2020

PhilRice R&D Highlights

Plant Breeding and Biotechnology Division

Contents

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Division head: **Oliver M. Manangkil**

EXECUTIVE SUMMARY

The Plant Breeding and Biotechnology Division (PBBD) develops promising rice lines using conventional and modern technologies entered into the National Cooperative Trials (NCT). Lines that pass the NCT generate new varieties adapted to different rice-growing agroecosystems. 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.

Essentially, breeding done at PBBD is directed to achieve desired traits such as high grain yield, resistance to abiotic and biotic stresses, and grain qualities acceptable to consumers. Restricting factors such as biotic and abiotic stresses affect rice growth and development under different environmental conditions. These factors significantly affect yield, which affects the ability to cater to the steady increase in rice demand.

Three out of the five core projects lodged in the division were focused on developing elite lines for the stressed and unstressed environments. These projects contributed to the development of rice varieties for different ecosystems by incorporating desirable traits to selected elite lines and modern varieties. The fourth project focused on the management and maintenance of the division’s laboratory, which complemented and supported the breeding activities and implementation of the IMS policy of the Institute, while the fifth project supported the mainstream breeding projects towards the development of rice varieties (both inbred and hybrid) adapted to various ecosystems. The two projects funded by the Rice Competitiveness Enhancement Fund (RCEF) Seed Program served as an integral part of varietal development and inherent support to the NCT. All five extra-core projects complemented and contributed to the breeding projects and activities of the division.

In an effort to improve and broaden the genetic diversity of parent lines and varieties, several procedures were employed to generate useful and efficient parents. Incorporating genes important in stressed environments to lines bred for favorable ecosystem including mechanisms of resistance and their genetics of traits, and varietal identification or characterization is one of the crucial steps of crop improvement. Also, it is imperative to rapidly identify and characterize plant varieties to provide valuable information for their introduction and possible improvement.

A total of 239 rice germplasm accessions were established in the crossing block every season in two staggered batches to allow a greater chance of flowering synchronization. Qualified rice accessions were ranked, and those in the top three positions were recommended to breeders working on yield enhancement. All data gathered in the characterization were provided to rice breeders to identify potential donors in rice improvement. By showcasing the potentials of available pre-breeding materials, their utilization in rice breeding will thereby increase. This can lead to the exploitation of new and diverse sources of variation needed to develop new gene pools for genetic enhancement of rice with a high frequency of valuable genes, wider adaptability, and a broad genetic base. Also, Philippine rice germplasm offers a genetically diverse source for yield-related traits. Mining of genes/quantitative trait loci (QTLs) found in the Philippine rice germplasm will enable us to utilize these traits to breed and improve rice varieties.

Mutant populations from different varieties, cultivars, and lines were generated and produced mutants with better yield and qualities than the original parents. Commercially-released modern varieties such as PSB Rc 10, and NSIC Rc 152 and Rc 150 with high-yielding ability and adaptability were mutated to improve their inferior quality traits, such as high chalky character, high amylose content, and poor milling qualities. NSIC Rc 240, a high-dense rice variety, was also induced to select for consistency in grain size and shape.

In search of a different donor for male sterility, different nucleus substitution approaches were made. From three nucleus substitution approaches, the highest pollen sterility, with 100% CS pollen and 0% spikelet fertility, was observed in inter-specific and inter-subspecific crosses. However, the pollen sterility of these crosses during the WS fluctuates into partial fertile pollen (60% to 70%) with 5% to 56% spikelet fertility. Obtaining complete sterile plants from any of the three nucleus substitution approaches was difficult, especially when harmonizing two different parent lines with diverse cultivar backgrounds.

Five new varieties were released for commercialization while 30 elite inbred lines for stressed environment were undergoing testing in NCT. Three elite inbred lines were completed for unstressed environment in the NCT Phase II. Moreover, 77 NCT-ready elite breeding lines with multiple stress tolerance (sal-sub tolerant, heat-sub tolerant, drought-heat tolerant, sal-sub-drought tolerant) were identified while three special-purpose zinc-dense lines were developed.

All these lines, when released, will be reproduced in bigger areas by PhilRice thru the Business Development Divisions in all stations across the country. The majority of breeder seed requirements are multiplied by PBBD thru nucleus and breeder seed production studies. Distinctness Uniformity Stability Test (DUST) characterized promising lines tested in NCT that generated passport data as bases in the purity and identification of all varieties for seed production. This year, 215 viable and pure seeds of 12 newly released inbred varieties were produced under the nucleus seed production. A total of 9,909kg of breeder seeds from 29 irrigated lowland (IL) varieties with 89% efficiency and 2,353kg of breeder seeds from 20 adverse (ADV) varieties with 73% efficiency were produced.

The use of modern breeding tools and technologies through various extra-core and external projects greatly helped attain different breeding objectives. This included the calibration and preventive maintenance of 144 and 3 laboratory equipment, respectively. Also, various research studies on hybrid rice were implemented to support the country's goal of rice security.

PROJECT 1

Pre-Breeding and Genetic Enhancement

Oliver E. Manangkil

Breeding methods still rely on conventional methods such as backcrossing and induced mutation and exploit molecular approaches with effective phenotyping techniques to characterize the resulting lines efficiently. Germplasm sources include traditional or indigenous cultivars known to harbor novel genes and modern varieties for further improvement.

Pre-breeding provides this unique opportunity to expand genetic diversity and becomes the foundation for sustainable development of new varieties, which offer options that farmers can plant or materials that breeders can manipulate to continue developing better and more adaptive rice varieties.

The studies focus on supporting the current breeding activities for the different favorable and adverse ecosystems. Its emphasis is on providing donor germplasm with essential morpho-agronomic traits, including yield, maturity, physiological traits, single or pyramided lines with genes or QTLs for resistance or tolerance to stresses, new parental of hybrids, high-quality table rice, and specialty rice with high nutritional value. Results of the 2020 dry season (DS) were affected by force majeure. Activities for characterization, evaluation, screening, and analysis of materials were conducted in the WS samples.

