

## FACT SHEET

### APPLICATION FOR APPROVAL FOR CONFINED FIELD ASSESSMENT OF GENETICALLY TRANSFORMED RUBBER TREES (HEVEA BRASILIENSIS)

NBB REF NO: JBK (S) 602-1/1/17

The objective of the Biosafety Act is to protect human, plant and animal health, the environment and biological diversity. Under the Biosafety Act, the National Biosafety Board (NBB) is currently assessing an application for approval submitted by Malaysian Rubber Board.

#### 1. What is the application for?

To establish a confined field trial of genetically modified rubber trees (*Hevea brasiliensis*) in Penawar, Kota Tinggi, Johor Darul Takzim.

#### 2. What is the purpose of the release?

To evaluate expression of transgenes in leaf and latex of genetically modified rubber (*H. brasiliensis*) trees at different stages of growth, under field conditions. Periodical leaf and latex sample collection for testing of transgene expression.

#### 3. How has the LMO been modified?

*Agrobacterium* mediated genetic transformation. The common thread in the *Agrobacterium* mediated genetic modification on *H. brasiliensis* is the insertion of a transgene (gene of interest) along with nptII - the gene that confers resistance to kanamycin, into the *Hevea* genome at callus stage. The technology aims to express and harvest the transgene product from the latex vessels of genetically modified rubber trees. Tapping of the rubber tree facilitates continual and non-destructive harvest of latex and the transgene product that is expressed therein. The genetic modifications are not expected to result in phenotypical changes to the rubber tree.

#### 4. Characteristics of LMO

##### a) Details of the parent organism

Although *H. brasiliensis* originated from the Amazon basin in South America, the plant has been adopted for cultivation in many countries in the tropical region, including Malaysia. Rubber elastomer and wood obtained from the tree serve as feedstock for a wide range of downstream applications.

*H. brasiliensis* is a monoecious flowering plant. The inflorescence is a panicle of separate staminate and pistillate flowers borne in the axils of basal leaves of new shoots. Pistillate flowers are terminal to the central stem and other major branches of the inflorescence. Smaller and more numerous staminate flowers make up the rest. Both flowers are shortly stalked and scented. Neither flower has petals but rather five triangular lobes. Staminate flowers have two rings of five stamens each borne on a stalk. Pistillate flowers have a compound ovary with three locules topped by three sticky, sessile stigmas. Within an inflorescence, a few staminate flowers open first and fall off after one day. Pistillate flowers then open for a period of three to five days after which the rest of the staminate flowers open. This mechanism ensures a high degree of cross-pollination. Pollination occurs primarily through insects, mainly bees, midges and thrips. Fertilization occurs within 24 hours after pollination. Unfertilized pistillate flowers quickly wither and die. There appears to be no evidence of self-incompatibility although cross-pollination usually results in better fruit set.

In Malaysia, flowering of *H. brasiliensis* occurs in February to April and (a lesser flowering) in September and October. After pollination, fruits mature in 6-7 months and dehisce explosively, scattering seeds in the vicinity of the mother trees.

Isozyme marker studies revealed that pollen can be disseminated as far as 1.1 km from the mother plant in plantations. The boundary of confined field trial is separated from normal rubber planting by a minimum distance of 1.5 km.

Vegetative propagation is a common practice in rubber cultivation where scions of plants are grafted onto selected root-stocks, hence creating identical copies of the mother plant. Hence this technique opens up the potential to generate unlimited copies of a single plant for rubber cultivation. There are no known wild relative of the genus *Hevea* in Malaysia. Other than *H. brasiliensis* there are no other species that are cultivated in Malaysia.

The rubber tree (*H. brasiliensis*) has no known toxic effects on human, animal and other organisms. Sporadic cases of allergy to latex products have been reported, mainly in the Western society.

*H. brasiliensis* trees cultivated primarily for rubber require periodical commercial fertilizer application throughout the productive phase i.e. twenty years, while trees cultivated for wood are maintained as forest plantation until they are felled after fifteen years.

