CONSOLIDATED REPORT OF GR2E RICE

APPLICATION FOR FIELD TRIAL

EXECUTIVE SUMMARY

On February 28, 2017, Philippine Rice Research Institute (PhilRice) submitted Golden Rice GR2E application for field trial to the Bureau of Plant Industry (BPI) under the DOST-DA-DENR-DOH-DILG Joint Department Circular (JDC) No. 1 Series of 2016. After reviewing and evaluating the accomplished risk assessment forms and technical dossier submitted by the proponent, the Scientific and Technical Review Panel (STRP) recommended the grant of a biosafety permit to conduct field trial.

The Department of Environment and Natural Resources – Biosafety Committee (DENR-BC), after a thorough scientific review and evaluation of documents and forms related to Environmental Risk, considered GR2E application for field trial safe to the environment and biodiversity. Likewise, in the assessment of Environmental Health Risk, the Department of Health (DOH) – BC found that the application for field trial of the regulated article is acceptable subject to monitoring based on compliance with the Environmental Health Impact Assessment (EHIA). Furthermore, the Socio-economic, Ethical and Cultural (SEC) expert, after evaluating the SEC considerations, also recommended for the issuance of biosafety permit for field trial.

BACKGROUND

In accordance with Article V. Section 10 of the JDC, no regulated article shall be released into the environment for field trial unless a Biosafety Permit for Field Trial has been secured. Only regulated articles that satisfactorily passed the process of contained use or confined test supervised and officially endorsed by DOST-BC may be subject of application for a Biosafety Permit for Field Trial.

The BPI Biotech Office provided the assessors the complete dossier submitted by PhilRice. Upon receipt of the individual risk assessment reports from the assessors, the said office prepared this consolidated risk assessment report for the information of the public.

FIELD TRIAL RISK ASSESSMENT

The objectives of the field trial proposed by PhilRice are the following:

1) to generate relevant agronomic and phenotypic data from GR2E PSB Rc82 lines and PSB Rc82 recurrent parent under Philippine field condition to complete the environmental risk assessment of GR2E rice; and
2) to confirm expression levels of β-carotene in samples of milled GR2E rice grains produced from the field trial.

The intended eventual use of GR2E rice, as reported by the proponent, is to produce rice grain for human consumption and food manufacture, and rice by products for use in livestock animal feeding. GR2E rice with elevated provitamin A is intended as a complementary approach to combating vitamin A deficiency (VAD) in the Philippines.

History of Safe Use

The species to be released is rice (*Oryza sativa* L.). The lines to be released consist of variety PSB Rc82 containing event GR2E and the control which is near-isogenic, non-transgenic PSB Rc82 rice.

Rice has a long history of safe use as food and has not been found to be allergenic nor a source of toxicants. It has been grown extensively worldwide and about half of the world's population consume rice. The transformed rice GR2E is intended to increase the production of provitamin A in the rice endosperm and, as confirmed by the assessors, it is unlikely to cause any potential human, animal or plant disease.

Characteristics of the Host Plant

Rice is grown all throughout the Philippines, particularly, in irrigated lowland and rain-fed shallow, deep-water, upland areas and tidal wetland, as classified by OECD (1999). STRP affirmed that rice is distributed worldwide and how it is cultivated by populations dependent on rice has defined the habitat and ecology of the plant and other agronomic characteristics.

Oryza sativa is basically an autogamous plant and propagated through seeds produced by self- pollination. After the spikelet opens at flowering in rice, the pollen is dispersed and germinates on the surface of the stigma. Only one pollen tube reaches an ovule to initiate double fertilization. The STRP added that pollen can maintain germinability only for several minutes after being shed from the anther under favorable temperature and moisture conditions, while the ovules keep their viability within three (3) to five (5) minutes according to OECD (1999).

The STRP confirmed that the cultivated rice varieties generally do not have weediness characteristics (Basu et al., 2004). The STRP also noted that rice tends to become weedy in areas where wild and cultivated rice plants grow sympatrically. In these areas, wild and cultivated rice plants can hybridize, producing plants that compete with the cultivars and produce inferior seed, thus decreasing the yield from the rice crop. However, weedy rice can also develop in areas without native wild rice populations.

In the case of *O. sativa*, the weeds are known as red rice due to the coloured pericarp associated with these plants. The STRP concurred that red rice is viewed as a major economic problem when it occurs in rice fields as it causes losses in yield through competition with the cultivars as well as decreasing the value of the harvested grain through its colour. Other rice species growing in and around rice fields are known as weedy rice and can also produce red seeds.

The STRP agreed that characteristics of weedy rice contributing to its potential weediness include similar growth attributes with cultivars due to common progenitors, high seed shedding rate, dormancy and persistence, adaptation to different habitats and relatively higher outcrossing ability. In view of the above, populations of weedy/red rice tend to be genetically diverse and highly heterogeneous and often have intermediate characteristics between wild and cultivated characteristics.