Mutational Improvement of Quality Characters in Rice Contributing to Food Security in the Philippines

Lenie R. Pautin, Thelma F. Padolina, Rustum C. Braceros, and Eleanor S. Avellanoza

An induced mutation is one of the potential tools for generating more variability, which has produced suitable mutant varieties, especially for agronomic and quality improvement. The majority of the treatments used gamma-ray irradiation and some with an ion beam. Mutants from traditional cultivars and modern varieties were initially selected for good agronomic traits leading to improved yield. Specific screening methods were expanded to choose for unique and value-added traits.

Advanced mutant lines derived from traditional cultivars Dinorado, Ballatinaw, and Azucena were identified with higher yield and other agronomic traits. The final selection of the promising lines will be based on the validated nutritional properties such as high anthocyanin content, low phytic acid, and excellent eating quality. Newly acquired traditional cultivars, Galo, Milagrosa, Elon-elon, and Pinalawan, now in the M4 generation, have the potential for improvement and are sources of value-added traits. In all these important mutants, evaluation for resistance to biotic and abiotic stresses was included.

Commercially-released modern varieties such as PSB Rc 10, and NSIC Rc 134, Rc 152, and Rc 150 with high-yielding ability and adaptability were mutated to improve their inferior quality traits, such as high chalky character, high amylose content, and poor milling qualities. NSIC Rc 240, a high-dense rice variety, was also induced to select for consistency in grain size and shape. The mutants of new modern varieties: NSIC Rc 218, an excellent eating quality variety, and NSIC Rc 27, a high-yielding upland variety, were selected for resistance improvement and adaptation to irrigated ecosystem, respectively.

Finding Durable and Novel Blast Resistance Genes Effective Against Phillippine's *Magnaporthe grisea* Pathogen Population

Jonathan M. Niones, Teodora E. Mananghaya, Jennifer T. Niones,
Juliet P. Rillon, and Rachelle P. Mallari

In the Philippines, rice blast disease has been frequently reported in upland rice-growing areas, where the condition is more favorable for disease development and rainfed lowland environments prone to drought. Moreover, rice blasts have recently been reported in lowland areas, bringing more concerns for its spreading threats to rice production. In a genetic analysis of US2/Malay2 cross combination resistance, two blast resistance quantitative trait loci (QTL) were identified in chromosomes 4 and 11 with LOD of 2.591 and 3.113. Out of 640 plants, 59 were selected on three mapping populations based on phenotype, of which 16 were advanced for Observation Nursery (ON) in 22020DS. The initial agronomic evaluation showed NILpi21-Owari [US2]/NSIC Rc 160/2*NR160 recorded the longest panicle (33.4cm) and highest spikelet fertility (92.6%). Six breeding lines outyielded the recurrent parent; the yield ranged from 12.8 to 13.9t/ha while NSIC Rc 240 recorded 12.6t/ha. The breeding lines were forwarded to an advanced yield trial in the 2020WS, and the post-harvest data collection is in progress.

General Evaluation of Donor Germplasm

Julieta F. Pariñas, Pinklet Athena C. Canilang, and Thelma F. Padolina

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 a set of desirable characteristics that meet the increasing market demands and to safeguard the environment through the creation of a diverse germplasm pool. In selecting the parent materials, considerations on aspects such as grain type and shape, plant height, and resistance to biotic and abiotic stresses are of primary importance. Hence, this study was implemented to ensure the availability of select pre-breeding materials in the PhilRice crossing block for use in various breeding programs and to conduct a general evaluation of rice germplasm accessions as needed. In 2020, 239 rice accessions were established in the crossing block every season in two staggered batches to allow a greater chance of flowering synchronization. From 2015, the characterization of selected rice accessions was based on the standard basis of selection, grain length, grain shape, and days to maturity. Qualified rice accessions were ranked, and those in the top three positions were recommended to breeders working on yield enhancement. However, all data gathered in the characterization were provided to rice breeders to identify potential donors in rice improvement. By showcasing the potentials of available pre-breeding materials, their utilization in rice breeding will thereby increase. This can lead to the exploitation of new and diverse sources of variation needed to develop new gene pools for genetic enhancement of rice with a high frequency of valuable genes, wider adaptability, and a broad genetic base.

Development of New Cytoplasmic Male Sterile Source Using Nucleus Substitution Approach

Imeldalyn G. Pacada, Abegail G. Pascua, and Claudenick A. Blacer

The exploration and use of Philippine rice genetic resources strengthen the foundation of the pre-breeding program. In this study, we explored the use of germplasm that could be used to develop a new cytoplasmic source to be utilized in hybrid rice breeding. From three nucleus substitution approaches, the highest pollen sterility, with 100% CS pollen, and 0% spikelet fertility, were observed in Inter-specific and Inter-subspecific crosses. However, the pollen sterility of these crosses during WS fluctuates into partial fertile pollen (60% to 70%) with 5% to 56% spikelet fertility. Obtaining complete sterile plants from three nucleus substitution approaches was difficult, especially when harmonizing two different parent lines with diverse cultivar backgrounds. Furthermore, the more genetically diverse the parent's background is, the lengthier it is to obtain sterile plants. Continuous backcrossing facilitated the process of getting sterile plants.

PROJECT 2

Development of Irrigated Lowland Rice Varieties

Emily C. Arocena

Breeding for irrigated lowland culture has become more critical as the need to obtain higher yields becomes more relevant in addressing the Philippine rice security and improving the lives of farmers. Moreover, the irrigated lowland areas are the most reliable ecology comprising 71% of the total harvest area of developing new intensive–culture rice crops. These areas are invariably market-oriented, and farmers are looking to make a better profit by investing in their farms in ways that will maximize the returns of their investments. The transplanting method prevails, but the utilization of a direct seeding system is becoming widespread, especially with the advent of mechanization. Incorporating the yield-enhancing traits for high yield potential, stabilizing yield by improving resistance to major biotic and abiotic stresses, and enhanced resilience to climate change are ways to increase the productivity and income of the farmers.

With the advent of modern and innovative technologies, variety development based on genetic enhancement and extensive phenotyping allowed the development of future rice ideotypes. The project aimed to develop appropriate inbred and specialty type rice varieties with high and stable yield, resistant to major biotic and abiotic stresses, with good grain quality and value-added traits, better resiliency to changing climate and shifting production constraints in highly intensive production systems, and implement a systematic approach for early generation screening and selection, and yield performance testing for multi-location trial.

