Adviesraad voor Bioveiligheid
Conseil consultatif de Biosécurité

Advice of the Belgian Biosafety Advisory Council on notification B/BE/21/V1 (poplars with altered wood composition) for deliberate release in the environment of genetically modified plants for research and development

10 May 2021
Ref. SC/1510/BAC/2021_0423

The notification B/BE/21/V1 has been submitted by the VIB to the Belgian Competent Authority (CA) in January 2021 for a request of deliberate release in the environment of genetically modified higher plants for research and development according to Chapter II of the Royal Decree of 21 February 2005.

The title of the notification is: **"Field evaluation of poplars with a modified wood composition"**. This release has the purpose to check whether the genetically modified (GM) poplars, adapted in the production of lignin, produce a good biomass for extracting sugars.

The notification has been officially acknowledged by the CA on 26 January 2021 and forwarded to the Biosafety Advisory Council, hereafter referred to as the Council, for advice. Within the framework of the evaluation procedure, the Council, under the supervision of a coordinator and with the assistance of its Secretariat, contacted experts to evaluate the dossier. Six experts answered positively to this request, including three from the common list of experts drawn up by the Council and the Division of Biosafety and Biotechnology (SBB). The Platform for Molecular Biology and Biotechnology of Sciensano took part in the evaluation of the detection method and the molecular data. The experts assessed whether the information provided in the notification was sufficient and accurate in order to state that the deliberate release of the GM poplar trees would not raise any problems for the environment, animal or human health in the context of the intended use.

On 9 March and 13 April 2021, the Council sent a list of requests for additional information to be provided by the notifier to the CA. Additional information was received on 2 April and 29 April 2021, and evaluated by the scientists in charge of evaluating the dossier.

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For the purpose of the scientific evaluation, the following legislation has been considered:

- Royal Decree of 21 February 2005 (Belgian Official Journal of 24.02.2005, p. 7129) modified by the Royal Decree of 19 February 2020 (Belgian Official Journal of 02.03.2020, p. 12666).

In parallel to the scientific evaluation, the CA made the dossier available on its website for a one-month public consultation as required in the abovementioned Royal Decree. The CA forwarded the list of questions to the Council. No questions of the public tackling biosafety issues of the GMOs were identified that needed to be taken into account by the Council in its evaluation of the dossier.

Summary of the scientific evaluation

1. Information related to the recipient or parental plants

Grey poplar (*Populus x canescens*) is dioecious (every tree is either male or female) and an obligatory outcrosser. Grey poplars begin flowering between the age of 5 and 8 years. Male and female flowers are borne in catkins. Male flowers ripen and shed pollen a few days before females, ensuring that pollen is in the air when the first females are receptive. Seeds can be dispersed over great distances, resulting in high rates of migration.

Grey poplar is sexually compatible with a few other *Populus* species present in Belgium, namely *Populus alba*, *Populus tremula*, hybrids of *Populus canescens* and *Populus tremuloides*.

The grey poplar used in the field trials is a female clone 717-1-B4. Hence, there is no production of pollen.

Besides sexual reproduction, also vegetative propagation through root suckers or branches can occur (OECD, 2001[1]) in *Populus* species. Vegetative propagation through branches is however very unlikely for grey poplar under natural conditions.

2. Information on the design and management conditions in the field trial

The small scale field trial will be designed as a short-rotation poplar coppice. Young rooted GM poplars will be planted during spring 2021. Before the start of the second growing season the trees will be cut down to stimulate the formation of many stems per plant. At the end of 2025 all biomass will be cut down and chopped to be processed into bio-ethanol. Occasionally branches will be cut down to be analysed in the laboratory. At the end of 2025 the rootstocks will be mechanically removed and destroyed, and the plot will be tilled with a rotary cultivator. Consequently, the plot will be monitored for suckers and it is proposed this will be done until there has been one year without any suckers**.** Potentially emerging suckers will be destroyed.

[1] OECD, 2001. Consensus document on the biology of *Populus* L. (poplars), ENV/JM/MONO(2000)10

3. Information related to the genetic modification

Two lines of the female clone 717-1-B4, transformed in their expression of CSE (Caffeoyl Shikimate Esterase), will be tested in the field experiment: they are identified as lines hpCSE#1 and hpCSE#2.

