Bioveiligheidsraad
Conseil de Biosécurité

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Secretariaat
Secrétariat

O./ref.: WIV-ISP/41/BAC_2018_0038

Title: Advice of the Belgian Biosafety Advisory Council on the notification **B/BE/17/V3** of VIB for deliberate release in the environment of genetically modified maize with altered growth characteristics

Context

The notification B/BE/17/V3 has been submitted by the VIB to the Belgian Competent Authority (CA) in October 2017 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: **Scientific field evaluation of maize with modified growth characteristics**. The purpose of the trial is to confirm the modified growth characteristics of the genetically modified (GM) maize under normal field conditions and to measure the effects of the combined modifications on biomass and seed production.

The notification has been officially acknowledged by the CA on 17 October 2017 and forwarded to the Biosafety Advisory Council for advice.
Within the framework of the evaluation procedure, the Biosafety Advisory Council, under the supervision of a coordinator and with the assistance of its Secretariat, contacted experts to evaluate the dossier. Three experts from the common list of experts drawn up by the Biosafety Advisory Council and the Biosafety and Biotechnology Unit (SBB) answered positively to this request.
The experts and the SBB assessed whether the information provided in the notification was sufficient and accurate in order to state that the deliberate release of the GM maize lines would not raise any problems for the environment, animal or human health in the context of the intended use.

On 4 December 2017, based on a list of questions prepared by the Biosafety Advisory Council, the CA requested the notifier to provide additional information. Answers to the questions were provided on 8 January 2018.

For the purpose of the scientific evaluation, the following legislation has been considered:
- Annex II (principles for the risk assessment) and annex III (information required in notifications) of the Royal Decree of 21 February 2005
- Commission Decision 2002/623/EC of 24 July 2002 establishing guidance notes supplementing Annex II to Directive 2001/18/EC.

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 Biosafety Advisory Council. The questions of the public tackling biosafety issues of the GM maize under consideration are taken in consideration in the opinion of the Biosafety Advisory Council. Answers to the questions of the public have been sent to the CA.

Summary of the scientific evaluation

1. Information related to the recipient or parental plants

Zea mays is an allogamous plant that propagates through seed produced predominantly by cross-pollination. Maize pollen can be collected by honeybees and other insects, however these pollinating insects play a minor role in the cross-pollination of maize plants which relies mainly on wind for the dispersal of its pollen (OECD, 2003[1]). Data on pollen dispersal in maize demonstrated that the levels of cross-fertilisation drop rapidly over the initial meters around the pollen source and that most of the released pollen is deposited within about 30 m of the source (Devos *et al*., 2005[2]). At distances farther than 30–50 m from the source, pollen dispersal is very low but not zero. However, vertical wind movements can lift up pollen and distribute it over distances up to kilometers under suitable climatic conditions. In Belgium (and in Europe) there are no sexually cross-compatible wild relatives with which maize can hybridise and form progeny (OECD, 2003). The only recipient plants that can be cross-fertilised by maize are therefore other cultivated maize varieties.

Seed dispersal of individual kernels of domesticated plants are mainly the result of field operations of harvesting the crop and transporting the grain from the harvested fields to storage facilities. Spilled maize seeds can overwinter, germinate and appear in the field as volunteers. However, maize is incapable of sustained reproduction outside the domestic cultivation area as it has lost its ability to survive in the wild due to its long process of domestication (OECD, 2003). Volunteers will only occur after a warm winter period (with no temperatures lower than 0°C for more than 6 to 8 hours) and will be characterised by a low probability of cross-pollination (Grüber *et al*., 2008[3]; Palaudelmàs *et al*., 2009[4]). Given the Belgium weather conditions, volunteers are not likely to occur.

[1] OECD, 2003. Consensus Document on the biology of *Zea mays* subsp. *Mays* (maize). Series on Harmonisation of Regulatory Oversight in Biotechnology (ENV/JM/MONO(2003)11), No. 27:1-49. http://www.olis.oecd.org/olis/2003doc.nsf/LinkTo/NT0000426E/$FILE/JT00147699.PDF

[2] Devos *et al.*, 2005. The co-existence between transgenic and non-transgenic maize in the European Union: a focus on pollen flow and cross-fertilization. Environmental Biosafety Research 4, 71-87.