Components of irrigated lowland variety development for direct-seeded, transplanted, and specialty types include the selection of parents, hybridization, and pedigree nurseries for trait discovery and line development and subsequent performance trials such as the ON, Preliminary Yield Trial (PYT), and General Yield Trial (GYT). These component activities were conducted to identify advanced lines with higher yield than the check varieties, comparable or better-desired specialty traits, and better agronomic performance for advancement to Multi-environment Trial (MET) and the NCT.

Varieties and breeding lines with the desired agronomic and specialty traits were selected for hybridization. There were 51 new crosses made, and 54 true F1s were identified for other line selection to be advanced to the pedigree nursery. The segregating generations in the pedigree nursery resulted in 4,306 F3-F7 breeding lines for further line evaluation and 233 uniform lines for advancement to ON. Twenty-one (21) were selected as potential anaerobic tolerant lines for use as a potential donor from the uniform lines. Furthermore, 12 promising rice lines with apparent resistance/tolerance to tungro and with resistance against 2-3 different bacterial blight (BB) pathogen races were identified at 21 dpi, and three candidate uniform lines were to be shared in the PBBD crossing block as a donor for tungro and BB resistance.

For direct seeding, the most advanced lines selected were PR 47874-26-1-2-2-2-3-B and PR 48575-20-3-2-1-2-1-B with excellent SV and AG tolerance and for advancement to ON. In contrast, six breeding lines exhibited resistance to lodging with a 1.24kg/cm^2 to 1.51kg/cm^2 push resistance rating.Two ON-direct-seeded (DSR) lines with fair to good AG tolerance were elevated to PYT-DSR, while three entries passed the yield standards and exhibited intermediate push resistance with no lodging incidence. In contrast, the good traits for direct seeding were elevated to GYT.

For transplanted culture, five top-yielding entries that passed the yield standards were advanced to PYT-TPR, while 13 PYT-TPR test entries with more than 10% YA over the corresponding maturity checks were elevated to GYT. From the GYT, 12 test entries with more than 5,000kg/ha yield and $\geq$10% yield advantage and fair to good phenotypic acceptability were identified as potential entries awaiting DS performance to confirm their potential for advanced testing the MET. Identified promising test entries in MET will be nominated to NCT for possible varietal release.

For specialty rice, there were 74 new crosses generated, 38 true F_1 crosses selected, and 1,553 F_3 lines selected for the pedigree nursery (PN). The selection of breeding lines resulted in 2,398 lines for further line evaluation, and 143 uniform lines were advanced to ON. There were 18 A, 11 G, 5 P, and 20 Zn/Fe ON promising lines identified for PYT; 28 A, 16 G, and 12 Zn/Fe lines for GYT, and 29 potential lines awaiting slots for MET evaluation.

Using marker-assisted selection (MAS) for aroma Bradbury gene markers, 813 lines were positive to the fragrant allele. These will be further evaluated in the PN for phenotyping and confirmation of the fragrant trait. There were 16 new marker systems already evaluated and established that can be used in addition to Bradbury fragrant markers for aroma detection.

Hybridization and Pedigree Nurseries

Emily C. Arocena, Oliver E. Manangkil, Wilhelmina V. Barroga,
Sherilyn B. Estrada, and Perfecto S. Ramos Jr.

Irrigated lowland ecosystem contributes about 71% of the total harvested rice in the Philippines. Rice breeding uses conventional and non-conventional methods to develop a new variety. Components of variety development include trait discovery, line development, and performance trials. The success of variety development depends on how rigorous the evaluation and selection are based on the breeding objectives. The study covered trait discovery and line development.

New superior crosses and breeding lines for advance evaluation under irrigated lowland ecosystem were generated.

Fifty-one (51) new crosses were generated using released varieties and breeding lines with high yield and biotic and abiotic tolerance backgrounds. In the F_1 nursery, 54 F_1s were found true hybrids and superior over their female parent for F_2 nursery. Pedigree lines were established and evaluated under DSR and Transplanted (TPR) methods. Selection among and within lines was based on phenotype, maturity, uniformity, reactions to abiotic and biotic stresses, and kernel quality. In 2020DS, 3,900 entries were established in the F_2 to F_4 nurseries, and 2,873 plants/lines were selected with phenotype based on breeding objectives. From F_5 to F_{10}, 602 lines were selected for advanced evaluation and 212 uniform lines for performance trials. In 2020WS, 3,470 entries were evaluated with 687 selections from direct-seeded F_2 to F_4, 144 from F_5 to F_7 nurseries for advance evaluation, and 28 uniform lines for performance trial in 2021DS. There were 28 DSR uniform lines selected as potential anaerobic tolerant lines for use as a potential donor and ON evaluation for 2021DS.

Early Seedling Vigor, Anaerobic Tolerance and Lodging Resistance Evaluation

Oliver E. Manangkil, Wilhelmina V. Barroga,
Perfecto S. Ramos Jr, and Ayra B. Rafael

Early seedling vigor, anaerobic tolerance, and lodging resistance are essential traits in direct seeding. During crop establishment, flooding affects germination and seedling survival when sowing coincides with the rainy period or when the field is not well-leveled. Strong wind causes lodging of rice plants which affects the color and flavor of the rice grains. The effects of flooding during crop establishment and lodging at flowering to maturity limit farmers' adoption of direct seeding, especially during the rainy season. The study identified breeding lines with high seedling vigor (SV), anaerobic tolerance (AG) under field and screen house conditions, and lodging resistance.

Under field conditions, 74 F_2 populations and 260 breeding lines were evaluated. There were seven populations and 15 breeding lines with excellent early seeding vigor and anaerobic tolerance, while 11 populations and 31 breeding lines with good ratings were identified. One hundred eight (108) breeding lines were evaluated under screen house condition, which resulted in five breeding lines with intermediate anaerobic germination tolerance. PR 47874-26-1-2-2-2-3-B, a promising entry, had excellent early SV and AG tolerance under field conditions and medium tolerance under screen house conditions. The line has intermediate resistance to bacterial leaf blight and resistance to blast. In addition, PR 48575-20-3-2-1-2-1-B had excellent early SV and AG under field conditions with a yield of 7,291kg/ha. Lodging resistance evaluation in the DS was not done due to force majeure. Most of the entries were lodged in the WS due to heavy rains, but six breeding lines exhibited resistance to lodging with pushing resistance of 1.24kg/cm^2 to 1.51kg/cm^2. These breeding lines are potential donor parents for hybridization and entry for performance trials.