These two lines, obtained through genetic transformation with *Agrobacterium tumefaciens,* have a modified lignin (a major constituent of wood) content due to the decreased activity of the CSE enzyme involved in the lignin biosynthetic pathway. In the GM poplar lines, the *cse* gene from *Populus* is partly inserted in sense, partly in anti-sense orientation with both parts being separated by an intron which contains *cat* (chloramphenicol acetyltransferase) gene conferring resistance to chloramphenicol. Transcription leads to the production of RNA with part of the *cse* sequence in a hairpin turn that will lead to a reduction of CSE. The *cse* sense-antisense construct is located between the promoter and the transcription terminator of the gene coding for the 35S RNA of the cauliflower mosaic virus, and thus expressed in all cell types throughout the plant. The *cat* gene is not expressed in the plant as it is part of an intron and is under control of a bacterial promoter and terminator. In addition, the transgenic lines contain a marker gene (neomycine phosphotransferase, *nptII*) that confers resistance to the antibiotics neomycine and kanamycine, and which was used to select genetically modified plants. The *nptII* gene is controlled by the nopaline-synthase (Pnos) promoter and a transcription terminator from the T7 gene from the T-DNA (tAg7).

Upon request of additional experimental data by the Council, the absence of vector backbone, in particular the *aadA* gene which confers resistance to spectinomycin and streptomycin, was demonstrated in the two lines. With these additional data, the information related to the genetic modification was considered sufficient by the Council.

4. Potential risks for the environment, animal or human health associated with the release of the GM poplars

No increase in persistence in the field or invasiveness into natural habitats compared to non-GM grey poplars is expected, as the modified lignin content is not known to confer a selective advantage to survivability. Due to the characteristics of the poplar cultivar used for transformation and through the measures taken during the release, vertical gene transfer through seed, pollen, branches or root suckers can virtually be ruled out:

- The GM poplars are not expected to flower, as the branches of the lignin-modified poplars will be harvested every 3 years. Nevertheless, monitoring will be carried out each year during the flowering season to check for flowering. If unexpected flower buds occur, they will be removed before seed set.
- There is no possibility of dissemination through pollen, as the grey poplar used in the field trials is a female clone 717-1-B4.
- Spontaneous regeneration from branches is considered unlikely, as clone 717-1-B4 does not easily form rooted scions even under optimal laboratory conditions.
- Root suckers observed during the trial period will be removed, as well as root suckers that might emerge after the field trial.

Horizontal gene transfer between plants and micro-organisms is considered as a rare event under natural conditions (Keese, 2008[2]). The possibility of horizontal gene transfer between the GM poplar plants and bacteria has been given particular attention due to the presence of (modified) recombination sites *att*B1 and *att*B2 of *E. coli* for phage λ in the GM poplar lines. It was questioned whether the presence of these *att*B sites could increase the uptake of plant DNA by lysogenic bacteria. The occurrence of such an event in an environment where phage *att*P1 and *att*P2 sites are present that could interact with the modified *att*B sites was considered unlikely. In case gene transfer from the GM poplars to micro-organisms would take place and gene expression would occur, negative effects on the environment and humans are not expected. The resistance genes (i.e. *cat* and *nptII*) occur naturally in microbes and are not anticipated to cause hazardous effects when occurring in plants for field experimentation (see EFSA, 2004[3], 2009[4]), and transfer of part of the *cse* gene will not confer a selective advantage to bacteria.

From data from former trials and literature, it can be concluded that the GM poplars are not expected to have significant effects on non-target organisms (invertebrates, vertebrates and soil micro-organisms) and humans. The impacts of lignin-modified trees on microbial pathogens, leaf eating insects and microbial soil composition have been shown to be negligible (see e.g. Brodeur-Campbell *et al*., 2006; Halpin et al., 2007[5]; Bradley *et al*., 2007[6]; Danielsen *et al*., 2012[7] & 2013[8]). Also effects on mammalian herbivores (e.g. rabbits) are expected to be negligible. The fence surrounding the entire field plot will restrict – although not entirely – entrance of mammals into the field plot, reducing their contact with the GM poplars. Given the restricted scale of the field trial, any potential effect to non-target organisms and biogeochemical processes - if these would occur - will be of a local and temporal nature. As clone 717-1-B4 does not produce pollen, a possible altered allergenicity of the transgenic pollen (pollen from poplar is known as a moderate allergen) does not form a concern for human health.

