2019

PHILRICE R&D HIGHLIGHTS

CROP BIOTECHNOLOGY CENTER

Contents

CENTER

Crop Biotechnology

Center Director: Roel R. Suralta

Executive Summary

Through the Department of Agriculture (DA) Administrative Order 21, the Crop Biotechnology Center was established to implement a rationalized, effective, and efficient AgBiotech R&D agenda and eventually generate improved agricultural technologies, productivity and enhanced commercial potential value, and activities for crops. The Center focuses on applying advanced technologies combined with experimental and computational methods to discover, quantify, and validate important traits that contribute to increased yield, value-added rice, and enhanced varietal development for grain quality and climate change resiliency. Outputs of the Center contribute to the Institute's strategic outcomes 3, 5, 6 and 7.

For rice-related researches, the Center currently has several studies dealing with molecular biotechnology techniques and approaches to improve research processes and methodologies. These studies are under the lone project on gene discovery and marker development for important agronomic traits. It examined the genetic composition of rice to accelerate genetic dissection of simple or complex traits (agronomic, quality, or resistance to biotic or abiotic stresses), and assessed species identity of other beneficial organisms. The final outputs of this project are quantitative trait loci (QTLs)/Genes/Markers for traits useful for different breeding objectives, advanced lines carrying pyramided QTLs/genes, trait-specific phenotyping protocol, modified and/or improved genetic information, and genetic database.

PROJECT

Gene Discovery and Development of Molecular Marker for Rice and Other Beneficial Organisms

AA Dela Cruz

This project had eight (8) studies using genomic and molecular information to support rice breeding toward development of promising lines with targeted traits such as drought tolerance, crack resistance, bacterial leaf blight (BLB) and tungro resistance, and deep-rooting. It supported the development of biotechnology products made or derived from microorganisms to help increase yield or prevent yield loss by establishing the genetic profile of the microorganisms, and develop protocols to develop high-quality agricultural products for future sources of growth. A thorough understanding of genes/QTL leads to the development of effective molecular markers for marker-assisted selection, which has been widely used in breeding to select for traits that are difficult or expensive to measure, exhibit low heritability and/or are expressed late in development. Outputs of the project supports rice breeding by expanding the genetic base of the rice germplasm through development of rice donor lines with improved traits. Collectively, outputs of this project contribute to Outcome 5 on the advanced rice science and technology as continuing sources of growth.

The first three studies focused on strengthening the understanding of the genetic mechanisms underlying root system development in response to drought and soil moisture fluctuation stresses. The first study, *Validation and fine-mapping of root plasticity QTL on lateral root development in response to water stress in rice*, used recombinant inbred lines (RIL) mapping populations of IR62266*/DHL96, Nipponbare*2/CSSL47, and IR64*2/CSSL47. The target trait contributes to increased water uptake and rice biomass production despite problems on soil moisture fluctuation. The study established the association of the total lateral root number to three QTL (*qTLRN12.1, qTLRN12.2, and qTLRN12.3*) while the total L-type root number (qLLRN) was found to be in particular regions in the rice chromosome 12. Another study aimed to identify QTLs associated with functional stay-green trait and develop RILs with the highest functional stay-green characteristics, which can play a significant role in increasing yield. There were eight promising RILs selected with high stay greenness with the average duration of 21 days after heading, which is 6-12 days longer compared with the original parents. Results of the screening showed that NSIC Rc 160 has higher grain yield than *Kutsiyam* but the greenness of *Kutsiyam* leaves can last more than 8 days than Rc 160. The third study aimed to find QTL or genes responding to soil moisture fluctuation brought about by erratic rainfall pattern, which adversely affects shoot and root growth on rice, using RIL derived from CSSL47/KDML105. Twelve (12) promising lines were identified with deep rooting traits under soil moisture fluctuation (SMF) stress based on the relation of total nodal root length and average nodal root length and two lines based on the relation of total nodal root length and shoot dry weight.

The fourth study focused on the evaluation of drought tolerance QTL effect in the background of popular high-yielding irrigated lowland rice varieties Rc 160 and Rc 222. Current results showed successful introgression of *qDTY2.2, qDTY4*, and *qDTY12.1* in rice, and initial screening of rice lines carrying one, two, or three QTL has been carried out.