[3] Grüber *et al*., 2008. Post-harvest gene escape and approaches for minimizing it. CAB International 2008 (http://www.cababstractsplus.org/cabreviews)

[4] Palaudelmàs *et al.*, 2009. Effect of volunteers on maize gene flow. Transgenic Res. 18, 583-594

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

The field trial will be conducted during three consecutive years (from April/May 2018 until October 2020). The surface of the area for cultivation will not exceed 1000 m^2. No glufosinate will be applied to control weeds.

Prior to complete formation, tassels from the GM maize will be removed by hand in order to prevent the dispersal of GM pollen. Monitoring of upcoming tassels will take place every two days until all tassels have been removed and will be maintained until September 15. Removed tassels will be transported in closed bags and inactivated.

During harvest, cobs of the GM maize will be collected by hand and transported in closed bags to the lab. Seeds will be stored for research or will be inactivated if no longer needed for research. Stems and leaves will be shredded on the field. Roots and the lowest part of the stem will be left in the ground.

The year following the field trial, monitoring for volunteers will be done. The field trial will be left fallow and any volunteer maize plants will be removed and inactivated.

3. Information related to the genetic modification

The GM maize GA20OX-1 X GA2ox_PLA1 with altered growth characteristics resulting from a cross between two previously field tested GM lines GA20OX-1 (see B/BE/11/V4) and GA2ox_PLA1 (see B/BE/14/V2), formerly named GAox_KLUH, is the subject of this field experiment.

GA20OX-1 contains the *GA20Oxidase-1* gene originating from *Arabidopsis thaliana*, which is under the control of ubiquitine promoter not functional in bacteria. The gene encodes for a GA20oxidase, which has its natural homologue in maize and which catalyses one of the steps of the biosynthetic pathway of gibberelline GA1.

GA2ox_PLA1 contains the *PLA1* gene, also known as the *KLUH* gene, originating from *Zea mays* and under control of a maize GA2oxidase promoter. The *PLA1* gene encodes for a cytochrome P450 monooxygenase, which is involved in cell proliferation. Under control of the GA2oxidase promoter, it leads to bigger leaves and increased seed yield (Sun *et al*., 2017).

In addition, both transgenic lines contain the *bar* gene from *Streptomyces hygroscopicus* that served as a marker for selection of transformants after *Agrobacterium tumefaciens*-mediated transformation. The *bar* gene produces the phosphinotricin acetyl transferase (PAT) enzyme, which acetylates phosphinotricin, also known as glufosinate, the active ingredient of the broad spectrum herbicides, thereby rendering it inactive.

The presence of the *GA20oxidase* and *PLA1* genes in GA20OX-1 X GAox_PLA1 were confirmed via PCR analysis.

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

The intended changes in the characteristics of maize as a result of the *GA20oxidase-1* and *PLA1* gene expression (increased leave length and width, plant height and biomass) are not known to confer a selective advantage to survivability.

Other observed changed characteristics, such as increased production of seed up 10 to 15% (Sun *et al*., 2017), may confer a selective advantage to survivability. However, due to the (a)biotic factors limiting maize's survival, it is also not expected that changes in these characteristics will increase the ability of maize to survive. Moreover, the measures taken (removal of tassels, manual collection of cobs, monitoring and removal of any volunteers) rule out the development and survival of the GM maize in the year(s) after the field trials.

Vertical gene transfer through pollen can virtually be ruled out due to the removal of the tassels.

The possibility of horizontal gene transfer between plants and micro-organisms is considered as a rare event under natural conditions (Keese, 2008[5]). The possibility of horizontal gene transfer between the GM maize plants and bacteria has been given particular attention due to the presence of the recombination sites attB4, attB1 and attB2. However, the occurrence of an active integrase-excisionase complex in an environment where attP4, attP1 and attP2 are present is estimated low. 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. The resistance gene (i.e. *bar*) occurs naturally in microbes, and the *PLA1* gene and *GA20Oxidase-1* gene, expressing proteins involved in plant cell proliferation and plant hormone production respectively, will not confer a selective advantage to bacteria.