#### **b) Details of the donor organism**

- i. B-Glucuronidase (GUS); donor organism – bacterium - *Streptococcus pneumonia*
- ii. A single chain variable fragment antibody specific to *Streptococcus gordonii* coat protein (scFv4715); donor organism – mammal – *Mus musculus*
- iii. Human atrial natriuretic factor (HANF); donor organism – human - *Homo sapiens*
- iv. Human protamine 1 (HP1); donor organism – human - *Homo sapiens*
- v. ix. Neomycin phosphotransferase II (kanamycin resistance gene); donor organism – bacterium - *Escherichia coli*.

#### **c) Description of the trait(s) and characteristic which have been introduced or modified**

##### **Genes of interest:**

1. Glucuronidase (GUS) – is a reporter gene encoding glucuronidase enzyme that hydrolyzes x-gluc - a colorless soluble substrate, into soluble blue colored product. This serves as an indicator of transgene expression in the target tissue of the transformant.
2. Recombinant antibody (scFv4715) specific to *Streptococcus gordonii* coat protein. Recombinant antibody (scFv4715) will bind to coat protein of *S. gordonii*, the causal agent of dental plaque/cavity.
3. Human atrial natriuretic factor (HANF) - a gene that encodes for a blood peptide hormone that plays a role in lowering cardiac blood pressure, particularly during open heart surgery.
4. Human protamine 1 (HP1) – a gene that encodes a small peptide that has been shown to induce blood clotting. HP1 is a therapeutic protein which is commonly used to neutralize the anticoagulant effects of heparin during cardiovascular surgery.
5. Neomycin phosphotransferase II – the nptII gene confers resistance to kanamycin. This gene is transferred along with the gene of interest as it facilitates screening of transformed callus in growth media supplemented with kanamycin.

#### **d) Modification method**

##### **Co-cultivation of *Hevea* Anther Callus with *Agrobacterium* Culture**

Large scale cultures of *A. tumefaciens* GV2260 that contained the gene constructs were grown on LB broth containing kanamycin (50 µg/ml) at 28°C until stationary phase. The OD<sub>600nm</sub> of the bacterial culture was adjusted to circa 0.6 using culture initiation (CI) media. *H. brasiliensis* GL1 anther callus was initiated from the anther walls (tapetum cells) in MS (ID)Z media. The callus tissue was then co-cultivated with *A. tumefaciens* GV2260 harbouring gene of interest to allow infection and insertion of the desired transgene into the rubber genome as described elsewhere. After co-cultivation, the callus tissue was transferred to fresh initiation medium containing cefotaxime and ticarcillin, to prevent overgrowth of *Agrobacterium*, while kanamycin in the selection media ensures growth of putative transformed callus. The plates were incubated in the dark at 25°C for 14 days (first selection); the callus cultures were subjected to several rounds of selections prior to embryogenesis and subsequent regeneration of plantlets.

pBIN19 for GUS, pGPTV-Kan for scFv4715, pGPTV-Kan for HANF, and pGPTV-Kan for HP1. Expression of scFv4715 in the original pGPTV-Kan is driven by CaMV 35S promoter while that of HANF and HP1 is driven by hevein promoter. pBIN19 and pGPTV-Kan are plant transformation vectors that have been commonly employed in plant transformation work elsewhere.

#### e) Characterization of the modification

Kanamycin resistance gene (nptII) serves as selectable marker during in vitro culture stage. In addition, the transgenes, GUS, scFv4715, HANF, and HP1, are useful for detection of transformed trees under field conditions.

Primer sequences employed for detection of each transgene using PCR:

HP1:

FP.hpheveinpro 5' TTTCCCGGGATGGCCAGGTACAGAT 3'

RP.hpheveinpro 5' TTGAGCTCGGCAGGAGTTTGGTGGATG 3'

NPTII:

FP.nptII 5' GAG GCT ATT CGG CTA TGA CTG 3' (position 52 – 72)

RP.nptII 5' ATC GGG AGC GGC GAT ACC GTA 3' (position 753 – 733)

scFv4715:

FPscFv4715 5' GGA TGG GAT TTG TTC TCT TTT CA 3'

