

# 2022

## PHILRICE R&D HIGHLIGHTS



## Plant Breeding and Biotechnology Division



**Philippine Rice Research Institute**  
Central Experiment Station  
Maligaya, Science City of Muñoz, 3119 Nueva Ecija

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# Plant Breeding and Biotechnology Division

Division Head: **Oliver E. Manangkil**

## EXECUTIVE SUMMARY

The Plant Breeding and Biotechnology Division (PBBD) develops promising rice lines through conventional and modern technologies, which are entered in the National Cooperative Tests (NCT) to generate new varieties adapted to different rice-growing agro-ecosystems. Two of the four core projects of the PBBD focus on developing promising lines for stressed and non-stressed environments. The two other core projects support mainstream breeding of inbred and hybrid varieties and manage and maintain the PBBD laboratories. Two projects financed by the Rice Competitiveness Enhancement Fund are an integral part of varietal development and provide inherent support to the NCT.

## CORE-FUNDED PROJECT 1

### PBD-211: Centralization

**Frodie P. Waing**

A hybridization block (HB) composed of varieties, elite lines, and accessions for utilization by various breeding projects was assembled. A total of 437 entries (223 in dry season or DS and 214 in wet season or WS) in three staggered batches at one-week interval to provide continuous source of parent lines for crossing. From the HB entries, 75 entries in DS and 67 entries in WS were selected and used in hybridization. The HB panel was genotyped using 30 functional markers through trait-based single nucleotide polymorphism (SNP) genotyping at Intertek. Results showed that the majority of the genes/quantitative trait loci (QTLs) related to grain quality, submergence and drought tolerance, bacterial leaf blight (BLB) and blast resistance, fertility, and grain shape were either not found or had very low frequency in the current elite germplasm. Second filial generation (F<sub>2</sub>) populations were established in the field nursery and in the rapid generation advance (RGA) greenhouse facility. A total of 101 populations in DS and 83 in WS from breeding projects.

## CORE-FUNDED PROJECT 2

# PBD-212: Pre-breeding

Christopher C. Cabusora

Component 1 of the project developed donor lines for earliness in maturity while improving their grain and nutritional qualities. Nine promising donor lines from seed mutation technique with maturity of about 96 days after seeding (DAS), improved head rice recovery, and increased anthocyanin content will be nominated to the hybridization block (HB) for use by rice breeders.

Component 2 conducted research and development (R&D) of cytoplasmic male-sterile (CMS) lines through nucleus substitution approach via series of backcrossing. The CMS lines are an important component of three-line hybrid production. This year, one CMS line with partially stained pollen was identified, yet a series of backcrossing and evaluation is still being conducted to improve its stability in terms of pollen sterility.

Component 3 utilized marker-assisted breeding to combine genes for resistance to bacterial leaf blight (BLB) and rice tungro disease (RTD). Thirty-five lines with stable resistance to BLB and RTD, five of which were confirmed to carry two Xoo9 genes and one RTD resistance gene.

Component 4 implemented efficient screening protocols for the abiotic stresses drought, submergence, salinity, and high temperature. In 2022 DS, 22 novel sources, which are pure lines from traditional varieties, and 33 mutant lines were identified as tolerant to drought. Eighty-three doubled haploids and two breeding lines were identified as saline-tolerant at seedling and reproductive stages, respectively. For submergence, 30 breeding lines with comparable survival (75% to 96%) to the tolerant check, FR13A, were identified; for high temperature, 11 heat-tolerant lines were identified. These lines will be the sources of abiotic tolerance to develop breeding lines with combined or multiple tolerance for a wider adaptation.

The generated donor lines will be characterized and evaluated further, and will be nominated to the HB. These will be used as sources of traits important to rice variety development.

## CORE-FUNDED PROJECT 3

# PBD-213: Inbred Rice

Norvie L. Manigbas

Hybridization for multi-abiotic stress tolerance, Irrigated Lowland (IL), and Specialty Rice (SR) produced 69, 139, and 83 new crosses, respectively. In Pedigree Nursery, 1,739 lines were selected from F<sub>3</sub>-F<sub>6</sub> and 600 lines from F<sub>6</sub>-F<sub>8</sub> for adverse environments. In IL, 8,097 lines were selected from F<sub>2</sub>-F<sub>5</sub> and 138 uniform lines in transplanted rice (TPR) and direct seeded rice (DSR), and 3,354 lines were selected in special rice (SP).