The fifth study, *Novel Gene Identification for Rice Grain Crack Resistance*, identified QTL that control the rice resistance crack/fissure. Major QTL found on chromosome 3 was fine-mapped in the interval between RM15291 and RM1588, narrowing down the QTL from 7.31Mb to 517Kb. Twenty-six (26) annotated loci were identified along the region of the QTL.

The study, *Association of GLH and RTSV resistance to Glh14, tsv1, and eIF4G single nucleotide polymorphism (SNP) type in selected popular Philippine traditional rice varieties and mapping of novel GLH and RTSV resistance genes*, identified potential alternative sources of Glh14 or tsv1 among PTRVs including potential sources of novel GLH or tungro virus resistance QTL/genes.

The study on the establishment of marker system in developing thermo-sensitive genic male sterile (TGMS) lines with bacterial blight (BB) and rice tungro disease (RTD) resistance focused on validating the marker system for development of TGMS rice with BB and tungro resistance. Among the candidate TGMS marker selected in the previous year, RM174 and RM71 were identified as the most promising, producing distinct banding pattern in reference donor parents.

The study on DNA fingerprinting of beneficial organisms for identification and product quality assessment focused on establishing rDNA fingerprinting protocols that allow effective counterchecking of genetic purity of beneficial organisms in both agar culture and in formulated agricultural products.
The optimization of rDNA sequencing protocols for bacterial, fungal, and cyanobacterial species identification was completed. Results of this study will help facilitate and ensure the development of products only containing the intended beneficial organism.

Validation and Fine-mapping of Root Plasticity QTLs on Lateral Root Development in Response to Water Stress in Rice

JM Niones, RR Suralta, TE Mananghaya, AS Cruz, R Mallari, and RJM Gonzaga

This study aimed to fine-map the genomic regions associated with L-type lateral root development using the recombinant inbred lines (RIL) mapping populations of IR62266*/DHL96, Nipponbare*2/CSSL47, and IR64*2/CSSL47. Nipponbare*2/CSSL47 progenies were subjected to root phenotyping using rootbox system. Parents were grown to both continuously waterlogged (CWL) and soil moisture fluctuation (SMF) treatments. Thirty-two (32) markers on Chromosome 12 were used in 160 RILs Nipponbare*2/CSSL47 for genotyping. Four QTLs were identified to be associated to lateral root development in response to soil moisture fluctuation stress; three QTLs (*qTLRN12.1, qTLRN12.2,* and *qTLRN12.3*) were associated to total lateral root number; one QTL in total L-type root number (qLLRN) on the regions of chromosome 12. The distance of target traits was narrowed from 3,850,000bp (14cM) to 302,500bp (1.1cM). Evaluation and validation effect of the QTL to biomass production using rootbox method is on-going.

Detection and Validation of QTL for Functional Stay Green Traits for Rainfed Lowland Environment in Rice

RR Suralta, JM Niones, AS Cruz, and RJM Gonzaga

Stay-green is the capacity of a plant to postpone senescence during post-anthesis. Under drought, functional stay-green plays a significant role and importance in higher grain filling to delay the decrease of chlorophyll content. This photosynthetic activity may extend the assimilatory capacity of the plant to higher grain yields. Plants possessing the stay-green trait are photosynthetically-active compared with genotypes not possessing this trait. This study aimed to identify a RIL with the highest functional stay-green characteristic and to identify QTLs associated with functional stay-green characteristics. Of the 275 lines, 80 lines of NSIC Rc 160/ *Kutsiyam* were subjected to drought stress in bucket system, to determine the stay-green capability in response continuously waterlogged (CWL) and progressive drought (PDR). At heading, SPAD and stomatal conductance were initially measured every two days until maturity. Genotypic data of 39 markers and phenotypic data of 80 RILs Rc 160/*Kutsiyam* were used in QTL analysis. Eight lines demonstrated high stay-greenness with the average duration of 21 days after heading lasting 6-12 days more than its parents. Rc 160 has a higher grain yield compared with *Kutsiyam* but the leaf-greenness of *Kutsiyam* stayed eight days more than Rc 160. Five lines surpassed both parents in terms of retaining its leaf greenness after heading, which translated to high grain yield under drought stress. One QTL in SPAD (qSPAD-1) detected on region of chromosome 1 between markers RM495 (216,956bp) and RM259 (7,446,627bp) was contributed by the Rc 160 allele. Genotyping and phenotyping of the additional lines and validation of identified promising lines with functional stay-green characteristic are on-going.