5. Information related to the control, monitoring, post-release and waste treatment`

The management measures proposed (e.g. removal of root suckers, monitoring for flowers, chopping of wood inside the fence) were considered as sufficient to prevent potential adverse effects to the environment, animal and human health during the field trial. To minimise the

[2] Keese, P. 2008. Risks from GMOs due to horizontal gene transfer. Environ. Biosafety Res. 7: 123-149.
[3] EFSA, 2004. Opinion of the Scientific Panel on GMOs on the use of antibiotic resistance genes as marker genes in genetically modified plants. EFSA Journal 48, 1-18.
[4] EFSA, 2009. Consolidated presentation of the joint Scientific Opinion of the GMO and BIOHAZ Panels on the "Use of Antibiotic Resistance Genes as Marker Genes in Genetically Modified Plants' and the Scientific Opinion of the GMO Panel on "Consequences of the Opinion on the Use of Antibiotic Resistance Genes as Marker Genes in GMPs on Previous EFSA Assessments of Individual GM Plants". The EFSA Journal 1108, 1-8.
[5] Halpin *et al*., 2007. Ecological impacts of trees with modified lignin. Tree Genetics & Genomics 3, 101-110.
[6] Bradley *et al*., 2007. Soil microbial community responses to altered lignin biosynthesis in *Populus tremuloides* vary among three distinct soils. Plant and Soil 294,185-201.
[7] Danielsen *et al*., 2012. Fungal soil communities in a young transgenic poplar plantation form a rich reservoir for fungal root communities. Ecology and Evolution 2, 1935-1948
[8] Danielsen *et al*., 2013. Ectomycorrhizal Colonization and Diversity in Relation to Tree Biomass and Nutrition in a Plantation of Transgenic Poplars with Modified Lignin Biosynthesis. PLoS ONE 8(3): e59207.

spread of transgenes into the environment after termination of the field trial, monitoring for root suckers will occur. The Council recommends to extend monitoring for root suckers until the moment that two years have passed after the last observed root suckers. In addition, the machinery used for chopping should be cleaned inside the fence before leaving the trial site, and the branches taken away to be analysed in the laboratory need to be registered.

Conclusion

Based on the scientific assessment of the dossier by the experts, the Biosafety Advisory Council concludes that it is unlikely that this small scale field trial with GM poplar with an altered wood composition will pose any risks to the environment, animal or human health.

Therefore, the Biosafety Advisory Council issues a **positive advice with the following conditions**:

- The notifier and the investigators must strictly apply the protocol, the monitoring plan and, if necessary, the emergency measures as described in the dossier.
- Additional conditions should be taken up in the monitoring plan:

1. Monitoring measures taken during the trial:
   - The notifier should keep records of dates and numbers of inflorescences removed from each transgenic line. This information is useful to check the adequacy of the monitoring frequency for inflorescences. Also dates, numbers and identity of branches taken away to be analysed in the laboratory should be recorded.
   - All harvested woody material should be chopped inside the fence and the machineries that are used to harvest and chop the wood should be cleaned at the trial site to prevent dispersal of plant material.

2. Monitoring measures taken after the trial:
   - The period to monitor the occurrence of root suckers should be extended until the moment that two years have past after the last observed outgrowing suckers.

Prof. G. Angenon
President of the Biosafety Advisory Council

Annex I: Compilation of comments of experts in charge of assessing the dossier B/BE/21/V1 (ref: BAC_2021_0229)

Adviesraad voor Bioveiligheid
Conseil consultatif de Biosécurité

Compilation of comments of the experts in charge of evaluating notification B/BE/21/V1

11 March 2021
Ref. SC/1510/BAC/21_0229

Coordinator: Bart Panis
Experts: An Vanden Broeck (INBO), Jan Van Doorsselaere (VIVES), Nicolas Van Larebeke (UGent), and experts from Sciensano, Service Transversal activities in applied genomics.
SBB coordinator: Adinda De Schrijver

INTRODUCTION

Dossier **B/BE/21/V1** concerns a notification of the VIB, for deliberate release in the environment of genetically modified higher plants (GMHP) according to Chapter II of the Royal Decree of 21 February 2005.