Marker-Assisted Selection for Pest and Disease Resistance Genes in Inbred Rice Variety Development

Arlen A. Dela Cruz, Rustum C. Braceros, Ma. Johna C. Duque, Mary Joyce G. Yapchiongco, and Thelma F. Padolina

This study aimed to provide an opportunity for the effective control of BB and tungro by enhancing the plant pest and disease resistance through the development of MAS-bred rice lines pyramided with various combinations of resistance genes. Individual plants were genotyped using molecular markers tightly linked to resistance genes *tsv1*, *Xa4*, *xa5*, *Xa7,* and *Xa21*, and *Glh14* resistance locus, and phenotyped for reactions to three different BB pathogen races *PXoo79, PXoo99,* and *PXoo340*, green leafhopper (GLH), and tungro viruses under screen house condition. In separate phenotyping setups, representative plants were inoculated with BB pathogens by leaf clipping method, or with tungro viruses by forced tube virus inoculation, or tested for reactions to GLH by antibiosis test. ELISA was also performed to quantify amounts of the tungro viruses in rice plants exhibiting slight tungro symptoms such as slight height reduction or yellow or orange leaf discoloration.

In 2020DS, 1,572 individual MAS-bred rice plants were genotyped from the materials established in the field and screen house. The genotypic screening for the presence of target resistance genes and phenotyping for reactions to BB and tungro were only partially completed due to the lockdown. Despite so, the data gathered were still used to select materials for advancement in the 2020WS. The screen house evaluation of MAS-bred rice lines for reactions to BB, tungro viruses, and GLH during the 2020WS resulted in selecting 12 promising rice lines with apparent resistance/tolerance to tungro and with resistance against 2-3 different BB pathogen races at 21 dpi. The agro-morphological characteristics of representative rice lines showing uniformity in the field or screen house setups were also evaluated. Three candidate lines were identified for sharing in the PBBD crossing for use in hybridization.

PROJECT 2

Field Performance Trial of Advanced Breeding Lines

**Emily C. Arocena, Oliver E. Manangkil, Gloria M. Osoteo,
Wilhelmina V. Barroga, Henry T. Ticman, Martha V. Chico,
Perfecto S. Ramos, Jr., and Sherilyn B. Estrada**

Uniform lines derived from the pedigree nursery with desirable morpho-agronomic traits such as yield, maturity, plant height, productive tiller, phenotypic acceptability, and reaction to field insect pest and diseases were evaluated under both irrigated transplanted and direct-seeded conditions. Promising lines identified based on yield performance, and agronomic traits from each of the ON, PYT, and GYT were nominated to the succeeding trial evaluation and nominated to MET or possible entry in the NCT.

The DS trial was put on force majeure due to the COVID-19 pandemic lockdown. Promising advanced lines were identified from the WS trial; however, monsoon and continuous rains brought by typhoons that occurred during the season affected the yield performance of the test entries. Despite these conditions, promising lines were identified for advancement in each of the succeeding nurseries. From ON-DSR, PR 48575-20-3-2-1-2-1-B and PR47887-10-1-1-2-2-3-1-B with more than 7,000kg/ha yield and fair to good AG tolerance suitable for direct seeding were elevated to PYT-DSR. From the ON-TPR, five top-yielding entries that passed the 5,000kg/ha yield and 10% yield advantage over the checks were also identified for advancement to PYT-TPR. From the PYT-DSR, selected three entries passed the yield standards and exhibited intermediate push resistance with no lodging incidence, good traits for direct seeding. These entries, together with the 13 top-yielding entries from the PYT-TPR, were elevated to GYT. From the GYT, test entries with more than 5,000kg/ha yield and with fair to good phenotypic acceptability were selected. These will be further evaluated for DS performance to confirm their potential for advance testing in the MET.

Evaluation and Utilization of Fragrance Markers for High-Yielding Aromatic Breeding Materials

Teodora E. Mananghaya, Rachelle P. Mallari, Henry T. Ticman, Martha V. Chico, and Dionicko R. Arceo

The demand for aromatic rice has increased significantly in recent years. However, the yield performance of traditional aromatic rice varieties is exceptionally low. The fragrant trait is recessive, and the breeding of aromatic rice needs extensive labor and involves large numbers. Thus, molecular markers associated with the fragrance trait will assist rice breeders in developing a high-yielding variety with good grain quality through a simple and inexpensive method. This study aimed to evaluate a marker system to be used in MAS for fragrance traits in rice and thus in an aromatic breeding program. The breeding materials were established in the field, and leaf samples from individually tagged plants were collected prior to a fragrance gene analysis. Of the 2,420 aromatic breeding materials genotyped in 2020 DS and WS using multiplex PCR fragrance gene markers (Bradbury), 813 (34%) were positive to the fragrant trait/allele, while 1,439 were detected negative to the trait. This result closely corresponded to the reported recessive inheritance of fragrance trait in rice. Breeding materials that showed fragrant trait/allele to Bradbury were screened using the INS 3 marker; however, all of the breeding materials tested were negative to the fragrant trait. Sixteen markers were already evaluated and established using Philippine rice germplasm background and can be used in marker-assisted breeding of aromatic rice varieties. DNA genotyping using other fragrance gene markers is in progress.