RPscFv4715 5' GGC TTC AGG TAC CCT TA 3'

HANF:

ANF-for 5' TTT CCC GGG ATG AGC ACC TTC TCC ACC 3'

ANF-rev 5' TTT GAG CTC TCA GTA CCG GAA GCT GTT 3'

HANF-For 5' ATG AGC TCC TTC TCC ACC AC 3'

HANF-Rev1 5' GTA CCG GAA GCT GTT ACA GC 3'

HANF-Rev2 5' GC CCA GTC CGC TCT GGG CTC 3'

Hevein promoter fragments:

FP.hevP 5' GG TCTAGA CCC ATT TCT TCC CAA TTC 3'

RP1.hevP 5' GG AAGCTT CCT GGC CCT ATG CTC TAT 3'

RP2.hevP 5' GG AAGCTT CGA GTT AAC CCT TGC GTT 3'

RP3.hevP 5' GG AAGCTT GCC CTC TTG GTT GTT GCC 3'

pBluescript KS+

T7 5' GAG GCT ATT CGG CTA TGA CTG 3'

SP6 5' ATC GGG AGC GGC GAT ACCGTA 3'

Glucuronidase:

Gus-For 5'-GGT GGG AAA GCG CGT TAC AAG-3'(position 400-420)

Gus-Rev 5'-GTT TAC GCG TTG CTT CCG CCA-3'(position 1599-1579)

#### f) Safety of the expressed protein

In so far, there are no reports on potential toxicity of the transgenes (genes of interest) on non-target organisms or environment.

### 5. Assessment of risks to human health

#### a) Nutritional data

The transgene products are not intended to enter the food chain. No parts of the LMOs are intended for human use, other than verification of leaf and latex samples for expression of transgene and transgene product.

#### b) Toxicology

Nil.

**c) Allergenicity**

Nil (other than what is described in response to question 4 above).

**6. Assessment of risks to the environment**

No known adverse effect to the environment.

**7. What is the emergency response plan?**

**a) First aid measures**

All personnel approved to handle the LMO are equipped with proper PPE (disposable gloves, lab coat, and foot wear). As the LMO and the transgene products are not known to have adverse effects on human, animals and environment, there are no additional first aid measures other than washing with soap and copious amount of water needed in case of contact with skin.

**b) Accidental release measures**

The confined field trial site is fenced (2 m height); therefore intrusion of all large animals that may feed on the plants is prevented. In case of accidental release, the CFT area will be cordoned off and efforts will be made to recover and destroy LMO or parts of LMO by burying on site.

**c) Handling and storage**

All LMOs and parts of LMOs will be handled according to the standard operating procedure by personnel equipped with PPE. The samples of LMO will be brought to laboratory in designated containers that are labelled accordingly and after testing, the remains and waste will be autoclaved prior to disposal.

**d) Disposal considerations**

Liquid cycle top-load autoclaving machines are available for the purpose of destroying LMO and waste prior to disposal.

Unwanted parts of LMO (ex. dried leaves, twigs) are left to decay onsite.

**8. How can I comment on this application?**

Any member of the public may submit their comments or queries on publicly notified information about the application. Before submission of comments or queries, the person should review the information provided. Your comments and queries on any possible impacts/risks to the health and safety of the people and the environment that may be posed by the proposed release are appreciated. The submission to the comments or queries should be prepared carefully as it will be given the same scrutiny as the application by the NBB. The submission of comments and clarifications of queries should contribute to the NBB's assessment. Even if the submission is not science-based, and focuses on cultural or other values, it should still be developed in the form of a well-founded argument.

Please note that the consultation period closes on **28 November 2014** and written submissions are required by that date. Submissions must be addressed to:

Director General, Department of Biosafety  
Ministry of Natural Resources and Environment  
Level 1, Podium 2, Wisma Sumber Asli  
No. 25, Periaran Perdana, Presinct 4, 62574  
Putrajaya, MALAYSIA.  
E-mail: biosafety@nre.gov.my.  
Fax: 03-88904935.

Please include your full name, address and contact details in your submission.