

DA-PHILRICE R&D HIGHLIGHTS

2021



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Department of Agriculture

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Plant Breeding & Biotechnology Division

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EXECUTIVE SUMMARY

The Plant Breeding and Biotechnology Division develops promising rice lines thru conventional and modern technologies that are entered to the National Cooperative Trials (NCT). Lines that pass the NCT generate new varieties adapted to different rice growing agro-ecosystems. It contributes to the strategic outcomes of the Institute, specifically on increased productivity, cost effectiveness, and profitability of rice farming in a sustainable manner; enhanced value, availability and utilization of rice, diversified rice-based farming products, and by-products for better quality, safety, health, nutrition and income; and advanced rice science and technology as continuing sources of growth.

Three out of the four core projects of the division focus on the development of promising lines for the stressed and unstressed environments by incorporating desirable traits to selected elite lines and modern varieties. The other core project supported the mainstream breeding projects towards the development of rice varieties (both inbred and hybrid) adapted to various ecosystems, and focused on the management and maintenance of the divisions laboratories, which complements the breeding activities and implementation of the IMS policy of the institute. The two projects funded by the Rice Competitive Enhancement Program serves as an integral part of varietal development and an inherent support to NCT while all four extra core projects and two externally funded projects complemented and contributed to the breeding projects and activities of the division.

A total of 105 inbred varieties from Basic Seed (67 varieties), Foundations Seed (13 varieties) and Register Seed (25 varieties) were inspected in DS and WS seasons with average field purity of 99.0%. Seed quality monitoring of carry-over seed lots of most BS varieties conducted showed 90% to 95% of seeds are vigorous and viable.

Centralized Hybridization, F1 and F2 Seed Generation

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Rice improvement largely depends on the breeders and their ability to understand a range of breeding-related disciplines. It involves different steps depending upon the objectives, breeding methodologies, and rice production systems. However, the first and foremost is how well the breeders identify genetic donors based on the target traits and objectives set. One of the most challenging tasks in carrying out a successful breeding program is the choice of germplasm. Rice breeders need to be sure that the source germplasm has desirable genetic variability to develop a variety with desirable characteristics that meets the increasing market demands and safeguard the environment by creating a diverse germplasm pool. A hybridization program must have clear objectives and a set of priority characters.

Thus, this project assembled a panel of elite breeding lines and accessions with unique, desirable, and valuable traits in the hybridization block/nursery for use in different breeding projects under PBBD and established F1 and F2 populations for breeders' evaluation and selection. A hybridization block composed of varieties, elite lines, and accessions for utilization of various breeding projects was assembled. Three hundred eighty-two entries (179 in dry season [DS] and 203 in wet season [WS]) were planted in three staggered batches at one week to provide a continuous source of parent lines for crossing. In addition, the centralized hybridization facility performed various processes, including spikelet clipping, pollen emasculation and panicle to panicle and/or plant-to-plant pair-crossing. Furthermore, the generated F1 crosses in both DS and WS were established in the field along with their pollen parent lines. For the F₂ nursery, 87 populations in DS and 138 populations in WS were assembled from various breeding projects. Each F₂ population was established in the field with at least 500 plants per population. Both F1 crosses and F2 populations were planted at the distance of 20cmx25cm between hills and rows. The amount of fertilizer used was based on the NCT-recommended rate. The experimental field was properly managed and maintained from seed sowing until maturity. The respective breeding team performed plant selection and harvesting of selected plants. The remaining unselected plants were properly turned over to the Business Development Division.

Pre-breeding

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Pre-breeding strategies are requirements before commencing any breeding program. Therefore, the setting of breeding objectives goes hand-in-hand with pre-breeding activities, such as mass screening, molecular assisted breeding, and induced mutation, to identify traits of interest for any plant breeding platform. The project is comprised of four components: (1) the induced mutation for nutritional, grain quality, and agronomic trait improvement, (2) the development of male sterile plants using the nucleus substitution approach through backcrossing, and (3) the utilization of molecular markers to combine bacterial leaf blight and tungro resistance and (4) the mass screen for abiotic stresses to fish out novel gene sources for tolerance.

In 2021, promising mutants derived from modern and traditional rice varieties possessing poor or undesirable traits were evaluated. The evaluation identified mutant lines with improved phenotype, grain quality, and resistance to rice blast. Marker assisted selection (MAS) strategy generated rice lines with combined tolerance to BLB and tungro, and which were planted in the hybridization block to be made available to breeders as donor/parent. Continuous backcrossing of wild rice derived lines, traditional varieties and modern varieties, generated cytoplasmic male sterile (CMS) plants with 70% - 100% male sterility, and are for further evaluation and validation in 2022. Mass screening for seedling tolerance to drought, submergence, salinity, and high temperature was conducted and mutants and traditional rice varieties with single, combined and multiple tolerance were identified. These generated breeding lines will be made available to breeders to be used as donor parents in the hybridization program.

- Identified 2 mutant lines with an amylose content of 75-82% lower than their wildtype, PSB Rc 10 (23.1%), and 2 mutants with amylose content of 17-18% lower than the widltype, NSIC Rc 152 (28.9%);
- Identified 2 mutants with 109-114% higher head rice recovery than the wildtype, NSIC Rc 134;

- Identified 2 mutant lines derived from traditional rice varieties Ballatinaw with improved phenotypic acceptability, low phytic acid and high anthocyanin;
- Identified 3 very early mutant lines (93-99 DAS) from PBS Rc10 and 16 mutant lines from NSIC Rc 240, Ballatinaw and NSIC Rc 134 with resistance to rice blast;
- Inter-specific cross, *Kalingking (Javanica)*/NSIC Rc302, generated cytoplasmic male sterile (CMS) plants with 100% pollen sterility at BC10F1. However, reduction in pollen sterility from 60–80% at the succeeding generation ($BC_{11}F_1$ and $BC_{12}F_1$) was observed;
- Identified 39 lines, from 11 cross combinations, pyramided with 3 bacterial blight resistance genes and 2 tungro resistance gene/ QTL; these rice lines are also exhibiting moderate to resistant reactions to the respective diseases under screenhouse conditions;
- Mass screen of 205 traditional rice varieties (TRVs) for abiotic stresses, identified 148 (72%) TRVs with tolerance to either drought or salinity at seedling stage, while 14 (6%) TRVs identified with combined tolerance;
- 110 mutant lines derived from NSIC Rc 222 and Jepun, identified with combined tolerance to drought, salinity, submergence and high temperature;
- From the 36 rainfed, saline and high-temperature tolerant rice varieties screened, 5 (14%) varieties: Sahod Ulan 29 (NSIC Rc 574), Sahod Ulan 32 (NSIC Rc 592), Sahod Ulan 22 (NSIC Rc 472), Sahod Ulan 33 (NSIC Rc 594) and PangMainit 2 (NSIC Rc 602) were identified with reproductive drought tolerance.