Varieties identified as tolerant to combined/multiple abiotic stresses were Drought+Heat tolerance (Drt + HT): Sahod Ulan 11, 13, 17, 20, 22, and PangMainit 2; Saline+Drought (Sal + Drt): Salinas 5, 6, 7, 8, 10, 17; Submergence+Saline (Sub + Sal): Salinas 33 and 34; Saline + Drought + Heat tolerance (Sal + Drt + Heat): Salinas 14, 25; Drought+Submergence+Saline+Heat tolerance (Drt + Sub + Sal + Heat): Salinas 21. For multiple abiotic stresses, 21 lines were identified with single tolerance, six with combined tolerance to salinity and submergence, and seven with combined heat and salinity tolerance.

Grain yields under irrigated (ILD), managed drought (DRD), irrigated (ILW), and simulated rainfed (RFW) ranged from 3.03-7.81t/ha, 0.05-3.03t/ha, 1.70-7.17t/ha, 1.25-5t/ha, respectively. Stress Tolerance Index (STI) of the test entries was 2.99-7.76, from which 131 (60%) lines had higher STI (5.96 to 7.76) compared with the tolerant check, PSB Rc14 (5.95). Under non-stress (NSTR) and complete submergence stress (SUB) conditions, grain yields ranged from 1.53-7.74t/ha and 0t/ha-6.18t/ha, respectively.

Mass screening of drought tolerance at seedling stage identified 2,432 putatively tolerant plants; 2,857 for salinity tolerance; for submergence, 2,647 plants survived. Under Heat Tolerance (HT) screening, 60 entries were identified as tolerant, 165 intermediates, and 4 were susceptible based on spikelet fertility.

In IL lines, eight F<sub>2</sub> populations and 11 observational nursery (ON) breeding lines (BL) were identified to have Excellent anaerobic germination (AG) and seedling vigor (SV); and 11 populations and 16 BL with good AG and SV under field conditions. Eight BL had intermediate AG tolerance under screenhouse conditions. For lodging resistance, 29 BL were identified with push resistance (PR) of 1.50-1.64kg/cm<sup>2</sup>.

Under ON-TPR, 48 BL yielded 4.32-7.91t/ha in DS; 18 lines had 4.46-6.87t/ha in WS. In ON-DSR, in DS, entries yielded 3.39 to 5.08t/ha, with 16 BL out-yielding checks at 5-15%; in WS, yields were 3.19-7.04t/ha, with 13 BL out-yielding the 5 checks at 10-35%.

In Preliminary Yield Trials (PYT-TPR), 115 lines yielded 1.43-6.11t/ha; 27 lines elevated to Multi-adaptation yield trial (MAYT) 2022 WS with yields of 4.03-5.95t/ha and Phenotypic acceptability (PA) of 3 to 5. In PYT-DSR, 12 promising entries yielded 10.63-12.98t/ha; push resistance measurements were 0.84-1.10kg/cm<sup>2</sup>.

Under ON-SP in DS, eight aromatic lines yielded 4.59-6.98t/ha; pigmented lines had 14 entries with 6.92-9.28t/ha yields; and six Zn/Fe lines with 4.24-7.73t/ha yields. In WS, the highest-yielding lines had 4.61t/ha for A group; 7.18t/ha for P; and 5.22t/ha for Zn/Fe. In PYT-SP, 52 lines yielded 1.73–6.08t/ha during DS, eight of which were elevated to MAYT in WS. During WS, only two entries yielded 4.15 and 4.54t/ha in Group I; seven lines yielded 3.28–4.42t/ha in Group II.

## CORE-FUNDED PROJECT 4

# PBD-214: Hybrid Rice

Frodie P. Waing

The cytoplasmic male-sterile (CMS)-based or three-line hybrid breeding system entails breeding of CMS or A line, maintainer or B line, restorer or R line, and ultimately, the development of heterotic F<sub>1</sub> hybrids from crossing the improved A with R lines. The A line is maintained by its corresponding B line, while R restores the fertility of A in the derived F<sub>1</sub> hybrid. Component breeding lines need to be improved before massive F<sub>1</sub> hybrid generation is pursued.

A total of 207 testcrosses were generated for prospecting of potential B and R lines. Molecular prospecting in terms of genes present using markers Rf3 and Rf4 for restoring ability and micronutrient (MNT) for maintaining ability identified 29 (11%) lines with Rf3 gene, 92 (36%) lines with Rf4, and 21 (8%) lines with both Rf3 and Rf4 genes while 14 (7%) with the MNT gene. A total of 779 experimental hybrids were produced from 59 male parents (MPs) with CMS with their corresponding B lines generated from simultaneous breeding of three CMS.