Evaluation of drought tolerance QTL effect in the background of newly released varieties

FP Waing, JD Caguiat, J2C Santiago, MAC Meman, JOS Enriquez, CU Seville, RR Suralta, and JM Niones

Drought is a recurring phenomenon that limits rice production areas in Asia. A fast and accurate breeding scheme could cope with the onset of drought (Pandey and Bhandari, 2009). One of the most effective ways to combat this phenomenon is the development of tolerant varieties through introgression of QTL linked to the drought-tolerant genes using molecular markers. This study aimed to evaluate and determine the effect of QTL for drought tolerance in adapted background, pyramid 2-4 QTL for drought tolerance and introgress into adapted background, and assess the performance of pyramided lines with QTL for drought tolerance under drought and non-drought stress conditions. Three populations of backcross inbred lines (BILs) were evaluated through marker genotyping and tolerance to drought stress under field and controlled conditions. Marker genotyping confirmed the presence of introgressed qDTY in BILs. SNP genotyping showed that BILs have high recovery of recurrent parent genome and most resembled that of Rc 160 and Rc 222. In terms of performance under drought condition, majority of the BILs performed better relative to Rc 160 and Rc 222. Results of this study demonstrated the successful application of markers in the introgression of abiotic stress tolerance especially for reproductive drought stress.

Novel Gene Identification for Rice Grain Crack Resistance

VG Dalusong, RP Mallari, and TE Mananghaya

Head rice recovery after milling is affected by several factors such as chalkiness, moisture content and cracking or fissuring of rice grain. Proper postharvest practices such as drying, storage, and milling can prevent losses due to broken grains. Increasing the resistance of grains from cracking through introgression of the gene that controls the trait will also increase the head rice recovery. Major QTL found on chromosome 3 was fine-mapped to identify and locate the precise position of gene/s involved in crack resistance. Results of analysis mapped the QTL on chromosome 3 in the interval between RM15291 and RM1588. The region of QTL on chromosome 3 was narrowed down from 7.31Mb to 517Kb. Twenty-six (26) annotated loci were identified along the region of the QTL. Using the identified QTL that controls the cracking of rice grains, the identification of genes for crack resistance is currently being done. It will also benefit the agricultural industry in breeding rice varieties with increased head rice recovery through molecular breeding.

QTL mapping analysis with emphasis on root plasticity traits under soil moisture fluctuation stress

JM Niones, RR Suralta, TE Mananghaya, AS Cruz, R Mallari, and RJM Gonzaga

Rainfed lowland fields are exposed to continuous cycle of soil moisture fluctuation due to erratic rainfall pattern and variability in soil moisture condition, which adversely affects shoot and root growth, and yield of rice. To respond to this heterogenous environmental condition, root plasticity – the plant’s ability to change its phenotype (root system) – an adaptation mechanism, can help the plants in maintaining higher growth and yield under abiotic stresses. One hundred fifteen (115) RILs CSSL47/KDML105 were evaluated for root system development using rootbox method. The progenies were subjected to soil moisture fluctuation (SMF) treatment while the parents were treated for with CWL and SMF. Forty-seven (47) markers, together with the phenotypic data, were used in QTL analysis. There were 12 promising lines identified with deep-rooting traits under SMF stress based on the relation of total nodal root length and average nodal root length and two lines in relation of total nodal root length and shoot dry weight. These lines will undergo validation using rootbox and basket method to identify deep-rooting rice lines. Four (4) QTLs were identified to be associated with SMF stress; three QTLs (q*TNRL-2, qTRL-2,* and *qTLRL-2*) found on the same region in chromosome 2 between markers RM12460 and RM13133, while the other one was found in chromosome 11 (qLLRL-11) between markers TG148 and RM27233. Distance between markers was observed, 11,875,517bp on chromosome 2 and 9,954,148bp on chromosome 11. Additional markers will be used to further narrow down the distance between markers, which will be subject to future studies.

