RISK ASSESSMENT REPORT

OF THE GENETIC MODIFICATION ADVISORY COMMITTEE (GMAC)

FOR

AN APPLICATION FOR APPROVAL FOR CONFINED FIELD EVALUATION OF TRANSGENIC EKSOTIKA PAPAYA AGAINST PAPAYA DIEBACK DISEASE

NBB REF NO: JBK(S) 600-2/1/6

APPLICANT: MALAYSIAN AGRICULTURAL RESEARCH AND DEVELOPMENT INSTITUTE (MARDI)

DATE: 1 AUGUST 2019

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 23 July 2019 for an application for approval entitled 'Confined Field Evaluation of Transgenic Eksotika Papaya against papaya dieback disease'. The application was filed by Malaysian Agricultural Research and Development Institute/MARDI (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 additional information and clarifications on certain details of the proposed field trial.

A public consultation for this application was conducted from 25 June 2019 to 24 July 2019 via advertisement in local newspapers. Comments were received from Third World Network (TWN) regarding the integrity of the nethouse structure, risk of gene flow, mechanism for conferring bacterial dieback resistance and risk of using marker gene *npt*II and *AHL lactonase* 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 one meeting 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.

II - Background of Application

This application is for approval to conduct a confined field evaluation of transgenic Eksotika papaya against papaya dieback disease. The purpose of the confined field trial is to evaluate T0 transgenic Malaysian Eksotika papaya variety with enhanced resistance to papaya dieback disease (PDD) under confined environment and to collect the transgenic T1 seeds for subsequent field trial. PDD is an important disease caused by a gram negative bacterium, *Erwinia mallotivora (E. mallotivora)* and has adversely affected Malaysian papaya industry through major production and economical losses.

To date, there are no effective strategies to control the disease besides proper management practice. To save our local papaya industry, especially Eksotika which has great commercial value, a versatile and effective strategy to combat this disease is proposed via genetic engineering approach. Strategically, a molecular technique to disrupt the quorum-sensing signalling of *E. mallotivora* has been identified and theoretically, it is possible to prevent the infection and survival of *E. mallotivora* in the papaya plant. An enzyme, *Acyl-homoserine lactonase* (*AHL-lactonase*), is capable to inactivate an acyl-homoserine lactones (AHLs) molecule that is crucial for bacterial quorum sensing. The inactivation mechanism is executed by hydrolysing the lactone bond of AHLs molecule. Thus, transgenic papaya plants expressing *AHL-lactonase* are capable of quenching the pathogen quorum-sensing signalling and may enhance the defence against dieback disease.

In previous project, two potential genes against papaya dieback were successfully isolated and characterised. These potential genes are *Acyl-homoserine lactonase* CHB37 and *Acyl-homoserine lactonase* SP24, which antimicrobial activities have been validated *in vitro* and transformed into embryogenic callus of Eksotika papaya using *Agrobacterium*-mediated transformation method. After challenged with *E. mallotivora*, 18 potential transgenic papaya plants which showed enhanced resistance against papaya dieback disease were successfully obtained. The evaluation was conducted in contained environment under

Transgenic Glasshouse condition. In this study a total of 54 transgenic papaya plants and 6 seedling control plants will be planted in Nethouse 3, MARDI.

The fruits from transgenic plants which show resistance against papaya dieback diseases characteristic will be harvested. 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 labelled and kept in dedicated fridge at Molecular Analysis Laboratory at Transgenic Glasshouse Complex, Biotechnology and Nanotechnology Research Centre, MARDI. The seeds from non-potential fruits are also collected and will be incinerated.

The trial site will be subject to post-harvest land use restriction for a period of six months. The post-harvest period begins immediately after the trial is terminated. During this period, all prohibited plants including volunteers of the regulated transgenic papaya plants will be removed from the trial site before flowering or anthesis and rendered non-viable.

Information about genetically modified Eksotika papaya

The parent organism is *Carica papaya L*. var. Eksotika which is exotic to Malaysia. It is widely grown as commercial cultivar in Malaysia. Global production of papaya rose gradually and achieved over 13 million metric tons in 2016 (FAOSTAT, 2017). It has extended history of safe use for human consumption.

The Eksotika papaya has been modified to show a dieback-resistant characteristic by manipulating bacterial quorum sensing mechanism. Quorum sensing is a communication process between bacteria, whereby the bacteria monitor their population cell density. During the quorum sensing process, small signal molecules known as auto inducers will be produced and secreted into the extracellular environment and its concentration will increase in proportion to bacterial cell growth (Miller et al. 2001). At low concentration of this signal molecule, it would not give negative effect to the plant. But once the signal molecules exceed threshold concentration it will activate the virulence genes and contribute to the increase in bacterial growth or populations that subsequently cause the disease to the papaya plant. Hence, by disrupting the essential communication mechanism, or also known as quorum quenching, the signal molecules will be degraded thus eliminating bacterial virulence and reduce the infection of the papaya plants and subsequently delays or inhibits the development of dieback disease. An enzyme, *Acyl-homoserine lactonase* (*AHL-lactonase*), is able to inactivate an acyl-homoserine lactones (AHLs) molecule that is crucial for bacterial quorum sensing. Therefore these genes were used in the transformation study. The *AHL-lactonase* genes from *Rhizosphere* soil bacteria were inserted by using sense technology to over express the production of AHL-lactonase enzyme in the transgenic papaya plant. By over expressing AHL-lactonase the transgenic papaya plants are capable of quenching the pathogen quorum-sensing signalling and may enhance the defence against dieback disease.