Performance of experimental hybrids was evaluated in the testcross nursery (TCN) and Breeding for Restoring and Maintaining Ability A line x Restorer line (BRMA AxR) performance trial to identify potential hybrids to be forwarded to the advanced yield trial nursery. In TCN during 2022 dry season (DS), 12 pollen donors were identified as potential restorers based on spikelet fertility, pollen evaluation and presence of Rf genes. In the wet season (WS), three of 144 hybrids evaluated were identified as potential R lines with high fertility rate (90-96%) and plant height (111.2-115.6 cm). For potential B lines, seven were identified based on spikelet sterility with 95-99% and heterosis of 26-60%.

Simultaneous crossing of AxR and AxB using three CMS lines with their corresponding B lines was done to produce CMS seeds, and R lines to generate experimental hybrids. Exactly 18 pollen donors out of 840 paired crosses evaluated were identified as possible restorers. Among the pollen donors, 56 plots were selected for pollen evaluation through microscopic evaluation.

Eight improved B lines with BLB-resistant genes, S5n genes, and exerted stigma were in the process of CMS conversion using the backcross method. Stability of the advanced backcrossing (BC) progenies was monitored by observing seed sets. Two CMS lines that were purified exhibited complete sterility among the evaluated progenies. Purified CMS and B lines were not fully evaluated for their fertility restoration to the identified R line due to genetic purity concerns.

Anthers from six RxR F<sub>1</sub> plants were subjected to anther-culture, with M55-anther culture double haploid lines (M55-AC DHL) as female parent and selected lines as male parent. In the AxR seed production setups of two promising hybrids, two methods of supplementary pollination were implemented, using drone and the conventional manual method. The estimated crop-cuts based on 5m<sup>2</sup> were 1.49t/ha in drone-assisted and 1.37t/ha for the pollination manual method.

## **TGMS-BASED TWO-LINE HYBRID RICE PROJECT**

The project is composed of nine interrelated studies: three on the development of female and male parents; two on generating new and promising experimental hybrids; one for field performance evaluation; and three studies for insect, diseases, and grain quality evaluation. The product of thermogenetic-male sterile (TGMS) and pollen parent line development is used to generate new promising experimental hybrids. Only the best-performing hybrids are nominated to the National Cooperative Tests (NCT). These component studies are working hand in hand to develop and evaluate thousands of experimental hybrids with the objective of identifying two-line hybrids with at least 15% yield advantage over inbred checks.

In the Los Baños testing site in 2022, Advanced Yield Trials (AYT) 205 (PRUP 15) has completed the necessary assessment for field, pest and disease resistance, and grain quality performance. It averaged 5,342kg/ha; M99 and NSIC Rc 222 had 5,184kg/ha and 4,236kg/ha. Comparing the biomass and yield components of PRUP 15 and hybrid check M99, this promising two-line hybrid has about 24 grains per panicle more than M99, while the 1000 grain weight, and harvest index are similar. This is the reason why PRUP 15 has a higher grain yield than M99.

## **RCEF-FUNDED PROJECT 1:**

# **Basic Seed Production (BSP)**

**Christopher C. Cabusora**

The project produced high-quality seeds of the most adapted inbred rice varieties, specifically nucleus and breeder seeds, the highest and purest of all seed classes. In 2022, 36 rice varieties were involved.

Produced were 4,800 panicles of nucleus seeds of three national varieties and 10,400 panicles of 13 regional rice varieties. Some 400 panicles each of the 20 replacement, newly released, and other popular varieties were also harvested. Produced were 490kg and 570kg breeder seeds of two national varieties; outputs for 20 regional varieties each ranged 105 to 380kg; for each of 12 other varieties, the amount was 115 to 250kg. A certification efficiency of 85% was obtained. Breeder seed stocks of the national and regional rice varieties, totaling 355 bags (at 5kg packing), are well-stored in the cold storage facility. Their viability and germination ranged from 89-98% and 82-94%, respectively.

