RISK ASSESSMENT REPORT

OF THE GENETIC MODIFICATION ADVISORY COMMITTEE (GMAC)

FOR

AN APPLICATION FOR APPROVAL FOR CONFINED FIELD EVALUATION OF DELAYED RIPENING TRANSGENIC EKSOTIKA PAPAYA

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

APPLICANT: MALAYSIAN AGRICULTURAL RESEARCH AND DEVELOPMENT INSTITUTE (MARDI).

DATE: 13 MAY 2013

I - Summary of Assessment Process

The Genetic Modification Advisory Committee (GMAC, please refer to Appendix 1 for details of GMAC), under the purview of the National Biosafety Board was given the dossier by the Department of Biosafety on 24 July 2012 for an application for approval entitled 'Confined Field Evaluation of Delayed Ripening Transgenic Eksotika Papaya'. The application was filed by Malaysian Agricultural Research and Development Institute (hereafter referred to as "the applicant"). GMAC members also took the opportunity to obtain further clarification on certain details of the activity.

After conducting an initial review, GMAC requested for a scientific meeting with the applicant. The Principal Investigator (PI) of the proposed confined field trial and the Biological Safety Officer (BSO) of the applicant attended the meeting and gave a scientific briefing on the 11 December 2012. GMAC members also took the opportunity to obtain further clarification on certain details of the proposed field trial. Additional information was also provided by the applicant as requested.

A public consultation for this application was conducted from 8 March 2013 to 6 April 2013 via advertisement in local newspapers. Comments were received from Consumer Association of Penang (CAP) and Third World Network (TWN) regarding the integrity of the nethouse structure, risk of gene flow, mechanism for conferring the delayed ripening trait and risk of using marker gene *npt*II and *ACC oxidase* gene. GMAC reviewed the input received and it was found that all the issues raised have been considered and taken into account in the risk assessment by GMAC.

GMAC had 5 meetings pertaining to this application and prepared the Risk Assessment Report and Risk Assessment Matrix along with its recommended decision, for consideration by the National Biosafety Board.

As part of the assessment process, GMAC members conducted a visit to the Nethouse of Malaysian Agricultural Research and Development Institute (MARDI), Serdang Selangor. Following this visit, GMAC has made some recommendations and set out some conditions for the applicant.

II - Background of Application

This application is for approval to conduct a confined field evaluation of delayed ripening transgenic Eksotika papaya. 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 in a 24 m x 18 m x 5.2 m confined environment under a nethouse structure (netting with size 50 mesh). The trial is of utmost importance since field evaluation is needed to confirm that the transgenic papaya lines are incorporated with the anticipated delayed fruit ripening characteristic and to collect biosafety data for subsequent open field trial.

Information about genetically modified Eksotika papaya

The parent organism is *Carica papaya* L. variety Eksotika and the donor organism is *Carica papaya* L. variety Eksotika*.* The cultivated papaya (*Carica papaya* L.) originated in Central America and is not indigenous to Malaysia. According to the applicant, there are several *Carica species* that can hybridise with *Carica papaya* but none of them are found in Malaysia.

The Eksotika papaya has been modified to show a delayed fruit ripening characteristic. *ACC Oxidase 2* gene obtained from Eksotika papaya was inserted by using antisense technology through *Agrobacterium*-mediated transformation method to reduce the production of ACC oxidase enzyme in the 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, *npt*II derived from *Escherichia coli* that conferred resistance to kanamycin was also inserted into the transgenic Eksotika papaya to facilitate screening and identification of the transformants during the growth of the transgenic plants.

Safety of the expressed protein

According to the applicant, 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 the 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.

Field trial location

The trial plot is not located in a residential area. It is located at a transgenic facility within the vicinity of the applicant's premise (a government research institute). The trial will be conducted in an insect-proof nethouse, built with a double door entrance. According to the applicant, 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 authorised personnel only. The key for entering the nethouse will be kept only by the Principal Investigator (PI). However, employees authorised by the project leader are allowed to enter the nethouse. Another key will be given to the Office of the Deputy Director to be used in case of an emergency. The nethouse facilities, including door lock, netting structure and fencing will be inspected regularly by the PI to ensure that all these containment features are intact.

According to the applicant, risk of cross-pollination is low since the trial plot is isolated with a perimeter of approximately 500 m from other untransformed papaya plants. 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 seed will be stored according to the biosafety requirement.

III - Risk Assessment and Risk Management Plan

GMAC evaluated the application with reference to the following documents:

(i) Roadmap for Risk Assessment of Living Modified Organisms, (according to Annex III of the Cartagena Protocol on Biosafety produced by the *Ad Hoc* Technical Expert Group (AHTEG) on Risk Assessment and Risk Management of the Convention on Biological Diversity).

(iii) The risk assessment and risk management plan submitted by the applicant.