Inbred Rice

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> The Inbred Rice project aimed to develop new rice varieties for irrigated lowland and multi-abiotic traits for rainfed and stressprone environments. The project consists of two components. The first component focused on the development of multi-abiotic traits rice varieties and breeding lines that can adapt to rainfed and areas affected by drought, submergence, salinity, and high temperature. The second component focused on the development of irrigated lowland rice varieties with high and stable yield, resistant to major insect pests and diseases, and with good grain quality traits. Development of specialty rices such as aromatic, glutinous, pigmented, and micronutrient-dense wasalso included.

> To accomplish the project objectives, 192 new crosses for multi-trait tolerance/resistance to abiotic and biotic stresses, 66 for irrigated lowland and 45 for specialty rices were generated in 2021. There were 14,055 segregating breeding lines from different pedigree nurseries evaluated and advanced. From these lines, 62 outstanding lines for multi-trait abiotic stress tolerance, 103 for irrigated lowland, 89 for specialty rices were selected. In addition, 4,923 breeding lines across two components were selected and forwarded in 2022 studies.

Field performance of 220 breeding lines under irrigated (DS and WS), managed drought and simulated rainfed conditions revealed 57 lines with grain yield equal or higher than the population mean yield and stress tolerance index (STI). Field screening for high temperature of 224 breeding lines with putative tolerance to rice tungro disease, drought, submergence and salinity, root plasticity and high temperature tolerance showed seven tolerant and 124 intermediate tolerant lines. Forty-four intermediate tolerant lines had ≥7 t/ha grain yield.

Breeding lines with single trait tolerance to different abiotic stress were also evaluated and identified. Seventy-eight recombinant inbred lines (RIL) and 18,864 plants from 10 segregating populations were tolerant to drought at seedling to early vegetative stage. Fortyfour RILs were identified with tolerance to moderately tolerance to

salinity while 12 were submergence tolerance both at seedling stage. For high temperature, 153 lines were identified tolerant and 203 intermediate tolerant.

Released varieties were also screened under different abiotic stress condition. Eight Salinas and 10 Sahod Ulan varieties were intermediate tolerant to high temperature. For drought at seedling stage, two Salinas and one PangMainit varieties showed tolerance, while five Sahod Ulan and four Salinas displayed tolerance to submergence. In addition, two Salinas showed tolerance under managed drought and two at seedling stage. Five Salinas survived field submergence stress in dry season, and four in wet season including five Sahod Ulan varieties. Genotyping for Sub1 gene showed Sahod Ulan 16, Sahod Ulan 17, and Salinas 3, positive for both SC3 and ART5 SSR markers.

Five F_2 populations and 15 breeding lines had excellent anaerobic germination (AG) and seedling vigor (SV) while 9 populations and 12 breeding lines had good AG and SV under field condition. Under screen house condition, eight breeding lines had intermediate AG tolerance. In terms of lodging resistance using push/pull gauge, 22 resistant breeding lines were identified with pushing resistance (PR) of 1.50–1.59 kg/cm2.

Across season evaluation in observational nursery (ON) for TPR and DSR resulted in identification of 71 outstanding breeding lines which out-yielded the check varieties by 10-78%. PR50227TPR-12-2-3-3 and PR51482ILR-6-2-1 topped ON-TPR while PR50780ILR-21-1-3-1-2-1-B and PR50697ILR-93-3-1-1-2-1-B in ON-DSR. For specialty rices, two breeding lines in aromatic, three lines in micronutrient, one line in glutinous and one line in pigmented group were selected based on yield and other desirable traits across 2021 dry and wet seasons.

Aside from ON, PYT also provided initial evaluation for breeding lines. For PYT-TPR, PR50227-TPR-12-1-3-3, PR49076-B-4-1-2, PR49097-B-3-3-1-2 and PR43809-B-2B-84-1 out-yielded four checks with yield advantage between 10.6% and 20.9%. PYT-DSR resulted in the identification of five breeding lines which out-yielded the four checks. PR47857-6-1-1-1-2-1-B, PR48591-9-3-1-2-1-2-B, PR47852-16-1-3-1-2-3-1-B, PR47855-14-1-1-1-1-2-1-B, PR47855-14-1-1-2-1-2-1-B showed 12.1-53.7% yield advantage over the checks. Also based on yield and yield advantage over checks across seasons, twelve aromatic and eight micronutrient dense breeding lines were selected. These lines had yield advantage of 12.4-157.9%.

Twenty-two MET-ready promising lines for irrigated lowland were identified. These lines were evaluated in General Yield Trial and

showed yield advantage over checks ranging 11%-105.3%. Two hundred-nine elite lines with multi-trait tolerance to abiotic stresses and with 3-5kg seeds were identified NCT-ready. In addition, 10 elite lines were submitted to MET and being evaluated in NCT-HT. These lines have combined abiotic stress tolerance and with 5t/ha and 3t/ ha yield under non-stress and stress condition, respectively.



Hybrid Rice

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> The PhilRice vision of a "Rice-Secure Philippines", anchored on the DA's vision of "A food secure and resilient Philippines with prosperous farmers and fisherfolks" cum "Masaganang ani, mataas na kita ", is meant to include availability, affordability, and accessibility to high-quality and nutritious rice at all times, and covering broad areas relating to rice cultivation, commerce, consumption, and competitiveness (PhilRice Strategic Plan 2017-2022). Aligned and in support to the DA's National Rice Program, PhilRice provides sciencebased approaches to produce enough rice for all Filipinos, and is focused in achieving seven strategic outcomes, one of which, the first outcome, is the "Increased productivity, cost-effectiveness, and profitability of rice farming in a sustainable manner". The PhilRice Hybrid Rice Project addresses this organizational outcome, aiming at domestically producing sufficient rice at a competitive cost, resulting primarily from higher grain yield of good quality rice per unit area of cultivation, coupled with reduced production cost per kilogram paddy rice and reduced yield loss attributable to pests, thereby, increasing farmers' profit.

