

FACT SHEET

APPLICATION FOR APPROVAL FOR CONFINED FIELD ASSESSMENT OF ANTISENSE TRANSGENIC PAPAYA FOR DELAYED RIPENING

NBB REF NO: JBK (S) 602-1/1/12

1. What is the application for?

The application is for Malaysian Agricultural Research and Development Institute (MARDI) to conduct a confined field trial. The title of the project is "Confined Field Assessment of Antisense Transgenic Papaya for Delayed Ripening". The application is to evaluate genetically modified *Eksotika* papaya for delayed ripening in a 24 m x 18 m x 5.2 m confined environment under a nethouse structure, netting with No.50 mesh size gauze.

2. What is the purpose of the confined trial?

The purpose of the confined field trial is to evaluate the delayed fruit ripening characteristic of the transgenic papaya transformed with antisense *ACC Oxidase 2* gene. The trial is of utmost importance since field evaluation is needed to confirm the transgenic papaya lines incorporated with the anticipated delayed fruit ripening characteristic and to collect biosafety data for subsequent open field trial.

3. How the *Eksotika* papaya is been genetically modified?

The *Eksotika* papaya has been modified to delay fruit ripening characteristic. *ACC Oxidase 2* gene obtained from *Eksotika* papaya was inserted by using antisense technology to reduce the production of ACC oxidase enzyme in transgenic *Eksotika* papaya. Hence, the production of ethylene gas that is responsible in fruit ripening was reduced and resulted in delayed fruit ripening. A selectable marker gene, *nptII* derived from *Escherichia coli* that conferred 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

Details of the parent organism

The parent organism is *Carica papaya* L. variety *Eksotika*.

Details of the donor organism

The donor organism is *Carica papaya* L. variety *Eksotika*.

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

ACC Oxidase gene had been introduced in an antisense orientation to reduce the activity of ACC oxidase enzyme in transgenic Eksotika papaya. Reduction of the ACC oxidase enzyme will reduce the production of ethylene gas, which results in delayed fruit ripening and subsequently improve the keeping quality of Eksotika papaya fruit.

5. Modification method

Characterization of the modification

The inserted gene was introduced into Eksotika papaya through *Agrobacterium*-mediated transformation method. The transgenic Eksotika papaya fruit will have a longer shelf-life.

Safety of the expressed protein

The inserted gene was obtained from the same organism, Eksotika papaya, which is widely grown as a commercial cultivar for human consumption in Malaysia. This gene is present in ethylene biosynthesis pathway and found in all papaya plants. Hence, the transgenic papaya to be evaluated in this confined field trial expresses the same protein as in the non-genetically modified papaya and can be considered safe for human consumption and the environment.

6. Assessment of risks to human health

At this stage of the evaluation process for the transgenic papaya plants proposed for confined trial, the acute toxicological data is not available. This data will be obtained through this proposed confined field evaluation. However, based on finding on field evaluation of transgenic papaya for delayed ripening carried out by The University of Queensland Australia, no toxicity or allergenicity was detected.

7. Assessment of risks to the environment

Environmental considerations: The trial plot is not located in a residential area. It is located at a transgenic facility within the vicinity of a government research institute. The trial will be conducted in an insect-proof nethouse, built with a double door entrance. The door will be locked for the duration of the experiment except when personnel are working on site and access to the nethouse is restricted to only authorized personnel. Key for entering the nethouse is kept by the project investigator (PI) only. A clear signage indicating entry restriction will be placed at the entrance. The nethouse facilities including door lock, netting structure and fencing are to be inspected regularly by the PI to ensure that all these containment features are intact.

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.

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.

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

8. What is the emergency response plan?

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.

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.

Handling and storage

Fruits will be harvested for delayed fruit ripening analysis at the Post-harvest Laboratory, Horticulture Research Centre, MARDI. All fruits for fruit ripening studies will be harvested by hand at the early colour break stage and removed from the trial site. The harvested fruits will be transported from trial site to the laboratory in a durable bag as the primary container and then placed in a sealed, leak-proof and water resistant secondary container. The seeds which show delayed ripening characteristic 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 4°C refrigerator in the Molecular Analysis Laboratory at the Transgenic Glasshouse Complex, Biotechnology Research Centre, MARDI for the subsequent field trial.

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.