GMAC took cognizance of the following as suggested within the AHTEG guidelines:

(i) That the risk assessment exercise be specific to the details of this particular application;

(ii) That the risk assessment exercise be specific to the receiving environment in question; and

(iii) That any risk identified be compared against that posed by the unmodified organism.

A Risk Matrix was prepared based on an assessment mechanism developed by Office of the Gene Technology Regulator, Australia (OGTR, 2005). In applying this matrix, GMAC identified potential hazards, and then added a value/rank for the likelihood of each hazard as well as its consequences. The likelihood of each hazard occurring was evaluated qualitatively on a scale of 1 to 4, with 1 for 'highly unlikely', and 4 for 'highly likely'.

The consequences of each hazard, if it were to occur, were then evaluated on a scale of 1 to 4, with 1 for 'marginal' and 4 to denote a 'major consequence'. A value was finally assigned for the overall risk from the identified potential hazard. The general formula: Overall Risk = Likelihood x Consequence was employed. GMAC also proposed risk management strategies for potential hazards, where appropriate. This methodology of assessment follows the procedure of Risk Assessment in Annex III of the Cartagena Protocol on Biosafety.

The Risk Assessment was conducted over a series of 5 meetings. To start with, the possible pathways to risk/hazard arising from the field trial were identified and listed. The potential hazards were identified in three main areas:

(i) **Effects on human health**

Issues pertaining to toxicity of the expressed proteins and potential allergenicity were examined.

(ii) **Effects on animal health**

Issues pertaining to allergenicity, toxicity, anti-nutritional, compromised nutritional content, effect on performance and survivability, horizontal gene transfer of marker genes to soil microorganisms were examined.

(iii) **Effects on the environment**

Issues pertaining to unintentional release, weediness, gene transfer to bacteria, accumulation of toxin, cross pollination and toxic effects on non-target organisms were examined.

Based on the above, a final list of 19 potential hazards were identified. All of these hazards were rated as having an Overall Risk of 1 or "negligible".

GMAC also took extra caution and further discussed pre-emptive mitigation procedures for hazards where the Overall Risk was estimated to be above the minimal, and also for a few hazards that required further evaluation and data acquisition. Some of these risks are expected to be managed effectively with the risk management strategies proposed (please refer to section IV of this document).

Although the applicant has applied for an approval to conduct confined field trial only, GMAC had conducted a thorough assessment and widened the scope of the risk assessment to include the risk posed to human and animal health and effects to the environment. Pertinent potential hazards are highlighted below along with the appropriate management strategies:

(i) Expression of antisense *ACO2* sequences perturbs gene regulation and/or metabolic pathways in GM Papaya

To date, there is no known study on anti-nutritional properties of GM papaya. Moreover, the papaya from this confined field trial will not be consumed. To minimize the risk of consumption of the GM papaya, GMAC requires the applicant to limit access to the GM papaya to authorised workers only. All parts of the GM papaya plants, including all the fruits shall be destroyed upon completion of study.

(ii) Expression of antisense *ACO2* sequences leads to changes in metabolic pathways in GM Papaya, resulting in enhanced fitness

Pleiotropic effects may be presented, resulting in unpredictable phenotypes. The target gene being manipulated is primarily involved in fruit ripening. There is a possibility of the creation of new weed strain if there are escapes from the nethouse. The risk is low but requires risk mitigation, and is contingent upon strict administrative policy for access control and adherence to Standard Operating Procedures (SOPs) for transport, storage and disposal of GM material.

(iii) Transfer of antisense *ACO2* and marker gene sequences to wild type (non-GM) Papaya.

Hermaphrodite papaya plants are predominantly self-pollinating. However, pollen from hermaphrodite papaya plants can pollinate nearby female flowers via insect pollinators and result in transfer of transgenes to wild type *C. papaya*. There is possible ingression of transgenes into wild population. This will result in severe consequences if expression of transgenes can result in toxicity/allergenicity and /or weediness. The risk is low but requires risk management, and is contingent upon secure containment of plant material and pollen in the nethouse. Bagging must be practiced by applicant to contain pollen. All

bags should be autoclaved and discarded according to procedures for GM materials. The Institutional Biosafety Committee (IBC of MARDI) should ensure that the nethouse is inspected regularly to maintain the integrity. There must be adherence to the SOPs by personnel handling, transporting and disposing material containing GM pollen. There should be no wild type papaya present in the vicinity.

(iv) Transfer of heterologous genes to other related species

Papaya can hybridise with closely related species. Intergeneric and interspecific crosses have been successfully produced by artificial pollination. However, there are no closely related species of papaya in Malaysia.

(v) Unintentional or illegal release of GM Papaya into the environment by workers.

Unintentional or illegal removal of viable GM material can happen through negligence or ignorance of workers handling, maintaining or disposing GM plants. There must be a recording system for storage, archiving and destruction of all GM material including seeds, fruits and mature plants. Access to the facility should be restricted to authorised personnel.