Association of GLH and RTSV Resistance to *Glh14, tsv1,* and *eIF4G* SNP Type in Selected Popular Philippine Traditional Rice Varieties and Mapping of Novel GLH and RTSV Resistance Genes

AA Dela Cruz, MJC Duque, VG Dalusong, and MJ Yapchiongco

The diversification of sources of tungro resistance genes is essential for a continuous breeding program, thus, this study was conducted to help facilitate the advancement of plant breeding by finding alternative or novel sources of tungro resistance genes among Philippine traditional rice varieties (PTRVs). Specifically, the apparent green leafhopper (GLH) or rice tungro spherical virus (RTSV) resistance observed among promising PTRVs was verified under screen house condition and representative plants were molecularly screened for presence of QTL/genes such as *Glh14, tsv1* and *eIF4G*. From the 133 accessions screened in 2019, 94 and 51 PTRVs were identified as potential alternative sources of *Glh14* and *tsv1*, respectively. In comparing the *eIF4G* gene sequences of 18 selected PTRVs, the SNPs observed within exon 9 region of the gene were similar with the susceptible SNP type detected in the TN1 check cultivar. This implies that eIF4G, a gene coding for a translation initiation factor and situated within the tsv1 gene may not be the one involved in the observed apparent RTSV resistance.

Among the promising PTRV included in this recent set of potential sources of novel tungro resistance genes, Nalwak can be a potential material for mapping of novel GLH resistance QTL. The mapping of RTSV resistance QTL in PTRV *Dinorado* has also been initiated this year using 59 F2 plants and 38 polymorphic SSR markers. Initial analysis detected presence of seven putative QTLs, which were mainly located on rice chromosomes 8 and 11.

Establishment of Marker System in Developing Thermo-Sensitive Genic Male Sterile (TGMS) Lines with BLB and RTD Resistance

AA Dela Cruz, RT Miranda, and IR Besas

Hybrid rice can increase production even in less land as it can yield 15-30% higher than inbred varieties. Two-line system or the environment sensitive genetic male sterility (EGMS) is simpler and more efficient for hybrid rice seed production, while Thermo-Sensitive Genic Male Sterile (TGMS) is one system that is being used in hybrid rice production. This technology however, is faced with two major rice diseases: BLB and tungro in the irrigated lowland environment, which may cause significant yield loss. Resistance genes have been known to be linked to molecular markers *Xa4, Xa7,* and *Xa21* for BLB and RM5495 and RM8213 for tungro, which can facilitate easier and faster introgression to susceptible varieties through marker-assisted breeding. This study aimed to establish marker-aided system in developing new and improved TGMS lines with resistance to BLB and tungro. We used RM174 and RM71, previously selected markers that had distinct banding pattern in reference to donor parents, validate F3 advanced breeding lines containing tungro and BLB resistance with PRUP 101 S and PRUP 102 S background. Among breeding lines, 122 F3 plants had banding pattern similar to the positive control while 140 F3 lines had banding patterns similar to RM71. All F3 plants contained *glh14, tsv1,* and *Xa* genes (positive to heterozygous). The present results are now being correlated to the pollen assay for sterility determination. This validates the markers' usefulness in tracking down introgressed TGMS genes in succeeding progenies of the advance lines using F3 and F4 population.

DNA Fingerprinting of Beneficial Organisms for Identification and Product Quality Assessment

TC Fernando, AA dela Cruz, MJC Duque, RT Aguilar, JA Cruz, RL Ordonio, and B Tabudlong

The formulations of bioinoculants for soil and plant health enhancement, or as biocontrol agents, intend to provide sustainable alternatives to synthetic fertilizers and chemicals used in crop production. In PhilRice, several beneficial organisms used for this purpose were identified. However, their identity should be verified before it can be used in agricultural product development. Traditionally, the methods employed to identify organisms is through morphological or microscopic examination or using biochemical test kits or assay. But there are limitations, and the latter is relatively expensive. Thus, this study was conducted to safeguard the purity of PhilRice effective microorganism collections useful for agricultural bioinoculant production and ensure their availability for future use.

The rDNA fingerprinting protocols for the molecular characterization of fungi, bacteria, and cyanobacteria were specifically optimized for effective counter-checking of identity and purity of organism in stock microbial cultures, and in formulated agricultural products. DNA extraction for each organism was done using the available extraction kits. PCR amplification was done and the resulting PCR products were sent to 1st Based DNA Sequencing, Malaysia for DNA sequence analysis. Compared with traditional methods such as morphological or microscopic examination and using biochemical test kits or assay, the protocols developed in this study are more practical and generally cost-efficient. This is particularly evident in the misidentification of an isolate from carabao dung. Data on rDNA fingerprinting profiles and DNA sequences of beneficial organisms are kept, serving as reference in species identification, along with the pure cultures of fingerprinted organisms. Results of this study will help facilitate the development of bioinoculant/biopesticide products containing only the intended beneficial organisms.