Further, it is not expected that the GM maize would have significant effects on organisms (invertebrates, vertebrates and soil micro-organisms) and humans, as no gene that affects organisms has been integrated. Given the restricted scale of the field trial, any potential effect to organisms and biogeochemical processes - if these would occur - will be of a local and temporal nature. As the GM maize will not produce pollen, a possible altered allergenicity potential of the transgenic pollen (allergy from maize pollen may occur in case of occupational exposure to high amounts of pollen grains, see e.g. Oldenburg *et al*., 2011[6]) does not form a concern for human health.

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

The management measures proposed are considered as sufficient to prevent potential adverse effects to the environment, animal and human health during and after the field trial. The removal of any appearing tassel in the transformed line will prevent gene flow by pollen spread. Careful manual harvesting of the cobs and storing them in closed bags will prevent seed dispersal. The seeds, after analysis, and the tassels will be destroyed. To prevent the spread of transgenes into the environment after termination of the field trial, monitoring for GM maize volunteers will be done.

[5] Keese, P. 2008. Risks from GMOs due to horizontal gene transfer. Environ. Biosafety Res. 7: 123-149.

[6] Oldenburg 2011. Maize pollen is an important allergen in occupationally exposed workers. Journal of Occupational Medicine and Toxicology

Conclusion

Provided that the trials are conducted as described in the dossier, the Biosafety Advisory Council concludes that it is very unlikely that this proposed small scale field trials with GM maize will harm human health, animals or the environment.

Prof. M. De Proft
President of the Biosafety Advisory Council

Annex I: Compilation of comments of experts in charge of assessing the dossier B/BE/17/V3 (ref: BAC_2017_0861)

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O./ref.: WIV-ISP/41/BAC_2017_0861
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1 December 2017

Compilation of comments of experts in charge of assessing the dossier B/BE/17/V3

Coordinator: Maurice De Proft
SBB coordinator: Adinda De Schrijver

INTRODUCTION

Dossier **B/BE/17/V3** 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 24 October 2017 and concerns a field trial transgenic maize with altered growth characteristics

Depending on their expertise, the experts were invited to evaluate the genetically modified organisms considered in the notification as regards their potential impacts on the environment, including human and animal health, and information relating to pre- and post-release treatment of the site.
The comments of the experts are roughly structured as in
- Annex II (principles for the risk assessment) of the Royal Decree of 21 February 2005
- Annex III (information required in notifications) of the Royal Decree of 21 February 2005
- Commission Decision 2002/623/EC of 24 July 2002 establishing guidance notes supplementing Annex II to Directive 2001/18/EC.

LIST OF COMMENTS RECEIVED FROM THE EXPERTS

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.
Items left blank have been evaluated by the experts but no comments or questions were raised.

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

(e.g. reproduction, survivability, dissemination, geographic distribution,...)

2. INFORMATION RELATED TO THE GENETIC MODIFICATION

(e.g. methods used for the modification, description of the vector,...)

3. INFORMATION RELATED TO THE GENETICALLY MODIFIED PLANT

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

3.2. Information on the molecular characteristics of the final GMO

(e.g. number of copies of the transgenes,...)

Regarding the insert copy number of B104(GA20OX-1) (cfr Annex 2):
I agree with the conclusion of the applicant regarding the number of insert copy number, but the way the applicant came to their conclusions is poorly described. In particular, the number of bands observed on the Southern blot in figure 1 is not interpreted by the applicant. A comparison of the expected (base on the map of figure 2) and observed (figure 1) fragments should have been made by the applicant instead of being left to the responsibility of the expert.

Q1 - Regarding the insert copy number of B104(GA20OX-1)
We ask the notifier to explain better how they come to their conclusions on the insert copy number. In particular, the number of bands observed on the Southern blot in figure 1 is not interpreted by the notifier. A comparison of the expected (figure 2) and observed (figure 1) fragments should have been made by the applicant instead of being left to the responsibility of the expert.