(vi) Release of GM Papaya into the environment by animals

Removal of viable GM material by animals such as rats will result in release of GM papaya into the environment. To mitigate this risk, the IBC of MARDI should ensure that the nethouse is inspected regularly to maintain the integrity and pest control measures are in place.

IV - Proposed Terms and Conditions for Certificate of Approval

Based on the 19 potential hazards identified and assessed, GMAC has drawn up the following terms and conditions to be included in the certificate of approval for the confined field trial:

(i) The IBC of MARDI shall ensure that the nethouse is inspected regularly to maintain the integrity of the nethouse and SOPs are complied with.

(ii) The National Biosafety Board shall be informed immediately if there is any containment breach or security breach in the confined field trial.

(iii) No parts of the GM papaya plants from this confined field trial shall be consumed or utilised for any other purpose other than this trial.

(iv) There shall be no planting of papaya plants within 1 km buffer zone from the nethouse, and all existing papaya plants in this zone shall be removed.

(v) Should the approved person receive any scientifically proven information that confirms any adverse effect of transgenic Eksotika Papaya, the National Biosafety Board shall be informed.

V - Other Regulatory Considerations

There are no other regulatory considerations.

VI - Identification of Issues to be Addressed for Future Releases

One additional issue has been identified that would be important during the assessment of an application for a larger scale or commercial release of transgenic Eksotika papaya, which is:

(i) Since there is no data on toxicity and allergenicity to human and animal, it is recommended that studies addressing these issues should be initiated.

VII – Conclusion and Recommendation

GMAC has conducted a thorough evaluation of the application for approval entitled 'Confined Field Evaluation of Delayed Ripening Transgenic Eksotika Papaya' and has determined that the confined field trial does not endanger biological diversity or human, animal and plant health. GMAC recommends that the proposed application for confined field evaluation be **APPROVED WITH TERMS AND CONDITIONS** as listed in section IV (Proposed Terms and Conditions for Certificate of Approval). GMAC also recommends that Section VI (Identification of Issues to be addressed for future releases) be forwarded to the applicant for further reference.

VIII - Bibliography

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Appendix I

GENETIC MODIFICATION ADVISORY COMMITTEE (GMAC) MEMBERS INVOLVED IN SPECIFIC RISK ASSESSMENT AREAS FOR THE APPROVAL FOR CONFINED FIELD EVALUATION OF DELAYED RIPENING TRANSGENIC EKSOTIKA PAPAYA

Genetic Modification Advisory Committee (GMAC) members divided the task of looking up more information for the Risk Assessment matrix based on three broad categories. The scope of research aspects for each group is as listed below. Each sub-committee had a nominated leader to coordinate the work and report back to the main GMAC. The respective leader contacted the sub-committee members and discussed the work process with their members. The groupings of GMAC sub-committee members and their assigned tasks are as below:

1. ENVIRONMENT

Issues pertaining to unintentional release, weediness, gene transfer to bacteria, accumulation of toxin, cross pollination and toxic effects on non-target organisms were examined.

- **Assoc. Prof. Dr. Mohd. Faiz Foong bin Abdullah (Universiti Teknologi MARA) (Leader)**
- Dr. Sim Soon Liang (Sarawak Biodiversity Centre)
- Dr. Martin Abraham (Malaysian Society of Marine Sciences)
- Madam Atikah binti Abdul Kadir Jailani (Department of Agriculture)
- Madam Jasbeer Kaur (Department of Chemistry)
- Dr. Tan Swee Lian (Academy of Science Malaysia)
- Dr. Mohamed Mohd Salleh (Former researcher at Malaysian Agricultural Research & Development Institute)

2. HUMAN HEALTH

Issues pertaining to toxicity of the expressed proteins and potential allergenicity were examined

- **Madam T.S. Saraswathy (Institute of Medical Research)(Leader)**
- Dr. Norwati Muhammad (Forest Research Institute Malaysia)
- Dr. Rahizzan Issa (Institute of Medical Research)
- Mr. Jamal Khair b Hashim (Ministry of Health)

3. ANIMAL HEALTH

Issues pertaining to allergenicity, toxicity, anti-nutritional, compromised nutritional content, effect on performance and survivability, horizontal gene transfer of marker genes to soil microorganisms were examined.

- **Prof. Dr Jothi Malar Panandam (Universiti Putra Malaysia) (Leader)**
- Dr. Ahmad Parveez bin Hj Ghulam Kadir (Malaysian Palm Oil Board)
- Prof. Dr. Helen Nair (Academy of Science Malaysia)
- Dr. Kodi Isparan Kandasamy (Malaysian Biotechnology Corporation Sdn. Bhd.)
- Dr. Fuzina Nor Hussein (Universiti Putra Malaysia)