Abbreviations and acronyms

AYT - Advanced Yield Trial
ABE - Agricultural and Biosystems Engineering
AEW - Agricultural Extension Worker
ATI – Agriculture Training Institute
AESA - Agro-ecosystem Analysis
AC - Amylose Content
BLB - Bacterial Leaf Blight
BLS -Bacterial Leaf Streak
BCA - Biological Control Agent
BS - Breeder Seeds
BPH -Brown Planthopper
BPI - Bureau of Plant Industry
CGMS - Cytoplasmic Genic Male Sterility
COF - Commercial Organic Fertilizer
CDA - Cooperative Development Authority
DAS - Days After Sowing
DAT - Days After Transplanting
DF - Days to Flowering
DM- Days to Maturity
DAR - Department of Agrarian Reform
DA-RFOs - Department of Agriculture-Regional Field Offices
DoF - Department of Finance
DOLE - Department of Labor and Employment
DTI - Department of Trade and Industry
DSR - Direct-seeded Rice
DS - Dry Season
FBS – Farmers’ Business School
FC - Farmers’ Cooperative
FSM - Farming Systems Models
FAA - Fish Amino Acid
FGD - Focused Group Discussion
FSP - Foundation Seed Production
FRK - Farm Record Keeping
GABA - Gamma-aminobutyric Acid
GT - Gelatinization Temperature
GAD - Gender and Development
GYT - General Yield Trial
GCA - Genetic Combining Ability
GIS - Geographic information system
GEMS - Germplasm Management System
GAS - Golden apple snail
GL - Grain length
GQ - Grain quality
GW - Grain Weight
GY - Grain Yield
GLH - Green Leafhopper
GOT - Grow Out Test
HR - Head Rice
HRA - Heat Recovery Attachment
HIPS – Highly-intensified Production System
HQS - High-quality Rice Seeds
HON - Hybrid Observational Nursery
HPYT - Hybrid Preliminary Yield Trial
ICT - Information and Communication Technology
IEC - Information Education Communication
IBNM - Inorganic-based Nutrient Management
ICM - Integrated Crop Management
IPM - Integrated Pest Management
JICA - Japan International Cooperation Agency
IRRI - International Rice Research Institute
IA - Irrigators’ Association
KP - Knowledge Product
KSL - Knowledge Sharing and Learning
LCC - Leaf Color Chart
LFT - Local Farmer Technicians
LGU - Local Government Units
LPS - Low Pressure Steam-operated
LE-CYPRO - Lowland ecotype Cyperus rotundus
MFE - Male Fertile Environment
MSE - Male Sterile Environment
MAS - Marker-assisted Selection
MRL - Maximum Root Length
MR - Milled Rice
MER - Minimum Enclosing Rectangle
MOET - Minus-one Element Technique
MC - Moisture Content
MAT - Multi-Adaptation Trials

MCRTP - Multi-crop Reduced Till Planter
MET - Multi-environment Trial
MYT - Multi-location Yield Trial
NAAP - National Azolla Action Program
NCT - National Cooperative Test
NFA - National Food Authority
NRAM - National Rice Awareness Month
NSIC - National Seed Industry Council
NSQCS - National Seed Quality Control Services
N - Nitrogen
NBSP - Nucleus and Breeder Seed Production Project
NFGP - Number of Filled Grains Panicle
ON - Observation Nursery
OSIS - One-Stop Information Shop
OBNM - Organic-based Nutrient Management
PL - Panicle Length
PW - Panicle Weight
PVS - Participatory Varietal Selection
PWD - Person with Disabilities
PHilMech - Philippine Center for Postharvest Development and Mechanization
PRISM - Philippine Rice Information System
PhilRice - Philippine Rice Research Institute
PSA - Philippine Statistics Authority
PTC - PhilRice Text Center
P - Phosphorus
PVS - Plant Variety Selection
K - Potassium
QTL - Quantitative Trait Loci
RCBD - Randomized Complete Block Design
RSP - Registered Seed Production
RBB - Rice Black Bug
RCEF - Rice Competitiveness Enhancement Fund
RCEP - Rice Competitiveness Enhancement Program
RCM - Rice Crop Manager
RHGEPS - Rice Hull Gasifier Engine Pump System
RPH - Rice Planthopper
RSTC - Rice Specialists' Training Course
RTV - Rice Tungro Virus
RBFHS - Rice-based Farming Household Survey
KQ - Kernel Quality
SV - Seedling Vigor
ShB - Sheath Blight
ShR - Sheath Rot
SMS - Short Messaging Service
SNP - Single Nucleotide Polymorphism
SWRIP- Small Water Reservoir Irrigation Project
SRB - Stabilized Rice Bran
SUCs - State Universities and Colleges
SB - Stem Borer
TESDA - Technical Education and Skills Development Authority
TDF - Technology Demonstration Farm
TRV - Traditional Rice Varieties
TOT - Training of Trainers
TPR - Transplanted Rice
URBFS - Upland Rice-Based Farming
WS - Wet Season
WCV - Wide Compatibility Variety
YSB - Yellow Stem Borer