Regarding the insert copy number of B104(GA2ox-PLA1) (cfr Annex 2):
The determination of the copy number is made by quantitative PCR comparing the transformation event with lines containing know numbers of gene copies and previously characterized by Southern blotting (see annex 2 page 6). However, in the absence of any data validating the reference lines accessible to the external expert (the Southern analysis of the reference lines is not displayed in the dossier), no evaluation is possible despite the acceptable rationale of the applicant.

Q2 - Regarding the insert copy number of B104(GA2ox-PLA1)
The determination of the copy number is made by quantitative PCR comparing the transformation event with lines containing known numbers of gene copies and previously characterised by Southern blotting (see annex 2 page 6). However, in the absence of any data (the Southern analysis of the reference lines is not displayed in the dossier), no evaluation is possible despite the acceptable

rationale of the notifier. We ask the notifier to provide these data.

Regarding the insert copy number in the plant materials intended to be field-tested: it is worth noting that the genotypes used for insert number determination are not the hybrids intended to be tested in the field but the homozygous parental materials used for producing the hybrids. The applicant makes the assumption that the sexual crosses producing the test materials will not affect this copy number. This assumption seems overall reasonable but, considering that in B104(GA20OX-1) two insert copies are located in close vicinity (as they cannot be out-segregated), the likelihood of intramolecular homologous recombination between these two copies – causing loss of one copy - cannot be completely ruled out. Furthermore, if happening between non-sister chromatids during meiosis and gamete formation, mispairing and recombination can lead to increased (up to 3) and decreased (down to 1) copy numbers in the meiotic products, i.e. in the gametes participating in the creation of the hybrids. As a consequence, in the absence of experimental evidence, it is not allowed to draw firm conclusions on the insert copy number in the hybrid field-tested materials derived from crosses using the B104(GA2ox-PLA1) parental line. I would be of the opinion that experimental evidence for insert copy number should be obtained from the field-tested materials, accordingly. A straight forward way would be to perform a Southern blot analysis of this material with the probe-enzymes combinations described in figure 2.

Note coordinator/SBB: Please note that for Part B notifications an 'estimate' of the insert copy number and not a 'firm conclusion', is considered as sufficient information (see Molecular Characterisation Guidelines Part B). An estimate can be obtained from the information on the single events that is available. Having said this, please note that Bijlage 3 – Totstandkoming van de B104(GA20OX1 x GA2ox-PLA1 140-01) x CML91 hybride planten, data on the presence of the insert are provided on the hydrids that will be tested in the field. In this annex it is clarified that double homozygote plants with 6 *bar* gene copies have been selected and further tested on the presence of the GA20OX1 gene and PLA1 gene.

Concerning Bijlage 3 page 4: VIB should provide molecular data on the crossing of the GMO B104 lines (double and single events) with CML91 in order to demonstrate that genuine hybrid seed will be planted in the field. This information is missing in the dossier?

Note coordinator/SBB: As it has been demonstrated that the GM B104 lines (double & single) contain the inserts, it is not considered necessary to test the GM B104 x CML91 lines again for the presence of the inserts.

3.3. Information on the expression of the insert

(e.g. parts of plants where the insert is expressed, (expected) expression of the insert during the lifecycle of the plant,...)

Comment on cross reference: Promoter activity of GA2ox (page 12 of main dossier) is described and refers to the previous field trial application B/BE/214/V2. Such cross referencing should be avoided as experts do not necessarily have access to the older dossiers. Each new application should be supported by a stand-alone dossier.

3.4. Information on how the GM plant differs from the recipient plant

Comment on endpoints measured: The applicant emphasizes the anthesis – silking interval as the most relevant phenotype expected to be modified by the genetic transformation, impacting fruiting and grain yield. On the other hand the plants will be emasculated to avoid pollen shed. How then to measure the anthesis-silking interval?
Later in the dossier (page 19, section F1) it is indicated that vegetative biomass is a major assessment endpoint. The applicant should be more clear about which phenotypic and agronomic characteristics are considered in these trials.

Note coordinator/SBB: Info on what the exact assessment endpoints are, will not aid us in doing the RA of a field trial and such information seems more important for the scientific outcomes of the trials.

