

FACT SHEET
APPLICATION FOR APPROVAL FOR CONFINED FIELD EVALUATION OF
GENETICALLY MODIFIED/TRANSGENIC EKSOTIKA PAPAYA
AGAINST PAPAYA DIEBACK DISEASE
NBB REF NO: JBK (S) 600-2/1/6

The objective of the Biosafety Act 2007 is to protect human, plant and animal health, the environment and biological diversity. Under the Biosafety Act 2007, the National Biosafety Board (NBB) is currently assessing an application for approval submitted by the Malaysian Agricultural Research and Development Institute (MARDI)

1. What is the application for?

This application is to conduct a confined field trial for genetically modified papaya. The title of the project is “Confined Field Evaluation of Transgenic Eksotika Papaya Against Papaya Dieback Disease”.

2. What is the purpose of the confined trial?

The purpose of the confined field trial is to evaluate the resistance of the genetically modified Eksotika papaya variety against *Erwinia mallotivora*, the causal agent of papaya dieback disease. Field evaluation is needed to confirm that the transgenic papaya lines have been incorporated with the anticipated disease resistant characteristic and to collect biosafety data for subsequent open field trial.

3. How has the Eksotika papaya been genetically modified?

The Eksotika papaya has been genetically modified to have anti-pathogenic characteristic against *E. mallotivora* through the insertion of the *AHL-lactonase* gene with a 35S Cauliflower mosaic virus (35S CaMV) promoter for constitutive expression. Insertion of the gene into the Eksotika papaya was done in a sense orientation through *Agrobacterium*-mediated transformation method. The AHL-lactonase enzyme produced has been found to specifically inhibit the growth of *E. mallotivora in vitro* by disrupting the important component for quorum sensing, Acyl-homoserine lactone. Besides the *AHL-lactonase* gene, a selectable marker gene, *neomycin phosphotransferase II (nptII)* that confers resistance to kanamycin was also inserted into transgenic Eksotika papaya to facilitate screening and identification of the transformants during the growth of the transgenic plants.

4. Characteristics of transgenic Eksotika papaya

a) Details of the parent organism

The parent organism is *Carica papaya* L. variety Eksotika, also known as Eksotika papaya. It can be grown in tropical and semi tropical climates. It is exotic to Malaysia and is widely grown

as a commercial cultivar in Malaysia. The global production of papaya reached over 13 million metric tons in 2016. The Eksotika papaya reproduces sexually. The papaya fruit production may occur following cross-pollination, out crossing and self-pollination

b) Details of the donor organism

Bacillus spp. is the source of the *AHL-lactonase* gene. It is a gram-positive bacterium widely distributed in the environment although most commonly found in soil.

Escherichia coli is the source of the *neomycin phosphotransferase II (nptII)* gene. It is a gram-negative bacterium commonly found in the environment and intestines of people and animals.

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

The AHL lactonase enzyme produced by the *AHL-lactonase* gene should prevent rapid spreading of the *E. mallotivora* pathogen by inactivating Acyl-homoserine lactones (AHLs) molecule that is crucial for bacterial quorum sensing, thus enabling the plant to have enhanced resistance towards the papaya dieback disease and slowing the disease severity.

d) Safety of the expressed protein

The transgenic papaya is not likely to become more toxic or allergenic than normal papaya as a result of the introduced proteins because none of the introduced proteins, or the native proteins with modified expression in the genetically modified papaya, have any known intrinsic toxicity or allergenicity. However, further evaluation of the transgenic papaya will be conducted in this confined field trial before it can be considered safe for human consumption and the environment

5. Assessment of risks to human health

At this stage of the evaluation process, the acute toxicological data for the transgenic papaya plants proposed for confined trial is not available. However, a comprehensive assessment of toxicology and allergenicity of the transgenic papaya will be carried out before being commercialized.

6. Assessment of risks to the environment

a) Environmental considerations

The confined field trial will be conducted in a restricted access, insect proof nethouse, equipped with containment features; with the parameter size of 21 m x 18 m x 5.2 m. The mesh size of the netting to be used is No. 50. The trial plot is not located in a residential area. It is located at a transgenic facility within the vicinity of the MARDI Headquarters in Serdang, Selangor.

b) Biological containment

The cultivated papaya (*Carica papaya* L.) originated in Central America and is not indigenous in Malaysia. There are several *Carica* species that can hybridise with *Carica papaya* but none

of them are found in Malaysia. Risk of cross-pollination is low since the trial plot is isolated with a perimeter of approximately 500 m from other untransformed papaya. In addition, only female and hermaphrodite transgenic plants will be planted in this trial. In order to prevent pollen from escaping, all the flowers will be tagged and bagged before anthesis. The fruit will be harvested at the colour break stage of maturity and the seeds will be stored according to the biosafety requirement.

c) Training of personnel

All personnel will be trained on the regulations and procedures for handling materials, as well as methods and objectives of genetic transformation in the experiment prior to their working on the project.

d) Maintenance

Technicians at the trial plot will carry out routine field maintenance after getting instruction by trained personnel involved in the project.

7. What is the emergency response plan?

a) First aid measures

In case of unintentional release, the MARDI Biosafety Officer will be immediately informed. All the corrective action will be taken, documented and IBC, MARDI will be notified.

b) Accidental release measures

In case of any unintentional release, the volunteer plants or prohibited plants can be controlled by herbicides or removed and destroyed by autoclaving or by incineration. The area of the unintended release will be treated in a manner so as to ensure that there is no release of additional materials. All the plants will be collected and kept in sealed autoclave bags as primary containers and then placed in a sealed, leak-proof and water-resistant secondary container prior to disposal by using autoclave or incinerator. If the disposal cannot be carried out on the same day, the waste will then be kept in the storage areas and clearly labelled. Access to this area will be restricted to authorized personnel only. The infected area will be disinfected using a chemical disinfectant, hypochloride (10%). In case where the netting structure is damaged by strong winds or other adverse environmental conditions, immediate action will be taken to repair or replace the net. If the damage cannot be repaired immediately, all the flowers from the transgenic papaya will be removed and destroyed.

c) Handling and storage

The fruits from the transgenic papaya plant showing resistance against papaya dieback diseases characteristic will be harvested. The fruit will be transported from the trial site to the laboratory in a durable bag as primary container and then placed in a sealed, leak-proof and water resistant secondary container. Then the seeds will be collected and kept in a sealed envelope as primary container and then placed in a sealed, leak-proof and water resistant secondary container. These seeds will be kept in Molecular Analysis Laboratory at Transgenic

Glasshouse Complex, Biotechnology Research Centre, MARDI until they are ready to be germinated for next field trial analysis to confirm the trait.

d) Disposal considerations

All plant materials will be chopped and dried at the trial site. Dried plant materials are to be packed in a sealed, durable bag for incineration by using an in-house incinerator located in the Transgenic Glasshouse Complex.

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 **24 July 2019** and written submissions are required before or on that date. Submissions must be addressed to:

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

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