Characterization and safety assessment of the GM product

GR2E was made through an *Agrobacterium*-mediated transformation. The STRP agreed that the genes integrated into *O. sativa* to express provitamin A were phytoene synthase from *Zea mays* and carotene dasturase I from *Pantoea ananatis*. A phosphomannose isomerase gene from *Escherichia coli* was also integrated as a marker. The genetic

modification involved the chromosomal rice genome and the transformed genome has a transfer DNA plasmid called pSYN12424. This resulted into a functional biosynthetic pathway capable of producing beta-carotene intended to increase the production of provitamin A in the rice endosperm.

The PCR assay can differentiate the GM crop from other crops. In the open environment, the color of the polished grains can be differentiated phenotypically because it is golden or yellow in color compared to conventional rice which is white (milled rice).

The STRP confirmed that Southern blot hybridization done by Cueto et al. 2016 provided proof of lack of integration of any plasmid backbone sequences, thus, vector backbone sequences are not present in the final genetically engineered GR2E rice. There was a single copy of the pSYN12424 T-DNA which was inserted at a single site. Also, the STRP added that data showed that at 3 backcrosses, the samples from the original germplasms were stable across multiple generations, following the Mendelian patterns of inheritance. Compositional patterns other than the expression of beta-carotene were comparable with the untransformed counterpart. Further, proof of stability of the inserted DNA is noted in Cueto et al. 2016 and Swamy and Samia, 2015. Taken together, the STRP concurred that the evidences indicate that secondary genetic effects would be highly unlikely.

On the level of expression of the transferred gene, concentrations of ZmPSY1 and CRTI were found only in developmental stages of the plant and not in the straw. On the other hand, the PMI protein was documented to be present in the straw. PMI is safe and exist in some plants such as soy. The level of expression is regulated by the use of the endosperm-specific rice glutelin promoter (GluA-2) for the patterns of expression of the ZmPSY1 and CRTI, while the constitutive maize ubiquitin promoter was used for the expression of the PMI protein. Thus, the STRP affirmed that the genetic change in the accumulation of provitamin A in the GR2E rice endosperm is not expected to affect or alter the reproductive biology of the crop.

Proposed Field Trial Site

The primary field trial site for GR2E rice is located in PhilRice Central Experimental Station (CES), Barangay Maligaya, Science City of Munoz, Nueva Ecija while its back-up site is

located in PhilRice Isabela Research Station, Barangay Malasin, San Mateo, Isabela. The field trial will be conducted in one season.

The assessors reported that the proponent has clearly described the physical location and geographical features of the proposed trial sites, including size, soil type, climatic type, distance to bodies of water, distance to populated areas, distance to center of agricultural activity, whether near protected areas or habitat of endangered species. The assessors also reported that a map of the field trial sites was provided by the proponent.

The STRP noted that the sites were representative of the receiving environment for GR2E rice for collecting relevant agronomic and phenotypic data to complete the environmental risk assessment. It was also noted that the field trial location are under the care and control of PhilRice which will facilitate compliant management of the proposed activities.

According to the STRP, determination of soil characteristics and the history of long-used of the sites for rice field experiments are given considerations when selecting field trial sites. The proponent reported that both trial sites have been used in previous Golden Rice field trials since 2012. The STRP affirmed that soil fertility analysis is regularly done in both sites and the results are within the acceptable range and for favorable rice cultivation.

In addition, the assessors reported that the site(s) is/are not prone to flooding and not close to existing populated areas, centers of agricultural activity, protected areas, or habitat of endangered species.

On the other hand, the proponent reported that the plants will be grown up to its physiological maturity or up to harvest stage. If the plant will be grown up to the seed stage, the seeds (10 mm in length) is possible to be dispersed through seed shatter. Dormancy will be influenced by ecotype and is reinforced by rainy weather. Furthermore, animals can also assist in seed dispersal. To mitigate the potential for any seed dispersal via foraging rodents or other animals, the proponent will erect an animal barrier surrounding the trial. In addition, to prevent the possibility of any seed dispersal via water movement, water will be retained within the testing site using a levee that will surround the test plots and the overall test site. Since cultivated rice dormancy is generally quite short, volunteers can be identified and destroyed during the period of post-harvest monitoring.

Furthermore, the proponent stated that the presence of vectors or agents of dispersal or dissemination will be controlled since access to the trial site will be restricted. The assessors strongly suggest that the proponent shall ensure that field trial sites will only be accessible to authorized personnel, rat fence shall completely surround the trial site, equipment and utensils to be used shall be completely cleaned on-site, ensure presence of bird-boys to shoo-away birds, and presence of levee to contain water in the trial sites. After the trial or release is completed, the assessors also recommended that no plant or crop shall be planted in the trial site for 30 days. Volunteer plants shall also be monitored. All movement of the materials to/from the trial site location shall be in closed packaging/containers by approved trained staff under the supervision of DA-BPI staff.

