

FACT SHEET

APPLICATION FOR APPROVAL FOR CONFINED FIELD EVALUATION OF TRANSGENIC MR219 RICE TOWARDS *Xanthomonas oryzae* pv. *oryzae* (Xoo) BACTERIAL AND *Pyricularia* spp. FUNGAL DISEASE AND GLYPHOSATE HERBICIDE RESISTANCE

NBB REF NO: JBK (S) 600-2/1/5

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 rice. The title of the project is “Application for field evaluation of transgenic rice: Genetic engineering of Malaysian rice cultivar towards *Xanthomonas oryzae* pv. *oryzae* (Xoo) bacterial and *Pyricularia* spp. fungal disease and glyphosate herbicide resistance using transgene stacking”.

2. What is the purpose of the confined trial?

The purpose of the confined field trial is to evaluate the transgenic rice performances (agronomic traits, glyphosate resistance, transgene flow, transgene stability) under confined field trial conditions. Field evaluation is needed to confirm the transgenic rice lines incorporated with the anticipated glyphosate resistance characteristic and to collect biosafety data for subsequent open field trial.

3. How has the MR219 rice been genetically modified?

The MR219 rice has been genetically modified to provide resistance to glyphosate and rice disease causing bacteria *Xanthomonas oryzae* pv. *oryzae* (Xoo) and fungi *Pyricularia* spp. through the insertion of *CP4-EPSPS* gene and *WRKY45* gene. Insertion of the genes into the MR219 rice was done through *Agrobacterium*-mediated transformation method. A selectable marker gene, *hptII* that confers resistance to hygromycin was also inserted into transgenic MR219 rice to facilitate screening and identification of the transformants during the growth of the transgenic plants.

4. Characteristics of transgenic MR219 rice

a) Details of the parent organism

The parent organism is *Oryza sativa L. indica* variety MR219, also known as MR219 rice, which is a Malaysian variety. The natural habitat of rice is tropical marshes but is now cultivated in a wide range of subtropical and tropical habitats. It is widely grown as a commercial cultivar in Malaysia. Productivity in rice production has reached 3.5 million tonnes in 2016. The MR219 reproduces sexually, mostly through self-pollination though inter-pollination with other rice varieties is possible.

b) Details of the donor organism

Agrobacterium tumefaciens strain CP4 is the source of the *CP4-EPSPS* gene. It is a gram-negative soil bacterium that causes crown gall disease in over 140 species of eudicots.

Oryza sativa spp. indica (rice) is the source of the *WRKY45* gene.

Escherichia coli is the source of the *hptII* 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 *CP4-EPSPS* gene introduced into the transgenic MR219 rice provides glyphosate resistance characteristics. The rice *WRKY45* gene provides *Xanthomonas oryzae* pv. *oryzae* (Xoo) bacterial disease tolerant and *Pyricularia* spp. fungal disease tolerant characteristics. The genes for bacterial and fungal disease tolerant (*WRKY45*) used in this genetic modification will not affect the phenotypes of the LMO. The glyphosate resistant gene (*CP4-EPSPS*) might increase the plant growth and yield of the LMO.

d) Safety of the expressed protein

The *WRKY45* gene was synthesized based on the sequence from japonica rice cultivar, which is widely grown as a commercial cultivar for human consumption in Japan and China. This gene is present in salicylic acid signalling pathway and found in all rice plants. Hence, the transgenic MR219 rice to be evaluated in this confined field trial expresses the same protein as in the non-genetically modified rice and can be considered safe for human consumption and the environment.

The *CP4-EPSPS* gene is synthesized based on the original gene sequence found in *Agrobacterium tumefaciens* CP4, with a predicted very low potential hazard. The *CP4-EPSPS* is involved in shikimate pathway which is present in bacteria, plant and fungi but absent in animal. Hence the CP4 EPSPS gene is predicted to have very low potential hazard (Coggins et al., 2003).

5. Assessment of risks to human health

At this stage of the evaluation process for the transgenic rice plants proposed for confined trial, the acute toxicological data is not available. This data will be obtained through this proposed confined field evaluation. No toxic or allergenic effect have been reported for recombinant *CP4-EPSPS* and *WRKY45* genes.

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 97.2 x 83.8 m x 3 m.

The entire experimental field trial area will be confined with 2 layers of metal net separated 1 m apart (the mesh size of netting is No. 38) and each metal net will be coated with two layers of nylon net (mesh size No. 32) of 3 meters height and two heavy duty metal doors with 24 hours security. The height of 3 meters is used to prevent any animal or human enter the confined field trial building. The use of netting with mesh size of No. 32 is to trap the pollen and prevent its release to the environment. The roof of the confined field trial house will be covered with 2 layers of metal net (No. 38) and 2 layers of nylon netting with net mesh size of No. 32. The confined field trial will be carried out at MARDI, Kampung Tambun Tualang, Arau, Perlis.

b) Biological containment

The cultivated rice (*Oryza sativa* L.) originated in Asia. There are several *Oryza sativa* cultivars that can hybridise with *Oryza sativa* cv. MR219. However risk of cross-pollination is low since the trial plot is separated at least 80 m from any nearby parent organism or closely related relative. No wild relatives of the transgenic rice are planted nearby the confined field trial site. The confined field trial site will be sprayed with herbicides other than glyphosate before and after the trial period to kill off all possible plant and seed (not limited to rice plant and seed). All 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 rice will be removed and destroyed.

c) Handling and storage

The seeds will remain within the seed husks. The seeds will be collected, dried and kept in a sealed envelope as primary container and then placed in a sealed, leak-proof and water resistant secondary container for next planting. 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 second field trial analysis to confirm the trait. The seeds from non-potential line will not be collected and will be destroyed using autoclave. The harvested seeds will be transported from the trial site to the MARDI HQ in a durable bag as primary container and placed in a sealed, leak-proof and water resistant secondary container.

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 16 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.

*** UPDATE:** The consultation period for this application has been extended until **4 August 2019** due to the public interest received regarding this application. Written submissions are required before or on that date. Please refer to <http://www.biosafety.gov.my/en-my/public-consultation/Pages/About-Public-Consultation.aspx> for guidance to provide your inputs.