The notification has been officially acknowledged on 26 January 2020 and concerns a field trial transgenic poplar with modified wood characteristics

Experts were invited to evaluate the GMHP considered in the notification as regards its potential impacts on the environment, including human and animal health, and information relating to pre- and post-release treatment of the site.

EVALUATION

The comments below served as basis for a list of questions that the competent authority forwarded to the notifier with a request to provide additional information. The comments highlighted in grey correspond to the questions/comments selected and sent to the notifier. The comments encompass comments to the technical dossier and comments on Annex 1 (Risk assessment) of the dossier.

TECHNICAL DOSSIER

B. INFORMATION RELATED TO THE RECIPIENT OR (WHERE APPROPRIATE) PARENTAL PLANTS

Have evaluated this section and had no comments/questions: 1 expert

Comment:
Just a minor comment regarding the geographic distribution of related plant species:
It is not completely correct that *Populus nigra* is rare. This is indeed the case for natural, autochtonous populations but not for cultivars of this species. In particular, *P. nigra* cv. Italica, also named the Lombardy poplar ('kaarspopulier'), is a cultivated poplar clone frequently planted

worldwide, also in Flanders. It is a male cultivar producing huge amounts of viable pollen (Vanden Broeck *et al.*, 2018). However, it is correct that *P. nigra* is not compatible with *P. x canescens* and will not be able to act as a pollen donor and male parent in case flower should occur in this field experiment. This is also mentioned in section E3 in the Dossier.

C. INFORMATION RELATED TO THE GENETIC MODIFICATION

Have evaluated this section and had no comments/questions: 3 experts

D. INFORMATION RELATED TO THE GENETICALLY MODIFIED PLANT

D.1. Information related to the traits and characteristics, which have been introduced or modified

Have evaluated this section and had no comments/questions: 3 experts

D.2. Information on the sequences actually inserted/deleted

Have evaluated this section and had no comments/questions: 2 experts

Comment:

In Annex 2, C.2 it is said that the absence or presence of DNA was verified via an alignment of the reads with the vector sequence. However, these data are not shown. Please provide data demonstrating the absence of donor material. Further, we would like the notifier to argue that the absence of the backbone sequence, including the *aad* gene, can be verified by the used NGS technology (MinION).

D.3. Information on the expression (of the insert)

Have evaluated this section and had no comments/questions: 1 expert

Comment 1:

Minor comment: the URL (http://www.nature.com/nrg/multimedia/rnai/animation/index.html) does not work

Comment 2:

It is stated '*Dit gen (Cm^R gen) staat onder controle van zijn originele bacteriële regulatiesignalen en komt om die reden niet tot expressie in de plant*'. However, no information is given in the dossier to verify this statement [the paper of Karimi *et al.* (2002) describing the pK7GWIWG2II is missing]. Please provide this information.

D.4. Information on how the GM plant differs from the recipient plant

Have evaluated this section and had no comments/questions: 3 experts

D.5. Genetic stability of the insert and phenotypic stability of the GMHP

Have evaluated this section and had no comments/questions: 1 expert

Comment:

p13: it is mentioned that 'De genetische stabiliteit van het donormateriaal is niet direct getest…'

Poplar is propagated using cuttings *in vitro*. It is known that RNA interference effects can vary over time, e.g. that phenotypes can disappear or are instable. Will it be investigated if the plants that will be put in the field still have reduced mRNA levels/CSE enzymatic activities?

Note coordinators: Information on genetic stability of the insert is not a requirement for a Standard Part B notification according to the BAC guidelines for Molecular Characterisation of GM Plants and this question will therefore not be asked.