## **RCEF-FUNDED PROJECT 2:**

# **National Cooperative Tests for Rice (NCT)**

**Oliver E. Manangkil**

The NCT confirmed the level of adaptation and stability of all promising lines to multiple stress factors - biotic and abiotic - intensified by climate change. The NCT has several phases depending on the rice ecosystem/environment such as irrigated lowland for transplanted and direct-seeded, rainfed drought for dry direct-seeded, upland dry direct-seeded, saline and flood/submergence-prone environments, high and low/cool temperature conditions, and specialty rice. DA-PhilRice, the Rice Technical Working Group (RTWG), and the Technical Secretariat of the National Seed Industry Council (NSIC) jointly coordinate and conduct the NCT.

As one of the R&D arms of the DA-PhilRice/Rice Competitiveness Enhancement Fund (RCEF) Seed Program, the NCT delivers the latest advances in technology. Testing the promising lines enables rightful decisions for effective varietal replacement in farmers' fields. The NCT has endorsed 23 promising lines for RTWG deliberation as possible varieties for adverse environments — six for cool-elevated (CE) areas and 17 for high-temperature (HT) conditions.

## EXTRA-CORE PROJECT 1:

# Gene-mining of Yield-related Traits in Philippine Rice Landraces

Joanne D. Caguiat

This study mined yield-related genes in Philippine landraces through full gene DNA sequencing, evaluated the haplotype variation between genes and analyzed the allele effects of the mined related genes, and correlated the expression levels of the mined related genes with DNA sequence and functional variation.

From the 240 diversity panel, a subset of 56 genotypes was investigated in detail for gene-based DNA sequencing of APO2, LAX1, WFP, MOC1, TGW6, and NAL1. Haplotypes/allelic variants were discovered in Philippine germplasm in these loci. Single nucleotide polymorphisms (SNPs) identified from the DNA sequences were analyzed in terms of their association with yield-related traits. Fifty-one SNPs were found to be significantly associated with the ten traits under investigation.

Gene expression levels for WFP, NAL1, and TGW6 are being analyzed. There were 890 RNA samples extracted and 566 cDNA samples generated while qPCR primers for NAL1 and WFP were optimized.

## EXTRA-CORE PROJECT 2:

# Genetic Improvement and Mechanism of Resistance to Stem Borer in Rice

Imeldalyn G. Pacada

This is a multiyear project. One of the two remaining activities is the evaluation of the reaction of advanced lines and segregating populations to natural infestation of white (WSB) and yellow stem borers (YSB) that respectively occurred in PhilRice Agusan and Central Experiment Station (CES). Parentage of these populations comes from the identified traditional varieties with stem borer resistance and selected released varieties. The other activity focused on the transcriptome profiling of WSB infecting resistant and susceptible germplasm.

High WSB infestation was recorded in PhilRice Agusan with 26.27% whitehead damage to the susceptible check (TN1). From the 199 F10 segregating

populations, two had highly resistant reactions, 167 resistant, 25 moderately resistant, and five moderately susceptible. Five advanced lines were resistant to moderately resistant to WSB. Low YSB infestation was recorded at CES; thus, all the F<sub>9</sub> segregating populations and advanced lines were harvested for future evaluation.

Four transcriptomes were de novo-assembled using Trinity and were assessed for quality and completeness using contig metrics, BUSCO, and alignment tools. Assemblies were annotated for genes involved in herbivory such as cytochrome P450 enzymes and Na<sup>+</sup>/K<sup>+</sup>-ATPase. Initial annotation showed transcripts coding for cytochrome P450 that were annotated from the genera *Plutella* (Diamondback moth) and *Cnaphalocrocis* (Rice leaf folder); and transcripts coding for Na<sup>+</sup>/K<sup>+</sup>-ATPase annotated from the genera *Ostrinia* (European corn borer) and *Maniola*.

### EXTRA-CORE PROJECT 3:

## OneRicePH: Development of Product Concepts for Target Rice Market Segments and Establishment of the Breeding Network (2022) – PhilRice Component

Oliver E. Manangkil

The project developed a baseline survey tool and established a database matrix of information related to ecosystem, geography, growing culture, and essential grain traits. The database matrix was used for updating product concepts for specific market segments for each target province. Data were collected from Kalinga, Ilocos Sur, Pangasinan, Isabela, Quirino, Bulacan, Aurora, Bataan, Nueva Ecija, Pampanga, Tarlac, Zambales, Quezon, Iloilo, Bukidnon, and Davao del Sur.

Fourteen seed kits composed of 312 elite lines and check varieties per breeding pipelines- irrigated and rainfed - were dispatched to OneRicePH Stage one network sites, ten of which were managed and established by PhilRice CES, Isabela, Negros, Agusan, and Bicol stations.