> Hybrid rice research is a worthwhile investment for the government owing to the following advantages when using hybrid varieties: (1) higher grain yield than inbred, (2) better use of resources, (3) more efficient use of inputs and (4) provide labor opportunities in seed production and seed distribution.

⁷ DA-PHILRICE R&D HIGHLIGHTS 2021 PLANT BREEDING AND BIOTECHNOLOGY DIVISION

The Hybrid Rice Project developed cytoplasmic male sterile (CMS)based three-line and thermo-sensitive genic male sterile (TGMS) two-line hybrids suitable for commercialization, that is, with high yield potential, high F₁ seed reproducibility, good agro-morphological traits, resistance to major insect pests and diseases, acceptable grain and eating quality traits and resistance to lodging. Widely adaptive, as well as location-specific hybrids are identified for maximum expression of yield potential, resulting in optimized field performance, and enhanced profitability of rice farming. The target of the project is well aligned with the main objective of the DA Hybrid Rice Program of increasing the productivity and income of Filipino farmers through the use of hybrid rice technology.

CMS-based three-line hybrid rice

The CMS-based or three-line hybrid breeding system entails breeding of CMS (cytoplasmic male sterile) or A line, maintainer or B line, restorer or R line, and ultimately development of heterotic F_1 hybrids from crossing the improved A with R lines. The A line is maintained by its corresponding B line, while the R line restores the fertility in the F_1 derived hybrid. Improvement of the component breeding lines is to be addressed first before massive F_1 hybrid generation is ensued.

To prospect potential maintainers and restorers in 2021, 255 pollen sources from various breeding programs were assembled in the testcross nursery (TCN). The 46 pollen parents testcrossed with 1-3 CMS (PR15A, IR80559A, IR58025A) generated 168 testcross F_1 progenies. The generated testcross F_1 progenies were phenotyped for pollen and spikelet sterility/fertility, and genotyped with markers for restoring fertility gene (*Rf3* and *Rf4*), and for maintaining ability gene (MNT).

The performance of the pollen parents (male parent) as determined from the performance of the testcross F₁progenies, in terms of pollen sterility/fertility evaluation and outcrossing rate, varied with the CMS (female parent) used. The four pollen parents: PR47932-B-B-1-B-B, PR47193-RT-555-1-1-1-1, IR126492-B-32-2-1-2-1, IR17K1051, were identified potential stable maintainers, when crossed with **PR15A** as CMS female parent; IR120990-B-14-1-1-3-3-2-B identified potential maintainer specific to CMS **IR58025A**; PSB Rc82 and PR43621-B-3-1 identified potential restorers specific for the CMS **IR80559A**. Confirmation of the observed performances is warranted before using the genotypes in the breeding program.

Other four pollen parents: PR47193-RT-792-1-1-1-1(crossed with **PR15A**, **IR59A**); PR47325-33-2-B-3 (crossed with **PR15A**, **IR59A**); PR44538-4-4-1-3-1-3-B (crossed with **PR15A**); NSIC Rc 300 (crossed with **PR15A**,

IR59A), were categorized sterile with spikelet sterility between 91–99%, of their testcross progenies, and were positive to *MNT* marker but negative to *Rf* gene markers. The use of markers did not delineate clearly the pollen parents as to their association with fertility restoring (for restorer) or sterility maintaining (for maintainer) ability, as majority of the pollen parents were positive to both the *Rf* gene and *MNT* markers, regardless of classification as maintainer, restorer, partial maintainer or partial restorer. The currently used marker system must be reviewed and assessed well for its effectiveness in differentiating the genotypes in association with fertility restoring or sterility maintaining ability in the F1 progenies.

An ideal commercially usable CMS line should have stable male sterility over environments; adaptability to target environment for which rice hybrids have to be developed; easy restorability, so that many elite lines can be used as male parents; good outcrossing ability to result in higher seed yield; good combining ability; resistance to major insect pests and diseases; and good grain quality so that rice hybrids can be developed with acceptable grain quality (Virmani, 1997). The project utilized CMS-WA (cytoplasmic male sterility- wide abortive) system of cytosterility in hybrid rice development.

Breeding for maintaining ability is a must do before proceeding to CMS conversion of putative maintainers. A robust CMS (e.g., A-line of M1 or M2 hybrid) is used to generate hundreds of pairwise (plant to plant) testcross progenies to identify completely sterile (CS) F1 progeny plants, and the corresponding A and B parental plants, which will serve as the seed source of the subsequent generations.

To assess the robustness in terms of pollen and spikelet sterility of CMS PRI5A and corresponding maintainer PRI5B, the 24 PRI5A/B plants generated from nine pair crosses were evaluated. Results indicated that five (20.8%) plants had 1-4 panicles with 1-5.3% seed set per panicle. Of the total 188 panicles from 24 plants, only 10 (5.3%) panicles had seed set of 1-4%. Averaging across 188 panicles, only 0.4±1.0 panicle produced seeds, which is translated to 0.36±0.95% seed set per sampled panicle. With these figures, it can be inferred and considered that PRI5A/B is a potentially robust CMS system, for as long as a systematic selection of CS CMS plants and corresponding B plants, and subsequent robust seed production is maintained.

Availability of a wide range of restorers is an essential prerequisite for exploitation of heterosis, the frequency of which varies between ecotypes and geographic regions (Virmani, 1997). Fertility restoration of CMS-WA (cytoplasmic male sterility- wide abortive) is governed

by two dominant genes with differential strengths of restoration, i.e., *Rf4*, the stronger restorer gene located on chromosome 7, and the weaker *Rf3* gene, located on chromosome 10. The effect of the restorer gene on CMS-WA cytoplasm is sporophytic, causing normal pollen and spikelet fertility in the F1 hybrids

Developing restorer lines (R) warrants breeding, evaluation, and selection of breeding lines as potential restorers with good fertility restoring ability, coupled with desirable agro-morphological traits, such as high yield potential, resistance to major insect pests and diseases, good grain and eating qualities.

Pair crosses were made between advanced R lines (pollen source) and the CMS tester PR15A. With the restrictions imposed by the pandemic, panicles for characterization were harvested from only seven (22%) of the 32 testcross hybrids with corresponding male parents established in 2021DS. Only one (14%) (PR1867-G147-12-2-3) of the seven restorers tested satisfied the standards, having restored 96% and 82% pollen and spikelet fertility, respectively, in the F1 progeny, and possessing the Rf4 restorer gene. In 2021WS, only one (G211-2-2-1-3) of the 19 restorers test-crossed with the CMS tester, yielded FI progeny exhibiting at least 80% spikelet fertility, and possessing the Rf3 and RF4-(H) restorer genes. With the current breeding materials, putatively possessing the restorer genes, the amplification of the Rf gene markers is not a guarantee of having a robust spikelet fertility restoring ability, a must trait for a desired good restorer. A highly variable spikelet fertility of the F1 testcross progenies between the developed restorers and CMS tester, ranging from 8-82% (n=7) and 34-80% (n=11), was observed during the DS and WS evaluation. respectively.

