

Regulatory Decision on the Application Field Trial of High Iron and Zinc Rice events IRS1030-031 and IRS1030-039

I. Brief Identification of the Genetically Modified Organism

Designation: Rice events HIZR IRS1030-031 and HIZR IRS1030-039

Applicant: Philippine Rice Research Institute (PhilRice)

Host Plant: *Oryza sativa* L. (Rice)

Trait Description: Increased iron (Fe) and zinc (Zn) trait in the endosperm

Trait Introduction Method: *Agrobacterium tumefaciens*-mediated transformation

Donor Organism(s): *Malus baccata* (Apfer), source of ferritin gene for increased iron in the rice grain; *Oryza sativa*, source of nicotianamine synthase 2 (*OsNAS2*) gene for iron enhancement in the rice grain; *Escherichia coli* source of hygromycin phosphotransferase (*hpt*) gene which serves as a selectable marker

Proposed Use: For field trial

II. History of Safe Use of the Host Plant

Rice (*Oryza sativa* L.) is the species that shall be released, with lines that consist of rice variety NSIC Rc238, a variety approved for release by Philippine National Seed Industry Council (NSIC) in 2011. It has a long history of safe use and is not a significant source of toxicants and allergens. Its transformation is only intended to increase the production of Fe and Zn in the rice endosperm and is unlikely to alter its potential to cause human, animal, or plant disease.

III. Characteristics of Host Plant

Rice is not known to grow wildly, hence cannot survive without direct human assistance. It is grown throughout the Philippines, in irrigate, rain-fed shallow, deep-water, upland, and tidal wetland (OECD, 1999).

Rice is an autogamous plant propagated through seeds produced by self-pollination with pollens only viable for three to five minutes. Upon opening, pollen is dispersed and germinates on the surface of the stigma, with only one pollen tube that reaches an ovule for double fertilization. According to the Association of Official Seed Certifying Agencies (AOSCA), rice seed requires a minimum isolation distance from other varieties of at least ten feet or about three meters to mitigate the cross-pollination and gene transfer to other rice varieties present in the field trial site. The Joint Assessment Group (JAG) expressed the possibility of the modified organism to

interbreed with its conventional counterpart. However, the chances of interbreeding are very small since rice is self-pollinating and its pollen is only viable for 3-5 minutes.

Oryza rufinogan is the only wild species in the Philippines capable of interbreeding which is not found at the field trial site as it is only reported to be in Bukidnon, Mindanao (Borromeo, et al., 1994). Furthermore, no adverse effects of interbreeding is expected due to intra or interspecific genetic interaction.

The dispersion of rice plants varies widely within the *O. sativa* species, with most cultivars having limited dispersal ability. Shattered seed can be dispersed by animals or movement of water. These shattered seeds and volunteers will be buried or killed through agronomic practices. The JAG added that rice seeds, although normally may fall on the ground during harvest, has no reports of having invasive behavior hence weediness potential is negligible.

IV. Characteristic and safety assessment of the GM product

Agrobacterium tumefaciens-mediated transformation was used to produce HIZR IRS1030-031 and IRS1030-039. Ferritin gene from *Malus baccata* (A*pfer*) and nicotianamine synthase 2 (*OsNAS2*) gene from *O. sativa* were introduced resulting to an increased level of Fe within the grain endosperm and increased level of nicotianamine that is involved in the transport of Fe and Zn to the grain. Hygromycin phosphotransferase (*hpt*) gene from *Escherichia coli* was also introduced as a selectable marker.

The modification will not alter the agronomic or phenotypic characteristics of rice, other than to result in the expression of increased Fe and Zn in the endosperm tissue. It is not expected to increase persistence of the GM crop or to have adverse effect on non-target organisms or to make the rice more susceptible to pests and/or diseases.