D.6. Any change to the ability of the GMHP to transfer genetic material to other organisms

Have evaluated this section and had no comments/questions: 2 experts

D.7. Information on any toxic, allergenic or other harmful effects on human health arising from the genetic modification

Have evaluated this section and had no comments/questions: 3 experts

D.8. Information on the safety of the GMHP to animal health, particularly regarding any toxic, allergenic or other harmful effects from the genetic modification, where the GMHP is intended to be used in animal feedstuffs

Have evaluated this section and had no comments/questions: 3 experts

D.9. Mechanism of interaction between the genetically modified plant and target organisms (if applicable)

Have evaluated this section and had no comments/questions: 2 experts

D.10. Potential changes in the interactions of the GMHP with non-target organisms resulting from the genetic modification

Have evaluated this section and had no comments/questions: 2 experts

D.11. Potential interactions with the abiotic environment

Have evaluated this section and had no comments/questions: 2 experts

D.12. Description of detection and identification techniques for the GM plant

Have evaluated this section and had no comments/questions: 2 experts

Comment:
The primer attachment sites need to be presented schematically (as requested in the Detection Protocol).

D.13. Information about previous releases of the GM plant, if applicable

Have evaluated this section and had no comments/questions: 2 experts

E. INFORMATION RELATING TO THE SITE OF RELEASE

Have evaluated this section and had no comments/questions: 3 experts

F. INFORMATION RELATING TO THE RELEASE

Have evaluated this section and had no comments/questions: 3 experts

G. INFORMATION RELATED TO CONTROL, MONITORING, POSTRELEASE AND WASTE TREATMENT

G.1. Any measures taken

Have evaluated this section and had no comments/questions: 2 experts

G.2. Information on methods for post-release treatment of site

Have evaluated this section and had no comments/questions: 3 experts

G.3. Information on post-release treatment methods for the GM plant material, including wastes

Have evaluated this section and had no comments/questions: 2 experts

G.4 Information on monitoring plans and techniques

Have evaluated this section and had no comments/questions: 2 experts

G.5. Information on any emergency plans

Have evaluated this section and had no comments/questions: 2 experts

Comment:
On p27 it is said "*Een derde mogelijke calamiteit is dat er bij het transport van het verhakselde transgene hout een ongeval zou plaatshebben met het voertuig en het verhakselde hout op de straat zou vallen. Ook al gaat het in dit geval niet meer om GGO's en kunnen er uit dit materiaal ook geen GGO's meer ontstaan, we zullen toch altijd enkele lege vaten voorradig hebben om het verhakselde hout snel te kunnen opruimen.*"
When stating '*Ook al gaat het in dit geval niet meer om GGO's en kunnen er uit dit materiaal ook geen GGO's meer ontstaan*…' we presume one refers here to natural regeneration and not to *in vitro* techniques. Can this be clarified?

G.6. Information on methods and procedures to protect the site

Have evaluated this section and had no comments/questions: 2 experts

ANNEX 1. ASSESSMENT OF ENVIROMENTAL & HUMAN HEALTH IMPACT

1. Information on the likelihood for the GMHP to become more persistent than the recipient or parental plants or more invasive

Have evaluated this section and had no comments/questions: 2 experts

Comment:
I think that insufficient data are available to make the statement that the genetic change introduced will not affect the success of the tree in question. But this is probably not very important in this context.

2. Information on the selective advantage or disadvantage conferred to the GMHP

Have evaluated this section and had no comments/questions: 2 experts

Comment:

I think that insufficient data are available to make the statement that the genetic change introduced will not affect the success of the tree in question. But this is probably not very important in this context.

3. Information on potential of gene transfer to other sexually compatible plant species under conditions of planting and its consequences

Have evaluated this section and had no comments/questions: 3 experts

4. Any change to the ability of the GMHP to transfer genetic material to microorganisms and the adverse environmental effects thereof

Have evaluated this section and had no comments/questions: 2 experts

Comment:

I am not completely convinced that horizontal gene transmission is indeed extremely unlikely. Possibly this happens more often than we generally think. Also the fact that the attB1 and attB2 sequences are mutated might not prevent recombination with attB-like sequences occurring in micro-organisms. Do we have information on the occurrence of attB-like sequences in microorganisms in general?