PROJECT 3

Development of Rice Varieties Adapted to Stress Environments

Norvie L. Manigbas

Field performance of elite breeding lines under irrigated (i.e., control), managed drought, and simulated rainfed conditions resulted in the identification of 70 best-performing lines with yields of approximately 6t/ha; and 1t/ha under irrigated and managed drought, respectively; and 4t/ha and 3t/ha during WS under irrigated and rainfed, respectively. These lines were ready for MET for abiotic stress locations. Submergence tolerance breeding identified 11 promising lines for another season of evaluation. Drought tolerance screening at seedling to early vegetative stage identified a total of 102 (44%) tolerant lines that can withstand water deficit for 24 days from drought imposition until plant re-watering. The identified tolerant lines had up to 138% plant recovery advantage compared with tolerant check (PSB Rc 14) with 43%. For $F_{2:3}$ segregating population coming from 13 potential crosses, 4,634 (46%) were resistant to drought. Under mass screen for reproductive drought, 280 (30%) test entries yielded higher than the lowest yielding check, IR64, while 105 (11%) test entries had yields higher than the mean yield of the top-performing check, PSB Rc 68. Overall, 247 test entries (27%) showed at least 5% yield advantage of the population yield mean; these breeding lines were seed increased in 2020WS for 2021 ON under drought and irrigated conditions. Evaluation of salinity tolerance at seedling stage across breeding lines identified 190 (65%) putatively salt-tolerant distributed as 5 (2%) highly tolerant, 68 (23%) tolerant, and 117 (40%) moderately tolerant. However, 4,288 (37%) plants from 11 $F_{2:3}$ segregating populations also showed tolerance to salt stress. Out of 378 breeding materials evaluated under complete submergence setup, only 49 (13%) lines expressed tolerability under submergence stress having 75-100% plant survival and tolerant check (FR13A) with 94% plant recovery. Also, 11 $F_{2:3}$ segregating population identified 3,947 (36%) tolerant plants, which confer result through genotyping. For multi-trait searching, a parallel approach was implemented, which identified 25 (39%) Philippine Traditional Rice Varieties (TRVs), two (7%) anther culture (AC)-derived lines from Mestiso 55, and 6 (14%) heat-tolerant materials with dual tolerance to drought and salinity stress. Also, two (3%) TRVs showed triple abiotic tolerance to drought salinity and submergence. These breeding materials will be further evaluated to ensure performance stability and, once validated, can be a potential gene source for multi-traits. In the multi-location test for rainfed environment, two lines, PR49035-B-5-4-3-2-3 with 5.46t/ha and PR49036-B-1-4-2-1-3 with 5.4t/ha, exceeded the 5t/ha threshold in the favorable rainfed lowland environment at PhilRice CES. They were significantly different from entries with a yield of less than 4.0t/ha that included all six check varieties. The grain yield performance of the 28 lines and varietal checks in the Sta. Maria's site showed that PSB Rc 14 (1.6t/ha) produced the highest yield but was comparable with almost all lines with grain yields ranging from 0.3 to 1.5t/ha. The lowest yielding lines PR44485-25-

B-B-7-B (0.2t/ha) and PR49036-B-1-4-2-1-3 (0.2t/ha) were significantly different. The yield performance and multiple comparison analysis of 28 lines and checks in Camarines Sur showed that 24 entries were not significantly different (2.2-3.4t/ha) while three lines and one check (N22) at 1.5t/ha to 1.9t/ha were significantly different to highest yielding lines: NSIC Rc 222 (3.6t/ha) and PR49010-B-14-3-3-3-3 (3.2t/ha). In the 2019WS-NO, there were no significant differences noted in grain yield. PR49036-B-1-4-2-1-3, the highest yielding entry (5.6t/ha), was significantly different from 11 entries that obtained a grain yield of 1.9t/ha to 4.2t/ha. Search for combined traits for submergence and salinity tolerance was done by screening 64 TRVs that resulted in identifying 2 and 48 TRVs with 3% and 75% tolerance, respectively. Meanwhile, two and seven TRVs were observed to have 3% and 11% combined tolerance to salinity and drought. A total of 384 pure lines were selected and will advance for uniformity evaluation in 2021DS. Under high-temperature conditions, 121 elite breeding lines were screened where 18 were identified tolerant, 86 intermediate, and 26 susceptible. Fifty-five (55) of these lines had a yield potential of 4.3t/ha to 8.8t/ha. Currently, 10 elite lines were being evaluated in the NCT. The project incorporated the role of men and women researchers and field workers involved in the implementation of the project by actively and equally taking part in the field preparation, seed preparation, data gathering, harvesting, seed processing, and seed storage. Women and men were allowed to be excused from their activities whenever their respective children need attention and care during the pandemic.

Development of Rice Varieties Adapted to Stress Environments

Norvie L. Manigbas, Nenita V. Desamero, Jonathan M. Niones, Christopher C. Cabusora, Luvina B. Madrid, Via Ann C. Marcelo, Raña Mae A. Sumabat, RJ D. Buluran, and Kimberly Rose P. Balmeo

Scanning of TRVs and doubled haploid lines (DHLs) for abiotic tolerance identified 58 (90%) TRVs and 23 (79%) DHLs with tolerance to single and combined abiotic stresses. The conventional breeding approach produced segregating populations and fixed lines with tolerance to abiotic stresses ready for selection and further evaluation and improvement. Field performance of elite breeding lines under irrigated, managed drought, and simulated rainfed conditions identified 70 best performing lines with yields of approximately 6t/ha and 1t/ha under irrigated and managed drought, respectively, and 4t/ha and 3t/ha during WS under irrigated and rainfed, respectively. These lines were ready for MET for abiotic stress locations. Submergence tolerance breeding identified 11 promising lines for another season of evaluation before nomination to the National Cooperative Test for further testing.

Mass Screen for Abiotic Stress (Reproductive Drought)

Jonathan M. Niones, Via Ann C. Marcelo, and Nenita V. Desamero

Water stress, such as soil moisture fluctuations, has a strong negative influence on yield and overall rice plant responses. Breeding for drought-tolerant cultivars needs to target a relatively high yield. Still, the efficiency of screening methods depends on yield and its combination with secondary traits correlated with yield. This study aimed to evaluate and select drought-tolerant lines under reproductive stage screening. A total of 1,109 entries, including 926 test entries and five replicated checks (IR64, PSB Rc 14, PSB Rc 68, NSIC Rc 192, and NSIC Rc 222) were evaluated through two cycles of drought that coincided with heading to maturity. Correlation analysis identified leaf rolling score, leaf canopy temperature, phenotypic acceptability, and maturity as negatively significant to yield ($r = -0.38 – 0.54$). Due to extreme drought stress (10% SMC), only 30% (285) of the test entries produced yield, wherein 247 (27%) obtained a 5% yield advantage over the population mean (6.72g/plant), which will be advance to observational nurseries under drought and irrigated conditions in 2021.

Mass Screen for Abiotic Stress

Norvie L. Manigbas, Nenita V. Desamero, Christopher C. Cabusora,
RJ D. Buluran, Luvina V. Madrid, and Raña Mae Sumabat

Abiotic stress-tolerant varieties are a sustainable strategy to cope and adapt in the climate change-vulnerable rice production areas. To address this, PhilRice implemented a rice breeding program geared towards developing varieties with tolerance to various abiotic stresses such as drought, salinity, submergence, or complete transient flooding. The program employs different breeding approaches such as in vitro culture, in vitro and seed mutation with gamma irradiation, marker-aided selection, and the classical hybridization and selection method.