We are a government corporate entity (Classification E) under the Department of Agriculture. We were created through Executive Order 1061 on 5 November 1985 (as amended) to help develop high-yielding and cost-reducing technologies so farmers can produce enough rice for all Filipinos.

With a "Rice-Secure Philippines" vision, we want the Filipino rice farmers and the Philippine rice industry to be competitive through research for development in our central and seven branch stations, coordinating with a network that comprises 59 agencies strategically located nationwide.

We have the following certifications: ISO 9001:2008 (Quality Management), ISO 14001:2004 (Environmental Management), and OHSAS 18001:2007 (Occupational Health and Safety Assessment Series).

PHILRICE CENTRAL EXPERIMENT STATION Maligaya, Science City of Muñoz, 3119 Nueva Ecija
Tel: (44) 456 -0277 • Direct line/Telefax: (44) 456-0354

BRANCH STATIONS:

PhilRice Batac, MMSU Campus, Batac City, 2906 Ilocos Norte
Telefax: (77) 772-0654; 670-1867; Tel: 677-1508 Email: batac.station@philrice.gov ph
PhilRice Isabela, Malasin, San Mateo, 3318 Isabela
Mobile: 0908-875-7955; 0927-437-7769; Email: isabela.station@philrice.gov.ph
PhilRice Los Baños, UPLB Campus, College, 4030 Laguna
Tel: (49) 536-8620; 501-1917; Mobile: 0920-911-1420; Email: losbanos.station@philrice.gov.ph
PhilRice Bicol, Batang Ligao City, 4504 Albay
Tel: (52) 284-4860; Mobile: 0918-946-7439; Email: bicol.station@philrice.gov.ph
PhilRice Negros, Cansilayan, Murcia, 6129 Negros Occidental
Mobile: 0949-194-2307; 0927-462-4026; Email: negros.station@philrice. gov.ph
PhilRice Agusan, Basilisa, RTRomualdez, 8611 Agusan del Norte
Telefax: (85) 343-0768; Tel: 343-0534; 343-0778; Email: agusan.station@philrice.gov.ph
PhilRice Midsayap, Bual Norte, Midsayap, 9410 North Cotabato
Telefax: (64) 229-8178; 229-7241 to 43 Email: midsayap.station@philrice.gov.ph

SATELLITE STATIONS:

Mindoro Satellite Station, Alacaak, Sta. Cruz, 5105 Occidental Mindoro
Mobile: 0917-714-9366; 0948-655-7778
Samar Satellite Station, UEP Campus, Catarman, 6400 Northern Samar
Mobile: 0948-754-5994; 0929-188-5438
Zamboanga Satellite Station, WMSU Campus, San Ramon, 7000 Zamboanga City
Mobile: 0910-645-9323; 0975-526-0306

PhilRice Field Office, CMU Campus, Maramag, 8714 Bukidnon
Mobile: 0916-367-6086; 0909-822-9813
Liaison Office, 3rd Flor. ATI Bldg, Elliptical Road, Diliman, Quezon City
Tel/Fax: (02) 920-5129

DEPARTMENT OF AGRICULTURE
PHILRICE
PHILIPPINE RICE RESEARCH INSTITUTE

www.philrice.gov.ph
www.pinoyrice.com
DA-PhilRice
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