Note coordinators: For information on horizontal gene transfer (HGT) between higher plants and micro-organisms we refer to the study of EFSA (2004), where it is concluded on the basis of existing information that HGT is considered as a rare event under natural conditions.
Concerning the potential role of *att*B-like sequences in recombination: The possibility of HGT due to presence of (modified) recombination *att*B sites has been evaluated in the context of previous field trials (e.g. B/BE/20/V1, B/BE/17/V3). In its opinion the Council considered that an increase in the uptake of plant DNA by lysogene bacteria was considered unlikely (as many conditions need to be fulfilled). Most importantly, in case gene transfer from the GM maize to micro-organisms would take place and gene expression would occur, negative effects on the environment and humans are not expected.
We therefore consider that additional information on the occurrence of *att*B-like sequences in microorganisms is not necessary to come to a conclusion on risk.

5. Information on the environmental impact resulting from direct and indirect interactions of the GMHP with target organisms

Have evaluated this section and had no comments/questions: 2 experts

6. Information on the environmental impact resulting from direct and indirect interactions of the GMHP with non-target organisms, including herbivores, parasites, symbionts…

Have evaluated this section and had no comments/questions: 2 experts

Comment:

A possible environmental effect of cultivating trees with less lignin and more cellulose could comprise the fact that these trees might be more vulnerable to certain parasites, fungi or microbes. These infected trees might then spread these infections or diseases to other plants. Is there any reflection on these topics?

Note coordinators: From the studies done so far, there seems to be no indications that lignin modified trees are less resistant to pests compared to non-modified trees (e.g. Halpin *et al*., 2007; Danielsen, 2012; results from previous field trials with GM poplars).

7. Information on possible effects on human health resulting from potential direct and indirect interactions of the GMHP and persons working with, coming into contact with or living in the vicinity of the GMHP release

Have evaluated this section and had no comments/questions: 2 experts

Comment:

Adverse health effects on humans manipulating the genetically modified trees seems indeed improbable. However, these trees with less lignin and more cellulose might serve as better substrates for microbes that might possibly affect human beings. Is there any knowledge concerning human infections stemming from microbes living on wood?

Note coordinators: We acknowledge this a plausible pathway to harm, but see this question for information disproportionate for a trial where only a handful of people have access to the trial and will come into contact with GM poplar tree material. The measures put in place (e.g. fence) we consider sufficient to deal with any remaining uncertainties on effects to human beings. Furthermore, the notifier reports that so far no adverse effects have occurred with the manipulation of GM trees.

8. Information on possible effects on animal health and consequences for the food/feed chain resulting from consumption of the GMO and any product derived from it, if it is intended to be used as animal feed

Have evaluated this section and had no comments/questions: 2 experts

Comment:

Again I think that the most probable adverse effect might consist of an increase in the likelihood of infectious diseases stemming from a selection of a particular microorganism or an increased intensity of contamination through the presence of the genetically modified trees.

Note coordinators: The GM poplar trees will not be used as food/feed, hence we do not see the relevance of further addressing potential adverse effects related to animal feed.

9. Possible immediate and/or delayed effects on biogeochemical processes resulting from potential direct and indirect interactions of the GMO and target and non-target organisms in the vicinity of the GMO release(s)

Have evaluated this section and had no comments/questions: 2 experts

10. Information on environmental impact of the specific cultivation, management and harvesting techniques used for the GMHP where these are different from those used for non-GMHPs

Have evaluated this section and had no comments/questions: 2 experts

OTHER INFORMATION

Do you have any other questions/comments concerning this notification that are not covered under the previous items?

Comment:

In summary, although it should be recognized that absolute certainty does not exist in relation to genetic modifications, I think that the proposed field evaluation of poplars with a modified wood composition does not lead to significant risks to human beings or to the environment.

Note coordinators: Agree

REFERENCES

Danielsen *et al*. (2012). Fungal soil communities in a young transgenic poplar plantation from a rich reservoir for fungal root communities. *Ecology and Evolution*, 2, 1935-1948.

Halpin *et al*. (2007). Ecological impacts of trees with modified lignin. *Tree Genetics & Genomes*, 3, 101-110.

Vanden Broeck *et al*. (2018). Variability in DNA methylation and generational plasticity in the Lombardy poplar, a single genotype worldwide distributed since the eighteenth century. *Frontiers in Plant Science*, Vol. 9, 1635. doi: 10.3389/fpls.2018.01635.