Effective and cost-efficient mass screens for abiotic stresses are vital for successfully implementing the breeding program for abiotic stress tolerance. In 2020, drought tolerance screen at seedling to early vegetative stage identified 44% or 102 out of 231 tolerant lines that could withstand gradual soil water deficit of 20% to 39% soil moisture content for 24 days from drought imposition until plant re-watering. The identified tolerant lines had a 2% to 138% plant recovery advantage over the tolerant check PSB Rc 14 that recorded a 43% plant recovery. With $F_{2:3}$ segregating populations from 13 crosses, 46% or 4,634 of 10,104 plants screened were identified as putatively tolerant to drought. The salinity tolerance screen of 292 breeding lines at the seedling stage yielded 192 (66%) putatively tolerant lines with five (2%) highly tolerant, 68 (23%) tolerant, and 119 (41%) moderately tolerant. Of the 11,599 plants from 11 $F_{2:3}$ segregating populations, 4,288 (37%) plants exhibited tolerance to salinity stress. The complete submergence tolerance screening of 378 breeding lines identified 49 (13%) putatively tolerant lines, displaying 75% to 100% plant survival compared with the 94% survival of the tolerance check FR13A. The submergence screen of 10,937 plants from the 11 $F_{2:3}$ segregating populations extracted 3,947 (36%) putatively tolerant plants, which were candidates for genotyping to validate the presence of the *sub1* gene. The identified tolerant lines/plants were maintained in the screen house for seed recovery.

To extract breeding lines with multi-stress tolerance, parallel screenings were conducted. The results identified the traits in 25 (39%) Philippine traditional rice varieties, two (7%) anther culture-derived lines from Mestiso 55, and six (14%) heat-tolerant lines exhibiting dual tolerance to drought and salinity stress. In addition, two (3%) TRVs showed triple stress tolerance, i.e., to drought, salinity, and submergence. These breeding materials are subject to further evaluation for field performance and stability of tolerance to abiotic stresses, which eventually may serve as a potential gene source for multi-abiotic stress tolerance.

Multi-Stress Tests of Breeding Lines in Rainfed Lowland

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Rainfed lowland rice areas exhibit multiple stresses such as flooding, drought, and salinity within the same season. Although progress has been made in developing lines tolerant to single stress, varieties adapted to multiple stresses are yet to be established. This study aimed to evaluate and identify lines with adaptation to a minimum of two stresses. Twenty-two (22) lines and six check varieties were tested in three trial sites in Camarines Sur (CS), Ilocos Sur (IS), and Negros Occidental (NO) during the 2019WS and 2020WS, while the Nueva Ecija (NE) test site was used as the control for rainfed lowland. Four distinct mega-environments were identified, wherein the most favorable three test sites (20 WS-NE, 20 WS-NO, and 19 WS-CS) comprised the mildly stressed mega-environment while the 2nd mega environment was composed of 19 WS-NE. Both 19 WS-IS and 19-NO represented unfavorable environments exhibiting both drought and salinity during the trial. The yields of varieties across these environments were analyzed using GxE analysis and identified **PR49036-B-1-4-2-1-3** (2.58t/ha) as the highest yielding variety that was most adapted to the 1st mega-environment; however, it possessed the most unstable yield across sites, based on AMMI Stability Value. **PR49035-B-5-4-3-2-3** (2.41t/ha) was adapted to 19 WS-NE; PR49029-B-9-4-3-1-2 with adaptation to 19 WS-IS; and PR40330-4-2-B-4-3-1 was suited to 19 WS-NO. Moreover, PSB Rc 14 (2.21t/ha) and **PR49012-B-4-2-1-3-1** (2.16t/ha) were identified as high-yielding and stable based on stability indexes.

Combining Submergence, Stagnant and Salinity Tolerance into High Yielding Rice Varieties

Nenita V. Desamero, Christopher C. Cabusora, Raña Mae A. Sumabat, RJ D. Buluran, and Kimberly Rose P. Balmeo

The screening of traditional rice varieties for submergence and saline tolerance as novel sources of genes resulted in identifying putative tolerant lines with either single or combined tolerance. Pure lines derived from the screening will be subjected to further characterization and evaluation before its use as donor parents. Segregating populations generated from crossing submergence and saline tolerant lines with high-yielding varieties/cultivars were also screened for submergence at the vegetative stage, resulting in putative tolerant plants for further selection and evaluation. Tolerant plants were positive to the Sub1 gene by molecular analysis using SC3 and ART5 markers. Fixed recombinant inbred lines were evaluated for yield under non-stress and submerged conditions, from which promising lines were selected for another season of evaluation before nomination to NCT for submergence or multiple stress.

Breeding for Heat-Tolerant Rice

Norvie L. Manigbas, Luvina B. Madrid, and Ferdinand B. Enriquez

Climate change has brought additional pressure to crops, especially rice. Air temperature will continuously rise, including the occurrence of drought and typhoon. An increase in temperature will make rice more vulnerable, especially at 35°C during flowering. High temperature leads to failed fertilization resulting in increased spikelet sterility and eventually 10% to 15% yield decline. Breeding for heat-tolerant lines in the Philippines was conducted to develop rice genotypes that can tolerate and adapt to high temperatures. The generated breeding lines were screened, evaluated, and selected in Cagayan and Nueva Ecija, where temperatures reached at least 35°C. Breeding lines were planted in a staggered manner so that their flowering coincided with the hottest period of the year, between April and

May. Micro-meteorological Instrument for Near Canopy Environment in Rice (MINCER) and Automatic Weather Station were used to monitor temperature, relative humidity, and other meteorological data. High-temperature tolerance at flowering was evaluated based on spikelet fertility. Thousands of breeding lines were screened under high temperature conditions in Nueva Ecija and Cagayan, but due to the COVID-19 pandemic, only advance and stable lines were evaluated. In the DS, 121 elite breeding lines were screened where 18 were identified tolerant, 86 intermediate, and 26 susceptible. Fifty-five (55) of these lines had a yield potential of 4.3t/ha to 8.8t/ha. Currently, 10 elite lines were being evaluated in NCT.