Of the 96 restorer lines evaluated in the advanced yield trial (AYT), 11 (11%) potential R lines were selected, of which, nine (82%) possess *Rf4* genes and two (18%) with both *Rf3* and *Rf4* genes. Evaluation of the selected 11 advanced potential R lines for major insect pest and disease resistance, through induced method at PhilRice CES and Isabela, indicated intermediate to resistant reaction to leaf blast, intermediate reaction to Blast and bacterial leaf blight (BLB), and susceptibility to tungro virus, sheath blight, green leaf hopper (GLH), and brown plant hopper (BPH).

The milling potential of the 11 selected advanced lines recorded 74.8% (Fair) to 78.7 % (Premium) brown rice, 67.3 % (Grade 3) to 71% (Premium) total milled rice, and 38.1% (Grade 3) to 61% (Premium) head rice. The physico-chemical properties of the selections were 3.9% (G1) to 13.9%

(G3) chalkiness, 7.7% (very Low) to 25.2 (high) amylose content (AC), 2.44 (high intermediate) to 6.3 (low) gelatinization temperature (GT). The AC/GT composition, which strongly influences the cooking and eating quality of rice, categorized the advanced lines as belonging to cluster 1B and 2A, classified as tender to moderately tender, respectively. All lines had long grain size, and intermediate to slender grain shape.

The initial breeding materials available for use in the project were not robust enough and not genetically pure, which posed difficulties in developing stable, completely sterile CMS lines, and in selection among potential restorers segregating for fertility restoration in the F1 hybrids. To address the challenges, the A/B, B, R and F1 breeding activities should be integrated, utilizing a common pool of germplasm. A simultaneous breeding strategy for the A-B-R genetic purification system in the component three-line hybrid parents is proposed for initial generation of genetically pure A/B, B, R, and identification and selection of heterotic AxR, plant to plant pair crosses. A system of rescuing the parentals A/B, B, R, exhibiting CS and acceptable fertility restoration in the F1 s of the corresponding pair crosses should be devised and implemented in an integrated manner. For the system to work effectively and efficiently, a consolidated and organized field lay-out for the establishment of breeding materials, monitoring, labeling, evaluation, selection, seed harvesting and maintenance, and recording system should be in place for integrated implementation of the breeding activities.

TGMS-based two-line hybrid rice

The development of thermo-sensitive genetic male sterile (TGMS)based two-line hybrid rice project involves line development of female and male parents, generation of new and advanced experimental hybrids, evaluation of the performance of these experimental hybrids under various levels of performance trials, and identification of best performing experimental hybrids for NCT nomination.

In 2021, the project focused on the f modification of shuttle breeding for female parent development, breeding for soft textured parent lines, modification of F1 seed production, and performance evaluation of new and promising experimental hybrids, and F1 seed production of two-line hybrid PRUP 14 in preparation for NCT nomination. Shuttle breeding is an integral part of the operation of the TGMS project for complete evaluation of behavior of TGMS lines for both Male Sterile Environment (MSE) and Male Fertile Environment (MFE). Only TGMS lines with complete sterility at MSE and with partial fertility at MFE will be

considered in developing new TGMS line. Using the original protocol for shuttle breeding, the TGMS segregating breeding materials is characterized for sterility at MSE site in Laguna, while the selected among the segregating population is transferred to MFE sites either in Benguet during the dry season (DS) or in Laguna during WS for fertility evaluation procedure. However, adhering to the guidelines regarding travel restrictions and safety protocols imposed by the Local Government Unit (LGU) and Inter-Agency Task Force (IATF) to limit the spread of COVID-19 in the community, and the mobility and accessibility of the project to MFE site in Benguet became a challenge.

In 2021 DS, selected sterile TGMS lines were transplanted in the screen house. These segregating breeding lines were shuttled along with breeding materials selected during 2021 WS in MFE site in Laguna. The MFE site in Laguna is now prioritizing the breeding phase of segregating identified while the MFE site in Benguet will be exclusively used for seed increase of the promising TGMS lines when the travel restrictions are lifted.

For development of male and female parent lines, the project aimed to increase breeding lines with low amylose content/gelatinization temperature (AC/GT) grains in the crossing block to have more materials with traits necessary in developing a two-line hybrid with excellent eating quality. This is in preparation for the upscaling of promotion of hybrid rice in CALABARZON and MIMAROPA, where the soft textured rice varieties are prioritized. Twenty-six crosses were made using NSIC Rc 160, Rc 218, and Jasmine for female development, while for male development, seven crosses were made using SN 705, NSIC Rc 160, and NSIC Rc 218 as the donor of low AC and GT. The segregating generation will be observed in 2022WS.

With the development of new experimental hybrids, crosses made starting 2021WS were focused on the TGMS lines developed starting in 2015 (TGMSON, RS ON, and TGON), to further improve the crossing block use in developing a two-line hybrid by utilizing the new TGMS lines. The genetic gain in breeding TGMS lines could improve further the new two-line hybrids developed using these parent lines. This is essential in developing a better performing two-line hybrid compared to the current public hybrids commercialized. Based on the experimental hybrids entered in Hybrid Observational Nursery (HON) during 2021 WS and 2022 DS, utilization of new TGMS lines in the development of new experimental hybrids had increased from 48% in 2021 WS to 67% in 2022 DS. This trend signifies the commitment of the project to prioritize the newly developed TGMS lines in developing new experimental hybrids.

As for the evaluation of the new and elite experimental hybrids, the project shifted to using the newly released public hybrids, Mestiso 99 (M99) and Mestiso 103 (M103) as checks. M99 and M103 were developed by incorporating the purple base gene in the pollen parent of these two hybrids. The source of the purple gene also improves the grain yield of these two public hybrids compared to their predecessors. M99 has at least a 5% yield advantage over Mestiso 20, while Mestiso 103 has a 3% yield advantage over Mestiso 19. M99 and M103 are also used as checks in the NCT hybrid rice performance trial.