Sixty-seven elite breeding lines were established in the 2022 WS for elite x elite crosses enhancing the genetic gain for favorable alleles with 141 F<sub>1</sub> lines generated. Progenies were subjected to rapid generation advance and marker-assisted selection to fast-track the development of breeding lines using IRRI

protocols. Three accessions of deep-water/floating rice from International Rice Research Institute (IRRI) were for characterization, seed increase, and initial testing in Liguasan Marsh.

For gene deployment using IRRI donors, 21 released varieties and 164 elite and advanced breeding lines from PhilRice were identified. Identification of at least 1,000 single nucleotide polymorphism (SNP) markers with highly informative content in Philippine varieties is nearly complete for the development of high-throughput genotyping platforms. IRRI SNP markers were also optimized and validated in PhilRice breeding lines to help accelerate the marker-aided selection (MAS) and marker-assisted backcrossing (MABC) activities.

Six varieties were identified as samples for measurement of in vitro GI in IRRI; 16 varieties were seed-increased as additional samples. Grain quality, amylose content, gel consistency, and pasting profile of six varieties and two IRRI rice samples were partially assessed. Some 70-1695kg breeder seeds were produced from 12 rainfed and/or saline varieties; 104-336kg eight foundation seeds and seven registered seeds were produced from upland, rainfed, saline, and/or submergence-tolerance varieties.

## EXTERNALLY-FUNDED PROJECT 1:

# Field Performance Evaluation and Selection of GUYA lines in the tropics

**Thelma F. Padolina**

Systematic seasonal evaluation of GUYA breeding materials is provided for yield performance and other agronomic traits tests at PhilRice CES and simultaneously shuttled to Benguet State University (BSU) for appropriate screening for low temperature stress, and blast disease reactions. The test lines were of different generations and are separately assessed for performance evaluation in the Advanced Yield Trials (AYT), Preliminary Yield Trials (PYT), and Observational Yield Trials (OYT).

Good materials were selected for further improvement as potential varieties for commercial cultivation. In all the nurseries, promising lines were selected. At PhilRice CES, the focus of selection was on yield potential, good agronomic traits and reactions to biotic stresses under tropical conditions; at BSU, low temperature tolerance and blast resistance were emphasized. Severe blast infections affected the majority of the test materials in BSU.

### **Advanced Yield Trials (AYT)**

Responses of the AYT entries and check varieties in both sites differed in terms of maturity and yield. At PhilRice CES, heading ranged from 69-85 days after sowing (DAS); and 82-99 DAS at BSU. Among the test entries, GUVA lines yielded 3,843-6,776kg/ha. No reactions to biotic stress were observed in CES.

Under BSU cold conditions, the checks yielded 74-2,450kg/ha; the GUVA lines had 159-1,660kg/ha. Two entries had comparable yields with Japonica 2. None of the test entries outyielded the check varieties.

### **Preliminary Yield Trials (PYT)**

In the dry season (DS), four check varieties and 23 parallel entries were tested in both sites. Under PhilRice CES, none of the breeding lines/combinations was better than the indica and japonica check varieties. At BSU, two test entries had better yield at least 20% over the overall mean (1,174kg/ha). In the wet season (WS), nine test entries showed promise by yielding 4,152-6,889kg/ha.

### **Observational Yield Trials (OYT)**

In the DS, 18 of 29 test entries were selected with appreciable characteristics. At PhilRice CES, one promising line yielded 13% higher than NSIC Rc 222. During the WS, three lines from rapid generation advance (RGA) technology outyielded all the check varieties.

## **EXTERNALLY-FUNDED PROJECT 2:**

# **Stress-tolerant, High-yielding Rice Varieties Suitable for AFACI Member-countries and Germplasm Utilization for Value-added (GUVA)**

**Norvie L. Manigbas**

The project improved the involvement and knowledge gained of the farmers in the salt-affected areas in Cagayan and Bicol Region. The farmers became more aware of the new varieties for saline-tolerant, received seeds for free from the Asian Food & Agriculture Cooperation Initiative (AFACI) project, and tested them on their farms. PhilRice Bicol and the local government units (LGUs) in Albay implemented a program for salt-affected rice environments, distributing seeds from the AFACI project to selected farmers.

We aim to replicate this success in other areas affected by salinity. Overall, the 2022 project was successfully implemented at each site, except at PhilRice Batac, where drought screening failed due to unsuccessful seed germination."