To demonstrate the lack of integration of any sequences derived from the IRS1030 or IRS1027 plasmid backbone, southern blot analyses of digested genomic DNAs obtained from IRS1030-031 and IRS1030-039 was performed. For these analyses, different probes, which together corresponded to the entire plasmid backbone region were utilized. Hybridizing fragments were not detected when the backbone probes were tested against samples of digested rice genomic DNAs from IRS1030-031 and IRS1030-039, confirming the lack of integration of any plasmid backbone sequences (Cueto-Reaño et al., 2020).

Consequently, the number of sites of insertion of the transfer-DNA (T-DNA) of pIRS1030 and the integrity of introduced genetic elements in each event was investigated using southern blot analyses. For these analyses, different probes, i.e., *Apfer*, *OsNAS2* and *hpt* were utilized to hybridize genomic DNAs digested with either *SspI*, *EcoRI*, or combination of *PstI* and *AseI* (Cueto-Reaño et al., 2020). Southern hybridization analyses showed that one copy of the pIRS1030 T-DNA was introduced at a single site within IRS1030-031 and IRS1030-039 rice genomes (Cueto-Reaño et al., 2020).

Furthermore, the stability of the inserted DNA across multiple generations of HIZR was assessed by polymerase chain reaction (PCR) analyses of genomic DNA samples prepared from the BC₃F₂, BC₄F₁ and BC₄F₂ generations of HIZR (Dueñas et al 2019a, 2019b). Based on the results of PCR analysis, the introduced DNA in HIZR was stably inherited across multiple generations as a single genetic locus according to Mendelian rules of inheritance.

The genetic modification resulting in accumulation of Fe and Zn in HIZR endosperm was not intended to alter the reproductive biology of rice, including rates of seed germination and pollen-mediated gene flow.

V. Proposed Field Trial

The purpose of the field trial is to generate data for environmental risk assessment, collect materials for nutrient composition analysis and protein expression analysis, and to harvest seeds that will be used for the future field trial if needed.

The field trial will be conducted for one (1) season in two (2) trial sites. The PhilRice-Central Experiment Station, Barangay Maligaya, Science City of Munoz, Nueva Ecija will serve as the primary site for the conduct of field trial while the experimental area at PhilRice Batac Research Station, Barangay Tabug, Batac City, Ilocos Norte will serve as the back-up site.

The trial sites were chosen to be representative of the potential receiving environment for HIZR for collecting relevant agronomic and phenotypic data required for environmental risk assessment. In addition, the location sites are under the care and control of PhilRice, which facilitates compliance management of the proposed activity.

To prevent the dispersal of regulated planting materials beyond the approved site of release, spatial or temporal isolation distance, establishment of physical barriers, and access to the sites limited only to authorized personnel shall be implemented. A period of post-harvest land use restriction and monitoring will be imposed to ensure the destruction of any volunteers arising after completion of the trial. All movement of the materials to/from the trial site locations will be in closed packaging/containers and shall be supervised by DA-BPI personnel.

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During the post-harvest period, the area under post-harvest restrictions shall not be planted with rice, or any sexually compatible species.

In cases of damage from natural and/or man-made causes, the PhilRice shall notify the DA-DA-BPI through the IBC of the steps and actions that it will take. Furthermore, contingency plan submitted by PhilRice shall be followed in case of *force majeure* or intrusions in the field trial site.

VI. Regulatory Decision

After reviewing the scientific data and information relevant to the field trial of PhilRice, the DA-DA-BPI has approved the conduct of the proposed activity in PhilRice-Central Experiment Station, Brgy. Maligaya, Science City of Muñoz, Nueva Ecija and PhilRice Batac Research Station, Barangay Tabug, Batac City, Ilocos Norte.

The DA-DA-BPI issued Biosafety Permit for Field Trial for the two rice events IRS1030-031 and IRS1030-039 with conditions that need to be complied with by PhilRice. Copy of the Biosafety Permit issued can be accessed through the DA-BPI Biotechnology Website.