PROJECT 4

PBBD Research and Analytical Laboratory Systems and Maintenance

Frodie P. Waing

Modern breeding tools and technologies are necessary to sustainably enhance the genetic potential of rice for yield and the ability to tolerate biotic and abiotic stresses while improving grain quality. Key laboratories served varied support functions in breeding operations to fast-track and increase breeding and line development precision. To continuously improve and maintain the current laboratory facilities, which require high-throughput equipment and accurate measurement, routine preventive maintenance, repair/services, and calibration are needed. Thus, this project aimed to (1) provide assistance in the improvement of the laboratory facilities for better quality research outputs, (2) establish equipment and chemical inventory database and other IMS-related laboratory management system, and (3) ensure the proper acquisition of chemicals and equipment and disposal of chemical wastes and unserviceable equipment.

Based on the updated general maintenance and calibration plan, a set of laboratory equipment was successfully calibrated and maintained following the set of standards. Moreover, all unserviceable equipment were evaluated. Monitoring and validation for the functionality of weighing balance, freezers, and ovens were regularly implemented. In compliance with housekeeping, routine clean-up and disposal of chemicals were properly implemented. The project facilitated the systematic laboratory management and implementation of the IMS policy of the Institute. This also complemented and provided support to the breeding activities, ensuring quality data outputs. However, due to the imposition of community quarantine resulting from the pandemic, critical processes, especially on calibration and PMS, were greatly affected. Catch-up plans were necessary to provide the need to continuously capacitate the laboratory systems for accurate, precise, and quality data analyses.

PROJECT 5

Basic Seed Production

Wilhelmina V. Barroga

Basic seed production is an integral part of varietal development, and it is inherent support to the NCT. It is the primary source of quality seeds for performance trials and breeder seeds of the National Seed Industry Council (NSIC) accredited rice varieties. It facilitates the rapid release and continuous availability of the original phenotype. Nucleus seed is the highest form of seeds from breeders and allows breeding institutions to reproduce breeder and foundation seeds. Registered seeds are produced from foundation seeds by seed growers for distribution to farmers as certified seeds. Currently, nucleus and breeder seeds of released varieties developed and bred by PhilRice, University of the Philippines Los Baños, and International Rice Research Institute (IRRI) were grown by the Plant Breeding and Biotechnology Division. Under the nucleus seed production (NSP), 68 varieties were established in 2020DS and 35 varieties with 14,000 panicles were submitted for 2020WS breeder seed production (BSP), while 30 varieties with 12,000 panicles were provided for 2021DS BSP. In 2020DS, 29 varieties composed of 18 irrigated lowland (IL) and 11 adverse (ADV) in BSP produced 6,045kg seeds for IL and 1,647kg for ADV varieties with efficiency passing rates of 89% and 73%, respectively. There were 32 varieties produced for BSP composed of 21 IL and 11 ADV varieties, which had 3,867kg and 706kg, respectively. Laboratory analysis of varieties for BS applied in National Seed Quality Control Services (NSQCS) is ongoing. Seeds produced cater to the seed requirements of RCEF. The seed component is vital in RCEF to attain its objective in increasing farmers' yield and income.

PROJECT 6

Centralized hybridization, F1 and F2 seed generation

Frodie P. Waing

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. A hybridization program must have clear objectives and a set of priority characters. Thus, this project aimed to (1) facilitate the generation of target crosses for irrigated and unfavorable breeding programs under PBBD and (2) establish F1 and F2 populations for breeders' evaluation and selection. The project had successfully implemented the hybridization process in an organized and systematized manner in a centralized facility. Through collaborative efforts, a centralized and harmonized breeding operation was implemented to support the mainstream breeding projects towards developing rice varieties (both inbred and hybrid) adapted to various ecosystems.

Gene Mining of Yield-related Traits in Philippine Rice Landraces

Joanne D. Caguiat, John Oscar S. Enriquez, Gelyn D. Valida, April T. Badajos, Dindo A. Tabanao, and Jonathan M. Niones

Philippine rice germplasm offers a genetically diverse source for yield-related traits. The mining of genes/QTLs found in the Philippine rice germplasm will enable us to utilize these traits to breed and improve rice varieties. To assess the diversity of yield-related loci in Philippine rice varieties, 11 allele-specific SNPs targeting genes *Grain Number 1 (Gn1), Grain Size 5 (GS5), SPIKE, Grain Number and Heading Date 7 (Ghd7), and Thousand Grain Weight 6 (TGW6)* were used for the molecular genotyping of 450 traditional and modern rice varieties. Only functional alleles of *Gn1a* (Habataki), *SCM2* (Habataki), and *Ghd7* (*indica)* were abundant in the panel. Genome-wide association (GWA) was performed to identify the causative loci controlling for yield-related traits in the association panel. Using Genotyping-By-Sequencing (GBS), 334,473 SNP markers were identified and used for association. Best Linear Unbiased Prediction values of phenotype data for yield-related traits were obtained and used for GWA. The Factored Spectrally Transform Linear Mixed Model (FaST-LMM) was used to associate false-discovery rates corrected using the Bonferroni method. Overall, 47 QTNs were identified: plant height-10, fertility-4, panicle length-15, panicle primary branching-1, secondary branching-2, grain number-1, thousand grain weight-4, tiller number-2, grain length-6, and grain width-2. Hypothesized location of genes/QTLs in the QTN's region was identified, and known genes such as *Gn1a* and *WFP* were found to be near the identified SNPs. Due to the pandemic, genotyping activities were delayed, which also affected GWAS and gene expression activities. Putative genes identified will be verified through DNA sequencing or through *in silico* methods.

Genetic Improvement and Mechanism of Resistance to Stemborer in Rice

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Before the identified TRVs can be utilized as a genetic resource for insect pest resistance, a series of investigations must be carried out. This includes the establishment of information in mechanisms of resistance and their genetics of traits. The information generated can be used as baseline data and a guide for developing breeding lines having resistance genes to stem borer.

In this project, 73 germplasms were continuously evaluated for five years in the field for natural stem borer infestation at PhilRice CES, Agusan, and Midsayap. This will provide information on which germplasm has a consistent resistant reaction. Based on continuous field evaluation, five germplasm were identified as having a resistant reaction, and these were subjected to antibiosis. A preference test, an exploratory research for white stem borer (WSB), was carried out to develop an alternative selection process for identified resistant germplasm and for developed advance breeding lines.