For F₁ seed production of elite hybrids, the project tested the modification of the isolation-free method. The modification is staggering the date of seeding of the male parents, while the female parent is seeded just once. These changes resulted in saving up seeds of the TGMS lines, while also producing F₁ seeds good for a year (200g). Through the new method of seed production, testing of elite hybrids in AYT can be conducted in the wet and dry seasons successively. This method also enables the team to send out F₁ seeds to the breeding support team for induced screening for insect pest and disease resistance. Based on the F₁ seed yield obtained during 2021 DS and 2021 WS, the new method has increased F₁ seed yield from the traditional isolation-free method with 60g/hybrid average yield to at least 200g/ha average yield using the modified isolation-free method.

The F_1 seed production of promising two-line hybrid PRUP 14 (AYT 191) was completed. The 50kg of F_1 seeds were produced during the 2021 WS seed production. These F_1 seeds produced will undergo Grow Out Testing (GOT) to determine the genetic purity of the seeds. Once verified, nomination to NCT will be done in 2022.

Basic Seed Production

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Basic Seed Production (BSP) project handles and ensures the production of high quality seeds of our rice varieties released for cultivation and commercialization. High-quality seeds specifically pertains to the physical and genetic purity of the rice varieties. The BSP project specifically produces nucleus and breeder seeds of the top most adapted varieties, the regional recommended varieties, other varieties that highly demanded and some newly approved rice varieties for irrigated lowland. The project is composed of two studies: The Nucleus Seed Production and the Breeder Seed Production.

Forty-three rice varieties were established to produce quality nucleus seeds. There were 29,600 panicles of nucleus seeds produced. Meanwhile, an average of 209kg of breeder seed class was produced from 26 national and regional recommended rice varieties. Of these 26 varieties applied for seed certification to the National Seed Quality Control Services (NSQCS), 25 (96%) passed the certification for breeder seed.

- 1,600 panicles of nucleus seeds produced for each of the top three rice varieties, namely NSIC Rc 222, Rc 216 and Rc 402;
- 800 panicles of nucleus seeds produced for each of the 16 regional recommended varieties (PSB Rc10, NSIC Rc 480, Rc 218, Rc 440, PSB Rc18, NSIC Rc 160, Rc 442, Rc 27, PSB Rc 82, NSIC Rc 358, Rc 438, Rc 436, Rc 400, Rc 158, Rc 226 and Rc 238);
- 5,200 panicles of nucleus seeds produced for the seven replacement varieties (800 panicles each for NSIC Rc 506, Rc 508, Rc 510 and Rc 514; 400 panicles each for NSIC Rc 414 and Rc 534; and 1,200 panicle for NSIC Rc 512);
- 400 panicles of nucleus seeds produced for each of the 17 newly released and other rice varieties (NSIC Rc 482 SR, Rc 484 SR, Rc 558, Rc 580, Rc 582, Rc 584, Rc 590, Rc 592, Rc 594, Rc 596, Rc 598, Rc 602 and Rc 604);
- Breeder seeds of the top three rice varieties produced: 625kg of NSIC Rc 402, 385kg of NSIC Rc 222 and 280kg of NSIC Rc 216 (305kg waiting for seed certification);
- An average of 268kg of breeder seeds produced from the 10 regional recommended rice varieties in dry season. The target is 11 varieties, however, NSIC Rc 584SR was downgraded to FS

and CS seed class. Additionally, an average of 154kg breeder seeds produced from the 16 regional recommended rice varieties in wet season. The production was slightly affected by BPH infestation.

 For other and newly released varieties, an average of 221kg of breeder seeds produced in dry season from NSIC Rc 122, Rc 300, Rc 506, Rc 510, and an average of 162kg breeder seeds produced in wet season from NSIC Rc 506, Rc 510, Rc 511, and Rc 580.

Extra-core Project 2

National Cooperative Tests for Rice (NCT)

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> The NCT is a multi-location testing, which confirms the level of adaptation and stability of promising lines to multiple stress factorsbiotic and abiotic, intensified by climate change affecting not only yields but also lead to adverse effects on grain quality. As one of the R&D arms of PhilRice/RCEF seed program, the NCT delivers the latest advances in technology. Exploring these promising lines enable rightful decisions for effective varietal replacement in farmer's fields aligned with the OneDA reform agenda.

> As the post-breeding stage, these promising lines whether inbred or hybrid were tested in field trials; evaluated under screenhouse and farmer's field conditions for insect pest and diseases resistance; and evaluated for milling recovery, physical attributes and physicochemical properties in identified laboratories. The NCT had endorsed 32 promising lines to the Rice Technical Working Group (RTWG) for deliberation as possible varieties for irrigated ecosystem. Five of which were PhilRice-bred- 2 irrigated lowland inbred and 3 special purpose rice with high zinc content. For adverse environment, 19 promising lines were endorsed. From these promising lines, 9 were PhilRice-bred intended for saline-prone (7) and upland areas (2).

- 9 NCT trials established in 54 of the 56 target sites in DS: 6 NCT 1, 19 Multi-location Adaptation Trial (MAT), 3 special purpose (SP), 3 saline, 3 cool elevated (CE), 3 high temperature (HT), 8 insect resistance screening, 7 disease resistance screening, and 2 grain quality evaluation (GQE);
- II NCT trials established in 69 of the 70 target sites in WS: 6 NCT
 I, 19 MAT, 4 SP, 4 saline, 3 CE, 4 upland, 4 rainfed lowland dry-seeded (RLDS), 4 submergence, 8 insect resistance screening,
 II disease resistance screening, and 2 GQE;
- 335 promising lines from different ecosystems/environments: 158 for DS and 177 for WS were established in the DUST plots.
- 32 promising lines were endorsed for Rice TWG deliberation as possible varieties for irrigated ecosystem. Five of which were PhilRice (PR) lines- 2 from MAT (PR39502-13-7-98 and PR40846-92-2-2) and 3 from special purpose with high zinc content (PR34627-B-44-2-1-2-1-2, PR34648-B-34-2-2-3-1-1-1, and PR34627-B-44-2-1-2-1-2-1);
- For adverse environment, 19 promising lines were endorsed to RTWG for deliberation. From these promising lines, nine were PR lines- 7 saline (PR41566-SubMSal132-2-2-1, PR48421-FR13A-IVM2012DS 1-7-4, PR42862-RB-BiLeg19-2-2-1-2, PR38537-B-2-4-2, PR40063-Y Dam Do-IVC2008DS 13-5-Sal1-DRT1, PR41570-B-38-3-BCg-1 and PR41905-Samba Mahsuri-Sub1-IVC2010DS 40-2-1) and 2 upland (PR40858-NSIC Rc9-M4R-370 and PR40858-NSIC Rc9-M4R-435);
- Viable and pure seeds of 15 2021 released varieties (irrigated lowland inbred – 8, SPR-non-glutinous – 5, SPR-micronutrient – 1 and RLDS – 1) were produced as initial seed stock.