Recommendation (STRP)

After a thorough and comprehensive risk assessment, the STRPs found that the mitigating measures to prevent unintended dispersal of seeds/plants are adequate, measures to prevent unintended pollen flow are adequate, experimental design and data to be gathered and analyzed are expected to attain the objectives of the field trial, field trial sites are adequately protected from unwarranted interventions of animals, and safety protocols during the conduct of the field trials are adequate and well-placed. Considering all of these, the STRPs recommended for the grant of biosafety permit for GR2E rice application for field trial.

<u>DENR-BC (for evaluation of Environmental Risk)</u>

The DENR-BC had a comprehensive review and evaluation of the submitted Project Description Report (PDR), and Site Inspection Report with geo-tagged photos of the field trial sites of the Beta Carotene-Enriched Golden Rice. The assessment of the DENR BC focused on the potential environmental impact of GR2E field trial application.

The following were the observations and recommendation of the DENR-BC:

1. The field trial of the regulated article will not cause any significant adverse effect on the environment (land and water) and biodiversity. The transgenic crop will not increase its weediness potential because the genetic modification resulting in the event GR2E is not intended to alter the reproductive biology of rice, with its seed germination rate, and pollen-mediated gene flow;
2. The novel proteins (ZmPSY1, CRT1, and PMI) that is expressed in the GR2E grains do not display significant amino acid sequence similarity with known allergens nor were there any primary sequence structural alerts for potential toxicity based on similar searches against existing databases of known and putative protein toxins. The beta-carotene that is expressed in GR2E is metabolized into vitamin A as needed by the organism and excess amounts resulting from the consumption of beta-carotene rich foods are easily flushed out of the body in the feces, urine and sweat. Hence, it is very unlikely that the accidental consumption of GR2E grains by wildlife species including birds, insects and other invertebrates could pose unintended adverse effects;
3. The GR2E has a very low chance of interbreeding or genetic exchange (0.08-0.92%) with its conventional counterpart or with other species found at or near the trial sites of planned release because rice is self-pollinating and its pollen is only viable for 3-5 minutes. Also, if any cross pollination were to occur between GR2E rice and sexually compatible plants, there would be no fitness advantage conferred to progeny plants that would increase their ability to persist, survive, or spread in the environment; and
4. The potential impacts to the environment during the site preparation, planting, data gathering, harvesting, and monitoring could be handled, as indicated in the material management plan as part of the Environmental Management Plan in the Project Description Report.
5. Based on the review and evaluation, the DENR-BC considered GR2E application for field trial safe to the environment and biodiversity. In the Standard Operating Procedure (SOP) of the Management of Confined Field Trials of Regulated Genetically Engineered Rice, incineration should not be an option, thus, only boiling and burying of plant material should be done. The Bureau of Plant Industry (BPI) shall ensure that the reproductive isolation will be maintained around the trial site and all the proper procedures, processes, and safeguards during the field trial are being observed by the proponent.

<u>DOH-BC (for evaluation of Environmental Health Risk)</u>

The DOH-BC assessed the potential environmental health impact of the GR2E field trial application through the proponent's submitted Environmental Health Risk Assessment

form, technical dossier, submitted baseline health information of the community and the workers, as well as the proposed monitoring plan for continuous monitoring and mitigation during pre and post-harvest activities.

After thorough review and evaluation of these documents, the DOH-BC found that the application for field trial of the regulated article is acceptable subject to monitoring based on compliance with the Environmental Health Impact Assessment (EHIA).

SEC Expert (for evaluation of SEC concerns)

The SEC expert evaluated the GR2E application based on the SEC questionnaire answered by the proponent. The SEC expert had concerns on the productivity of the said regulated article. One of the concern is if the GR2E product will exhibit increase in productivity. Productivity means the amount of beta-carotene produced by GR2E as a productivity factor compared to its conventional counterpart since the concern is not the comparison of ordinary yield per se. The proponent responded to the issues raised by the expert that GR2E rice is being developed as a humanitarian intervention to help address the serious public health problem posed by vitamin A deficiency in the Philippines. Also, there is no anticipation of any price premium for GR2E in the marketplace, which would be counterproductive to ensuring availability to the consumers with the greatest need. Thus, the per se production of beta-carotene in the endosperm tissue of GR2E cannot be considered a productivity factor as it confers no capturable economic benefit. The SEC expert expressed his satisfaction on the explanation given by Philrice.

Another concern of the SEC expert was how will the GM product require changes in farm management to attain higher productivity. The proponent responded that except for the production of beta-carotene in the polished grains derived from GR2E rice, all other agronomic characteristics, including yield, are unchanged relative to the based non-GM rice variety. In future, pending receipt of a permit for commercial propagation, varieties containing the GR2E event can be considered as essentially derived varieties with no anticipated changes required to farm management practices relative to the base non-GM variety. The SEC expert expressed again his satisfaction on the explanation given by Philrice.

With the completion of all the necessary requirements for socio- economic compliance in their application for field trial, the SEC Expert recommended that the proponent be given the go signal in proceeding with the field trial.