., 2000) inserted in sense or antisense orientation. The corresponding chimeric genes (p70-S-CCoAOMT-pA35S and p70-AS-CCoAOMT-pA35S) once introduced in the pBIBHygro binary vector generate respectively the pBIBHygro/S-CCoAOMT and pBIBHygro/AS-CCoAOMT transformation vectors.

For the p70-S-CCoAOMT-pA35S, p70-AS-CCoAOMT-pA35S, p70-S-CCR-pA35S, the selection gene is the hygromycine B phosphotransferase (hpt (or hph) gene fused to the promoter of the nopaline synthase gene (pNOS) from Tn7 and to the terminator of the gene 7 from the T-DNA (pAg7).

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 on 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 to 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 for the content and/or quality of lignin. Lignin is very important for both tree growth and development, particularly for water conduction and mechanical support. These different transgenic lines of poplars have been already evaluated in a previous field trial in France, for agricultural performances and for evaluation of the technological properties of wood for pulp and paper making. This release has the purpose to produce enough wood from lignin modified poplars in order to evaluate its properties for bio-energy production, in particular bio-ethanol. Both lignin/cellulose ratio and the accessibility to cellulose are critical for the production of bioethanol from ligno-cellulosic feedstock. The poplar trees will be grown as a short rotation intensive culture on a low-grade soil (marginal land) using sustainable low-input conditions. The release also intends to take advantage of the developments in the Ghent-BioEnergy-Valley, where a number of bio-energy initiatives have taken ground, including the start-up of a bioprocess pilot plant for bio-energy production. The release can also be seen as a partial repetition of the trial B/FR/07/06/01 (the current release only involves 4 lines, where the FR trial includes more lines) at INRA-Orleans in France, providing additional scientific value to the outcomes of this trial and vice-versa.

C2. Geographical location of the site
The University of Ghent Science and Industry park in Zwijnaarde, Belgium.

C3. Size of the site (m2)
The site is in total 6500 m2 of which a maximum of 2400 m2 will be planted with transgenic poplars.

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
There has been one previous release involving four of the five same CCR and CCoAOMT lignin modified poplars (notification number B/FR/99.02.15).

During this previous field trial, no significant differences between GM and wild type poplars with regards to reproductive aspects were observed. Lignin modified poplar flowering time and intensity did not appear affected by the genetic modifications.
However, lignin is involved in major biological functions for tree growth and development such as mechanical support, water conduction and pathogen defense. Field trials with lignin modified trees over more than 12 years have shown that important lignin modifications are very rapidly translated into changes in the function of conduction and/or support. It has also come out that some lines that were shown to grow normally in the greenhouse (i.e. in optimal growth conditions), where unable to do so in a nursery. Some transgenic lines were even unable to survive. Apparently there has to be a balance between the lignin modification that can be of

interest for certain applications on the one hand and the impact of the modification on tree growth and development on the other hand. The lines in this application have been shown to grow almost normal (CCR down-regulated) to normal (CCoAOMT down-regulated), and have been shown to release up to twice the amount of glucose in biochemical breakdown experiments, when compared to conventional poplars.
The experiences with lignin modified poplar appear to suggest that lignin modified poplars will have a fitness that is less or at the maximum comparable to their wild type counterparts.

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

Note especially if the introduced traits could directly or indirectly confer an increased selective advantage in natural environments; also explain any significant expected environmental benefits

The environmental impact from the release is expected to be zero, since the GM poplars are not going to flower and any suckers from superficial roots will be destroyed. This means that there will be no transfer of transgenes to native or cultivated poplars, or spread of the GM poplars themselves. When poplar is grown in short rotation intensive culture the trunks and branches will not become older than three years, and therefore they will not 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 targeted at non target species. In former trials no effects on non target species were identified. One could speculate on the effect of lignin modification on the degradation of leaves and wood under natural circumstances. But there are currently no data available on that.

And as outlined above, there is no expected selective advantage of the GM poplar. It is more likely that the GM poplar will have a selective disadvantage.

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. In consequence, there is 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 as short rotation intensive culture with a harvest of all trunks and branches after 3 years of growing, the GM poplars are not expected to flower. Suckers are also regularly monitored and destroyed once a year using a contact herbicide.
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

In this field trial there will be no data collection of new data on the environmental and human health impact of the release. However, in the similar field trial B/FR/07/06/01 there will be data collection on the effects on biodiversity.

G. Final report

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H. European Commission administrative information

I. Consent given by the Competent Authority:

Not known