Q3 – Regarding information on how the GM plant differs from the recipient plant
The applicant emphasizes the anthesis – silking interval as the most relevant phenotype expected to be modified by the genetic transformation, impacting fruiting and grain yield. On the other hand the plants will be emasculated to avoid pollen shed. How then to measure the anthesis-silking interval?

3.5. Genetic stability of the insert and phenotypic stability of the GMHP

Comment 1: The phenotypic stability in B104(GA2ox-PLA1) is described in very vague terms (“more” stable, “less” stable; of which plant traits?) and refers to previous trials with no quantitative data displayed. I cannot evaluate the conclusions of the applicant in the absence of quantitative data.

Note coordinator/SBB: According to the Guidelines for Molecular Characterisation of GM Higher Plants for a Standard Part B Consent, information on stability, especially genetic stability, is not required. To be in line with the guidelines, we should not ask for further quantitative data.

Comment 2: D5: referee finds it somewhat difficult to understand why GA2ox_PLA1 is being used (for which is shown that phenotype stability is an issue?) instead of 139; what is the explanation for the instability of GA2ox_PLA1 140?

Q4 – Regarding genetic stability of the insert and phenotypic stability of the GMHP
The phenotypic stability in B104(GA2ox-PLA1) is described in very vague terms, such as “more” stable and “less” stable. Can it be explained to which plant traits is referred to here and what is exactly meant with ‘more’ and ‘less’ stable.

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

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

3.8. 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

Comment: Not relevant

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

Comment: Not relevant

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

3.11. Potential interactions with the abiotic environment

3.12. Description of detection and identification techniques for the GM plant

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

Comment: Not applicable

4. INFORMATION RELATING TO THE SITE OF RELEASE

(e.g. description of the site ecosystem, presence sexually compatible species, proximity of protected areas,...)

Note coordinator/SBB: Q5 - Information on the introduction site
The technical dossier states (page 18, E3) 'De afstand tot andere maïsplanten zal minimaal zijn.' A more precise distance from maize fields should be provided.

5. INFORMATION RELATING TO THE RELEASE

(e.g. purpose of release, dates and duration of the release, methods for preparing and managing the release site, number of plants,...)

6. INFORMATION RELATED TO THE RISKS FOR THE ENVIRONMENT

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

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

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

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

Comment: Not relevant

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

6.6. 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

6.7. 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

Comment: Not relevant

6.8. 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)

Comment: Not relevant

6.9. 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

Comment: Not relevant

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

7.1. Precautions taken

Comment: Bijlage 6 page 2: it is stated that "… achtergebleven bladeren zijn niet reproductief en dus geen GGO…."; this statement is questionable and should better be omitted; the remaining leaves will (as for remaining stem and roots) be degraded and therefore they should not be considered as dangerous for the environment.

Note coordinator/SBB: In directive 2001/18/EC the term 'genetically modified organisms' is defined as follows: "an organism, with the exception of human beings, in which the genetic material has been altered in a way that does not occur naturally by mating and/or natural recombination". Within that definition 'organism' means: "any biological entity capable of replication or of transferring genetic material". Stems and leaves of genetically modified maize are genetically modified materials, but are as such not an organism, as they are not capable of reproduction or of transferring genetic material. In other words: they are GM, but not a GMO. As a consequence non-reproductive materials which are derived from a GMO, and which are not capable of transferring genetic material, neither fall within the scope of directive 2001/18/EC, nor within the scope of the Belgian Royal Decree of 21 February 2005, implementing this directive.

7.2. Information on methods for postrelease treatment of site

7.3. Information on postrelease treatment methods for the GM plant material, including wastes

Note coordinator/SBB:
Q6 - Information on post-release treatment methods for the GM plant material, including wastes
How will the plant material (waste) be inactivated?

7.4 Information related to monitoring plans and the detection techniques

Comment: Not relevant

Note coordinator/SBB: Q7 – regarding the detection protocol
The notifier is asked to verify why 2,5 µl of a 10X buffer will be used on a total volume of 20 µl. In order to be able to use a master mix for PCR-reactions, the notifier is also asked to mention the volume of the DNA-template solution in the total volume of the PCR-mix.

7.5. Information on the emergency plan(s) proposed by the notifier

7.6. Information on methods and procedures to protect the site

8. OTHER INFORMATION

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