Gene Mining of Yield Related Traits in Philippine Rice Landraces

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This study aimed to mine yield related genes and their useful alleles in Philippine rice landraces. The specific objectives were to (1) mine yield related genes in Philippine landraces through full gene DNA sequencing, (2) evaluate the haplotype variation between genes and analyze the allele effects of mined related genes, and (3) correlate the expression levels of the mined related genes with DNA sequence and functional variation.

In 2021, 47 significant SNP-trait associations for 9 yield related traits were identified through Genome Wide Association (GWAS). Also, a haplotype of established yield in Philippine rice varieties APO2, MOC1, WFP, NAL1, TGW6, and LAX1 from 56 varieties underwent full gene DNA sequence. TGW6 was the most diverse with 6 haplotypes and 29 site variations. NAL1 had 5 haplotypes, APO2 and LAX1 both had 4, and MOC1 had 3. Through candidate gene association analyses, significant associations between DNA polymorphism and yield related traits were found. Validation of association analysis result is warranted but this initially shows diversity of yield genes with possible novel alleles for breeding.

In addition, a panel of 376 entries were genotyped for presence of important GQ-related genes/QTLs using SNP markers and presence of favorable alleles were established.

Genetic Improvement and Mechanism of Resistance to Stem Borer in Rice

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> Stem borers are among the most damaging insects in rice production. Its infestation causes 30% and > 20% yield losses for deadheart and whitehead. The two stem borer species documented in the Philippines are yellow stem borer (YSB) Scirpophaga incertulas and white stem borer (WSB) Scirpophaga innonata. YSB is the most prevalent in Luzon, whereas WSB was documented in Visayas and Mindanao.

> This project evaluated the mechanism of resistance of identified six TRVs based on physiological classification and validated their resistance using antibiosis. Advance lines from the crosses between two TRVs and modern variety were also evaluated based on natural infestation of WSB in YSB in Luzon (Nueva Ecija) and Mindanao (Agusan), respectively.

The following are the results derived from the project:

- Antibiosis on larval survival of YSB (PhilRice CES), Dinorado (Acc # 12798-A) and Mukol (Acc # 12393) showed lower larval recovery of 8% than TKM6 (resistant check) and TNI(susceptible check) with 9% and 20%, respectively. For larval weight, Mukol showed comparable larval weight with TKM6. Lower larval weight among germplasm indicated possible antibiosis mechanism.
- Antibiosis on larval survival of WSB (PhilRice Agusan), lower larval weight was found in Inarciaga (Acc # 3920) with 11% compared with resistant check TKM6 at 46%. Lower larval weight was also found in Inarciaga (0.0460g) and followed by Dinorado (.0489g) compared with TMK6 (>.0489g).
- Antibiosis on larval survival of WSB (PhilRice Midsayap), no significant difference was observed in the six TRVs and their corresponding check. This is mainly due to the high temperature recorded during the experimental set up. The resistance

reaction was validated in the exploratory approach for WSB selection process using preference test, in which resistant reaction was identified in Inarciaga, C4-Dinorado (Acc # 1246), Mukol, and TKM6. The five advance lines were also evaluated and validated. Two of these lines showed resistant reaction while the other three demonstrated moderate resistant reaction. TKM6 displayed resistant reaction.

- In the first set up Dinorado, recorded the thickest lignin layer with 34.81µm, slightly thicker than TKM6 with 32.14µm. Both had significant comparable thickness of 46.73µm and 47.35µm, respectively, in the second setup. Inarciaga showed the highest mean lignin concentration value of 15.39%, a bit higher than TKM6 with 15.10% during the first setup. Meanwhile, Mukol and Inarciaga significantly got the highest lignin concentration of 20.22% and 17%, respectively, comparable with TKM6 (17.24%).

High lignin content was also found in Dinorado during the first set up with 5.30%, slightly higher than TKM6 with 4.90%. However in the second set up, TKM6 had higher lignin content than Dinorado with 4.78% and 4.45%, respectively.

- In the dry season, high WSB infestation was recorded in PhilRice Agusan experimental field where 32–72% damage was observed in susceptible check (highly susceptible reaction). Among the five uniform lines, two lines from each cross combination of RED 18/ PSBRc10 and RED 18 / NSIC Rc216, had highly resistant to moderately resistant reaction to whitehead. Among the 216 F7 population, 105 were highly resistant (3), resistant (58), and moderately resistant (44). Low WSB infestation in the field was observed in the wet season; thus, uniform lines and F8 segregating population was harvested and advanced to the next generation.
- At PhilRice CES during DS, massive tungro infestation was observed during vegetative/reproductive; thus, no whitehead rating for YSB was carried out. Plants were selected based on observed escape mechanism expressed among individual plants within population. From the uniform lines, 15 plants were selected from two cross combinations RED 18 /PSBRc10 (8) and RED 18 / NSIC Rc 216 (7). The same process was carried out in F₆ segregating population and only 17 plants from 260 entries were selected. During WS, remnant seeds of F₇ population from PhilRice Agusan were used. Low YSB infestation was observed; thus, uniform lines and F₇/F₈ segregating population was harvested and advanced to next generation.

Optimizing Yielding Ability of Hybrids and Parents through Growing-Environment Specific Adaptation and Crop Management for Enhanced Productivity in Rice

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> The project generally optimized the yielding ability of hybrid rice and parents through appropriate utilization of widely adapted and growing environment-specific varieties and development of optimized crop management system(s) for efficient hybrid rice production, resulting in enhanced yield and profitability of hybrid rice cultivation.