Safety of the expressed protein

According to the applicant, *AHL-lactonase* CHB37 and *AHL-lactonase* SP24 were isolated from *Rhizosphere* soil bacteria (*Bacillus cereus* and *Bacillus thuringiensis*) and these genes are well known and commonly used for transformation study. There are a few success studies done on transformation of *AHL-lactonase* from *Bacillus* species into crop plants such as tobacco, potato and elephant yam.

AHL-lactonase is an enzyme capable of inactivating the AHL molecules that is crucial for bacterial communication (quorum sensing). The hydrolysis of the lactone bond of AHL

molecules by AHL-lactonase protein would inactivating the communication mechanism known as quorum quenching (Dong et al., 2001). It is postulated that transgenic papaya plants expressing *Ahl-lactonase* are capable of quenching the pathogen quorum-sensing signalling, hence enhancing their defense against dieback disease.

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 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 1000 metres from other untransformed papaya plants. The field trial location is at a safe distance from drinking water supply zone. The nearest water supply source is at Semenyih Dam, 40 km from MARDI. A total of 54 transgenic papaya plants will be planted in nethouse and 6 seedling control plants will also be planted. Dead plants or those with stunted growth will be replaced from the laboratory stock. In addition, no related plants have been identified in the release site.

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).

(ii) 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 meeting. 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 was 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 *AHL-lactonase* gene sequences perturbs gene regulation and/or metabolic pathways in GM Papaya

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

(ii) Expression of *AHL-lactonase* gene sequences leads to changes in metabolic pathways in transgenic Papaya, resulting in enhanced fitness

Pleiotropic effects may be presented, resulting in unpredictable phenotypes. The inserted genes is being involved in enhancing the defence against dieback disease. 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 transgenic material.

(iii) Transfer of *AHL-lactonase* gene and marker gene sequences to wild type (non-transgenic) 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 transgenic 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 transgenic 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 transgenic Papaya into the environment by workers.

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

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

Removal of viable transgenic material by animals such as rats will result in release of transgenic 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:

Part A: Actions to be taken and reported to the National Biosafety Board prior to the start of the field trial

(i) A consent letter to conduct the confined field trial from the Local Council for the district where the site is located shall be provided.
(ii) Proper signage shall be present at the trial site informing of the presence of genetically modified papaya as according to the Confined Field Trial Guidelines. Access to the confined field trial site shall be limited to authorised personnel only.
(iii) Appropriate training shall be given to all personnel who will be handling the genetically modified papaya.
(iv) Pest and animal control measures shall be in place at the confined field trial site.
(v) The owner of the plot of land on which the confined field trial site is situated (MARDI or other parties) must consent, in writing, to a post-trial land use restriction period of 6 months.
(vi) An Emergency Response Plan shall be prepared and approved by GMAC to handle possible incident of breach in containment.

Part B: Actions to be taken and reported to the National Biosafety Board during the field trial

(i) The approved Standard Operating Procedures (SOPs) for transportation of all GM papaya and materials from the greenhouse to the confined field trial site shall be adhered to. Records shall be kept for all genetically modified papaya transported to the confined field trial site.
(ii) An isolation zone shall be established, whereby the confined field trial site must be separated by a distance of at least 400 metres from other papaya plants on all sides.
(iii) Regular inspections shall be carried out to ensure that there are no volunteers (papaya and wild relatives) in the vicinity of the isolation zone. Any volunteers found shall be collected and destroyed. A record of this inspection exercise and of the numbers of volunteers destroyed shall be maintained.
(iv) If a breach of the isolation zone should occur, the National Biosafety Board shall be informed immediately and random sampling and testing shall be conducted to ensure no outcrossing of genetically modified papaya.
(v) The Emergency Response Plan approved by GMAC must be implemented to handle any incident of breach in containment.
(vi) Records of all seeds and other plant materials that are removed from the trial site for storage or analysis off-site shall be kept. The SOPs for transporting such materials shall be strictly adhered to.
(vii) Bioinformatics data analysis on potential toxicity and allergenicity of the expressed proteins shall be submitted to the National Biosafety Board.
(viii) Appropriate and continuous training shall be provided to personnel who will be handling the genetically modified papaya plants.
(ix) Pest and animal control measures shall be implemented. Regular inspection of the net house shall be carried out to ensure its integrity at all times.
(x) The Biosafety related approved SOPs that have been approved under this application shall be strictly adhered to and personal protection equipment shall be used to avoid exposure.