To establish information for the possible mechanism and nature of resistance, morphological, physiological, and biochemical classifications were also determined. Published molecular markers linked to yellow stem borer (YSB) were also validated to detect the susceptible and resistant genotypes. The two identified resistant germplasm from antibiosis analysis, namely Red18 and Inarciaga, were used to generate breeding lines using three known modern varieties, namely PSB Rc 10, NSIC Rc 216, and NSIC Rc 298.

For validation and confirmation of resistance/tolerance reaction of 73 germplasm, PhilRice Agusan identified 16 resistant and nine moderately resistant during the DS. For morphological characterization, in comparison with TKM6 (resistant check), Inarciaga, Red18, C4-Dinorado, and Mukol germplasm had the same pubescent traits; Inarciaga, Red18, C4-Dinorado, and Mukol had erect flag leaf attitude. For trichome orientation, Inarciaga, Red18, C4 Dinorado, and Mukol were identified. For physiological classification, compared with TKM6, Dinorado had the thickest epidermal lignin/layer while Red 18 had the thinnest, while the germplasm Inarciaga had the highest mean lignin content. The germplasm Dinorado, C4-Dinorado, Inarciaga, and Hinumay were classified as having the highest klason lignin, while Dinorado was identified as having acid soluble in comparison with the other six TRVs.

Among the markers validated, RM211 showed distinct separation banding patterns between resistant and susceptible genotypes. For developing tolerant lines against stem borer, among F_5 population evaluated, 168 entries were identified to have a resistant reaction while 54 had moderately resistant. Among the five lines considered for homogenous selection, two lines already exhibited uniformity and had a high resistant reaction.

Collection and Evaluation of Farmers' Rice Lines in Irrigated and Rainfed Lowland Areas in the Philippines

Joanne D. Caguiat, John Oscar S. Enriquez, Xavier Greg I. Caguiat, Norvie L. Manigbas, and Sailila E. Abdula

Varietal identification or characterization has been one of the critical steps of crop improvement. It is imperative to rapidly identify and characterize plant varieties to provide valuable information for their introduction and possible improvement. This study aimed to establish the genetic identity of farmers' rice genotypes (FRG) and Released Inbred Varieties (RIVs). DNA fingerprint of the assembled rice panel was established using 93.3% or 6,623 out of 7,098 SNP markers distributed across the rice genome. Generally, narrow genetic diversity was observed among the farmers' rice genotypes (FRG). A total of 6% of the collected FRGs were identical, implying the similar preferences of farmers despite the different labels/names of rice varieties. Most of the FRGs possessing similar names, such as "Diamond," were found to be identical despite being collected from multiple provinces. About 7% were identified as similar to NSIC Rc 218 (known for its aroma and very soft texture when cooked), while 8% were found to be related to PSB Rc 10, a famous early-maturing variety. In comparison, 11% FRG had 95% to 98% similarity with NSIC Rc 222, popularly known to be one of the highest yielding released varieties. Efficient naming and varietal identification system of RIVs in the Philippines is important and should be reconsidered for the farmers to easily retain the real identity of these varieties used in the field. Relying on farmers' non-standard nomenclature system is not reliable since most of these were based on farmers' preference or the branding used by the marketing entity. There is a need to establish a standard reference system using molecular markers to back up the phenotypic evaluation or grow-out data. Furthermore, policies must be updated to address the utilization of non-accredited seeds and protect intellectual property.

Field Performance Evaluation and Selection of GUVA Lines in the Tropics

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The Germplasm Utilization for Value Added (GUVA) project, in collaboration with IRRI and RDA (Rural Development Authority)-Korea, had continuously provided Japonica and Indica-Japonica germplasm relevant to the breeding programs of PhilRice. In 2020, breeding focused on improving cold tolerance targeted for high elevation areas in the country. Yield improvement, grain quality, and blast resistance were equally considered in the process. Promising lines identified with these set objectives are potential nominations to NCT and pre-breeding materials or donor lines in the PhilRice crossing program.

FINDINGS

PhilRice Maligaya

Seventy-two (72) new sets of GUVA lines, including check varieties, were established in PhilRice CES in 2020DS. Partial performance evaluation was the only accomplished source for the 2020WS re-evaluation. Available seeds produced ranged from 1,690 to 3,200g. Two highlighted lines IR31012-B-39-1-2-3 and 15K1494 had the most seeds of at least 3 tons and were observed to have resistance to yellow stem borer and exhibited high phenotypic acceptability.

In the WS, the same 72 GUVA lines were re-evaluated in the preliminary yield trial (PYT). The majority of the test entries were sister lines, with 37 unique cross combinations. The yield ability was assessed using two replication data with resulting mean yields ranging from 1,615 to 4,232kg/ha. In contrast, the Japonica checks had 2,510 to 4,194kg/ha. There were 14 lines with an output of at least 3t/ha and four entries with 4t/ha and above, namely: 1 IR 130992-B-44-3-1-1(4,131kg/ha), IR 130996-B-55-3-2-3 (4,232kg/ha), IR 131022-B-23-2-3-1(4,024kg/ha), and IR 131073-B-36-3-1-B (4202kg/ha).

BSU, La Trinidad, Benguet

During 2020DS, duplicate entries were established in Benguet State University (BSU), La Trinidad. A preliminary yield assessment was done, and yields were dismally low owing to drought brought about by the lockdown. Nevertheless, the yields recorded were from 219.7kg to 1,884kg. Only 14 lines were highlighted with yields of more than 1t/ha. Leaf blast screening was not evaluated due to pandemic;

however, panicle blast was successfully assessed. The screening identified 23 resistant lines with an SES rating of 0 to 3, while 9 lines exhibited intermediate resistance with a score of 5. These entries also showed good seedling vigor and were acceptable phenotypically. However, symptoms indicating the seedling's cold tolerance were missed.

Blast resistance and cold tolerance screening were done at the seedling stage during the WS. Thirty-nine entries exhibited fair to high seedling blast reaction, with 11 entries on the resistant side. For cold tolerance, tolerant to moderate reactions were recorded from dough to maturity stage. Yield data is not yet available.