Component A: Assessing adaptation and stability of elite F1 hybrids and parents in diverse growing environments

Component objectives include:

- Identified widely adapted and growing environment-specific
 F1 hybrids and parents through multiple environment field performance evaluation;
- Predicted potential yield of hybrids and parents under various growing environments through application of DSSAT (Decision Support System for Agro-technology Transfer);
- Increase efficiency of F1 seed yield through accurate flowering synchronization of parents with application of Growing Degree Day (GDD) technique;

Sub-component A.1: Multi-Environment Field Performance Evaluation of Elite F1 Hybrids and Parents

- Seeds of at least 1kg produced for five elite hybrids and corresponding parent lines;
- Il elite hybrids, 4 hybrid checks (Mestizo 1, Mestiso 20, Mestiso 55, Mestiso 73), and NSIC Rc 222 evaluated in 5 sites during the 2021 DS (PhilRice Batac, PhilRice Isabela, PhilRice CES, Department of Agriculture-Western Visayas Agricultural Research Center, and Davao Del Sur State College;
- DSSC (8,713.30kg/ha) was the highest yielding environment

followed by PhilRice CES (6096.61kg/ha), PhilRice Isabela (5270.07kg/ha), PhilRice Batac (4755.10kg/ha), and DA-WESVIARC (4183.28kg/ha);

- Overall, 2021 DS results indicated that PR51570H obtained the highest yield (6,467kg/ha) among the elite hybrids and outperformed NSIC Rc 222 (6,201kg/ha);
- Average performance of the entries in rainfed environment was also evaluated. PR51570H (5,370.9kg/ha) simultaneously outperformed all the check varieties with 2.8% yield advantage over the highest yield hybrid check, Mestiso 20 (5224.96kg/ha) and 17.6% yield advantage over NSIC Rc 222 (4567.92kg/ha).

Sub-component A.2: Simulating Hybrid Rice Yield Using Decision Support System for Agrotechnology Transfer (DSSAT) Model Under Various Growing Environment Evaluate and calibrate the DSSAT model in predicting hybrid rice yield in the Philippines

- Predicted/simulated yield of the DSSAT models for two sites (Batac and Southern Philippines Agri-Business and Marine and Aquatic School of Technology) using the observed datasets (2019 DS) and compared with the observed experimental yield;
- Established 2021 WS validation sites: MYT and 2 farmers' field setup in llocos Norte were used for the sensitivity analysis;
- Sensitivity analysis showed that three hybrids attained the better goodness fit namely Mestiso 20 (0.71), Mestiso 32 (0.73), and Mestiso 55(0.61) based on their higher R2. Likewise, two of the maintainer line (IR68897B and IR79128B) also attained good fit based on their high R2 of 0.73 and 0.51, respectively.

Subcomponent A.3: Growing Degree Day (GDD)-based Flowering Synchrony between Parents

- Established AxR synchronization trials in DS) and WS with the following treatments using GDD and leaf count method (LCM):
 (1) GDD w/GA3, (2) GDD w/o GA3, (3) LCM w/GA3, and (4) LCM w/o GA3;
- For 2021 DS, GDD-based methods obtained better seed yield than LCM-based methods. Remarkably, the relative yield gain of GDD-based over the LCM was 55% under GA3 applications and 80% without GA3 application. Within the same methods, GA3 application increased the yield of GDD-based by 19% and the LCM by 38%;
- In 2021 WS, the relative yield gain of GDD-based over the LCM was only 27% with GA3 applications and 15% without GA3 application. Within the same methods, GA3 application increased the yield of GDD-based by 7% but resulted in a lower yield for LCM by -3%.

NextGen PLUS: Increasing Access to Adaptive Rice Varieties in the Philippines

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> The NextGen Project has developed breeding lines introgressed with resistance traits to tungro, bacterial leaf blight, blast, and drought using advanced breeding technologies. Over the past five years, commendable advances in rice genetics now offer us the opportunity to develop better and higher quality rice varieties for "mega" environments or to a more "localized and specialized" environment.

> This project employed genomic selection, line and variety augmentation, and rapid generation advance to accelerate breeding cycles and enhance breeding efficiency and progress made in the NextGen project. In this strategy, there will be a continuing release of novel inbred varieties with a succession of new traits. This could create an environment in which farmers find it attractive to purchase the next round of improved inbred varieties.

Component 1. Validation of Provincial Variety Profiles and Recommendation Domains

• Secondary, Focus Group Discussion (FGD), and SWOT data from 5 pilot provinces completed for validation and analysis.

Component 2. Development of New Adaptive Rice Varieties Line augmentation:

• 2backcrosscombinationsatBC2F1and10atBC1F1weregenerated for stacking of biotic and abiotic stress tolerance with confirmed introgression of genes/QTLs from donor lines with the following combinations: Xa4+xa5+Xa21+qDTY4.1; Xa4+xa5+Xa21+xa13; Xa4+xa5+qDTY4.1+2.2; Xa4+xa5+Xa23+qDTY4.1+2.2 and Xa4+xa5+Xa23+xa13. 156 F4 plants with gene combinations: xa13+Xa21 and Xa21+qDTY4.1+2.2 were forwarded in pedigree nursery for evaluation of phenotypic acceptability and generation advance;

- 4 backcross combinations at BC2F1 with gene combinations: sub1+qDTY4.1+qDTY2.2 and sub1+xa13 were generated and 4 backcross combinations at BC1F2 introgressed sub1+qDTY4.1, sub1+qDTY2.2 were evaluated for phenotypic acceptability in 2021WS for submergence tolerance;
- 4 backcross combinations at BC2F1 with gene combinations: ag1+xa13, ag1+qDTY4.1+dDTY2.2, ag1+sub1+qDTY4.1+qDTY2.2 and ag1+sub1+xa13 from three advanced lines and 4 IRRI donor line generated for anaerobic germination + submergence tolerance.

Varietal augmentation:

- Three backcross combinations at BC3F1 and BC2F1 in NSIC Rc 216 background introgressed with Xa4+xa5+qDTY4.1, Xa4+xa5+qDTY2.2, Xa4+xa5+Xa21+qDTY4.1+2.2, and Xa4+xa5+xa13+Xa21 and Xa4+xa5+Xa21; six backcross combinations at BC1F1 in NSIC Rc 402 background introgressed Xa4+xa5+gDTY4.1+gDTY2.2, Xa4+xa5+gDTY4.1. with Xa4+xa5+qDTY2.2 and Xa4+Xa23; and five backcross combinations at BC1F1 in NSIC Rc 440 background introgressed with Xa4+xa5+qDTY4.1+qDTY2.2, Xa4+xa5+gDTY4.1. Xa4+xa5+gDTY2.2, Xa4+xa5+Xa21 and Xa4+xa5+xa13 generated using NextGen breeding lines and IRRI donor lines;
- 738 F2:3 segregating plants from 2 crosses in NSIC Rc 402 and Rc 440 background screened for submergence tolerance and genotyped for the presence of sub1 gene;
- 4 backcross combinations at BC2F1 in NSIC Rc 216, Rc 402, Rc 440, Rc 438, Rc 480 and Rc 298 backgrounds augmented with ag1+sub1 using IRRI donor line Ciherang (ag1+sub1) for Anaerobic germination+Submergence tolerance;
- Genomic selection (GS): Genetic diversity and population structure of 466 assembled breeding lines from various breeding projects of the PBBD-PhilRice generated based on the obtained fingerprint data using 7K Infinium SNP Array (C7AIR);
- Dispatched and established seed kits containing 400 entries (149 PhilRice and 241 IRRI elite lines including 10 global checks) to 5 Multi-environment advanced yield trials (MAYT): Nueva Ecija, Isabela, Agusan del Sur, Laguna and Bukidnon. Twentyone (21) PhilRice elite lines were nominated to NCT for 2022;
- New set of elite hybrids (SL Agritech 6 entries, PhilSCAT -4 entries, 2 entries each from Long Ping, Leads Agriventure, Bayer and Corteva, and 1 entry each from Spring Autumn, PhilRice and IRRI) were composed and dispatched to 24 NCT hybrid sites in 2021 WS;
- PPTV: 205 (DS) and 216 (WS) seed kits under irrigated, rainfed,