(xi) No changes shall be made to the Biosafety related SOPs that have been approved under this application. Any changes proposed shall be submitted to and approved by GMAC.
(xii) Additional conditions may be imposed based on monitoring visit by Department of Biosafety and these conditions shall be complied with.
(xiii) Should the approved person receive any scientifically proven information that confirms any adverse effect of transgenic papaya, the National Biosafety Board shall be informed.

Part C: Actions to be taken and reported to the National Biosafety Board at termination of the field trial

(i) No parts of the genetically modified papaya plants from this confined field trial shall be consumed or utilised for any other purpose other than this trial.
(ii) At the termination of the field trial, all residual plant materials in the confined field trial site shall be rendered non-viable using methods approved by GMAC. The confined field trial site and the isolation zone are subjected to post-trial land use restrictions for a period of 6 months.
(iii) During this 2-year period, the confined field trial site and the isolation zone shall not be planted with any plants without prior approval from the National Biosafety Board. The confined field trial site and isolation zone shall be continuously monitored for growth of papaya volunteers, which shall be collected and destroyed. Proper records of these post-trial activities shall be maintained and the report submitted to the National Biosafety Board upon the expiry of the post-trial period.

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 Transgenic Eksotika Papaya against papaya dieback disease' 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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2. http://www.allallergy.net/fapaidfind.cfm?cdeoc=1124 assessed on December 2012.

3. Office of the Gene Technology Regulator. Executive Summary.Risk Assessment and Risk Management Plan for Application No. DIR 026/2002. (*Limited and controlled release of GM papaya*) (2002).

4. Office of the Gene Technology Regulator. Risk assessment and risk management plan DIR 026/2002. Field trial For Evaluation of GM Papaya to Delay Fruit Ripening and Test the Expression of the Introduced Genes (2002).

5. Office of the Gene Technology Regulator. The Biology and Ecology of Papaya (paw paw), *Carica papaya* L (2003).

6. Office of the Gene Technology Regulator. Risk Assessment and Risk Management Plan. Application for license for dealings involving an intentional release into the environment. DIR 026/2002. Field trial for evaluation of GM papaya to delay fruit ripening and test the expression of the introduced genes. Applicant: The University of Queensland. June (2003).

Appendix I

GENETIC MODIFICATION ADVISORY COMMITTEE (GMAC) MEMBERS INVOLVED IN SPECIFIC RISK ASSESSMENT AREAS FOR THE APPLICATION OF APPROVAL FOR RELEASE OF GENETICALLY MODIFIED EKSOTIKA PAPAYA FOR CONFINED FIELD TRIAL FOR RESEARCH AND DEVELOPMENT PURPOSE

Genetic Modification Advisory Committee (GMAC) members divided the task of looking up more information for the Risk Assessment matrix based on three broad categories which were environment, human health and animal health. Each sub-committee had a nominated leader to coordinate the work and report back to the main GMAC. The GMAC members involved in the risk assessment are as below:

- **Prof. Dr. Mohd. Faiz Foong bin Abdullah (Universiti Teknologi MARA) (GMAC Chairman)**
- **Dr. Kodi Isparan Kandasamy (Industry Representative) (Environment sub-committee Leader)**
- **Madam T.S. Saraswathy (Institute of Medical Research - retired) (Human Health sub-committee Leader)**
- **Prof. Dr Jothi Malar Panandam (Universiti Putra Malaysia - retired) (Animal Health sub-committee Leader)**
- **Dr. Rahizan Issa (Institute of Medical Research) (Notification Assessment sub-committee Leader)**
- Dato' Dr. Sim Soon Liang (Sarawak Biodiversity Centre)
- Prof. Dr. Abd Rahman Milan (Universiti Malaysia Sabah)
- Assoc. Prof. Dr. Chan Kok Gan (Universiti Malaya)
- Assoc. Prof. Dr. Choong Chee Yen (Universiti Kebangsaan Malaysia)
- Assoc. Prof. Sharifah Syed Hassan (Monash University Malaysia)
- Dr. Adiratna Mat Ripen (Institute of Medical Research)
- Dr. Norliza Tendot Abu Bakar (Malaysian Agricultural Research & Development Institute)
- Dr. Norwati Muhammad (Forest Research Institute of Malaysia)
- Dr. Saifullizam bin Abdul Kadir (Department of Veterinary Services)
- Dr. Teo Tze Min (Entomological Society of Malaysia)
- Madam Atikah binti Abdul Kadir Jailani (Department of Agriculture - retired)
- Madam Norizan Jaafar (Department of Chemistry Malaysia)
- Madam Shafini Abu Bakar (Ministry of Health)