saline ecosystem and special purpose rice (aromatic, glutinous and japonica) dispatched and established in multi-ecosystem sites across 16 regions.

Component 3. Development of Accelerated Variety Delivery Support Mechanisms

Basic seed production:

- 35-135kg breeder seeds of 13 varieties in DS and 35-85kg of 11 varieties for adverse ecosystems in WS produced;
- 260-420kg foundation seeds of 4 varieties in DS and 89-320kg of 4 varieties for adverse ecosystems in WS produced;
- 20-470kg registered seeds of 5 varieties in DS and 50-350kg of 5 varieties for adverse ecosystems in WS produced.

Capacity development of Regional Partners:

- An e-Learning module was developed (out of the four modules), in collaboration with IRRI Education and ATI. The e-Learning module will be turned over to ATI for its regional rollout starting 2022;
- Training/Retooling conducted in Region 3 (face-to-face) in September 15-17, 2021, Region 6 (virtual) in October 18-22, 2021 and Region 12 (virtual) in November 25-27, 2021.

Field Performance Evaluation and Selection of GUVA Lines in the Tropics

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In the Philippines, production of Japonica and Indica/Japonica rice is mostly in the highland areas wherein temperatures ranged from 15° to 18oC during vegetative stage and 19o to 22.9o C at reproductive phase. Low temperature as a major abiotic stress significantly affects the growth and development with remarkable impact to yielding ability. Moreover, blast disease becomes prevalent due to the humid environment resulting in low yield production of susceptible entries.

With the country's long collaboration with the International Rice Research Institute (IRRI) and thru Rural Development Administration (RDA) GUVA project, it was established to evaluate temperate elite lines possessing major traits of interest such as high yield, good grain quality, and multiple resistance to biotic and abiotic stresses. The project with PhilRice focused on high yield, excellent grain quality, blast resistance, and cold tolerance, with adaptability to tropical conditions as major objectives. Best lines can be candidates to NCT and/or pre-breeding lines for genetic enhancement.

In 2021, breeding lines were at different stages of development: 9 lines for the Advanced Yield Trial (AYT), 17 entries at Preliminary Yield Trial(PYT), and 94 at the Observational Yield Test(OYT). Trials were conducted simultaneously at PhilRice Experiment Station for non-stress conditions and at Benguet State University (BSU) for stress conditions.

Results for the year identified lines from AYT for nomination in the National Cooperative Test (NCT) trials. Four lines were identified based on yield and tolerance to cold and resistance to blast and with good phenotypic acceptability, namely: IR19K1062, IR19K1088, IR19K1015 and IR19K1030. These materials performed in PhilRice with yields ranging from 2.9 to 5.1kg/ha. However, in BSU the yield recorded 1665-2042kg/ha as compared with the check variety of only 823g/ha. The results in the test sites were affected by the continuous lockdown owing to the lack of care and maintenance of the area.

Among the 16 PYT entries, only three passed the standards under the stress site in BSU for the AYT advanced tests while among the 94 OYT entries, 20 entries were conditionally advanced for PYT. These entries will be further validated in 2022 DS before the next advanced trials in 2022 WS

Among the 16 PYT entries, only three passed the standards under the stress site in BSU for the AYT advanced tests while among the 94 OYT entries, 20 entries were conditionally advanced for PYT.

Further validation of these entries will be done in 2022DS before they are finally included in the next advanced trials in 2022WS.

Externally-funded Project 2

Development, Evaluation & Identification of High Zinc Rice Breeding Lines for Varietal Promotion in the Philippines

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> Improvement on the micro-nutrient content of the rice grain was initiated by Consultative Group for International Agricultural Research (CGIAR). This project on breeding for mineral and vitamin enhancement of rice in collaboration with International Rice Research Institute (IRRI) holds great promise for making a significant, low-cost, and sustainable contribution to reducing micro-nutrient malnutrition in the country. With the thousands of rice selections with elevated mineral content generated by IRRI, evaluation of these materials for local consumption and utilization in the breeding program would be beneficial. Yielding ability of the micronutrient-dense breeding materials is one of the major bases of selection to suit farmers' demand. However, several factors contribute to the full expression of this trait. The genetic make-up of the selected lines coupled with the biotic and abiotic stresses prevalent during the cropping season and the interaction with the environment dictates the yield performance of these selections.

> Breeding activities generated 11 new crosses, 10 of which were selected for plant selection. In the segregating generations, 13 populations were evaluated for plant selection and selected 509 panicles for further evaluation in the pedigree nursery. Twenty-one

Externally-funded Project 2

lines with good plant type, long dense panicles, uniformity and field resistance to pest and diseases from the pedigree nursery were elevated to observational nursery (ON).

In the four replicated trials conducted, promising lines were evaluated and identified to select high zinc lines for nomination to multi-location trial and to the NCT for varietal release. Promising advanced breeding lines were selected from the different nurseries for further evaluation in the subsequent trial. There were 6 lines selected from OYT, 5 lines from PYT, 12 lines from AYT and 9 lines from pre-NCT. Selection of promising lines was based on yield, yield advantage of >5% over the corresponding check and elevated >20mg/kg Zinc content. Eight entries were identified as the most widely adapted entry across seasons and locations with elevated 20.4mg/kg to 23mg/kg zinc. These entries were potential candidates for national evaluation under the micronutrient group.