2023 PhilRice R&D Highlights

CROP BIOTECHNOLOGY CENTER

Philippine Rice Research Institute
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Crop Biotechnology Center

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EXECUTIVE SUMMARY

The Center focused on applying advanced technologies combined with experimental and computational methods to discover, quantify, and validate important traits that contribute to increased yield, value-added rice, enhanced varietal development for grain quality, and climate change resiliency. It helped ensure safe and efficient operation of laboratories by keeping all critical laboratory contraptions calibrated and duly maintained, all laboratory personnel properly oriented, and all wastes managed. It also continuously contributed to the enhancement of knowledge and skills in various biotechnology fields of partner-agencies, academic institutions, and even the general public through collaborative research projects. It conducted training sessions, hosted on-the-job trainees, guided facility tours for PhilRice visitors, as well as shared relevant information and biotechnology updates on social media.

One of the Center's projects has identified rice varieties with potential resistance to rice tungro bacilliform virus (RTBV) and lines with resistance genes against brown planthoppers (BPH). Progress was also made in producing antisera for the immunological detection of RTBV and rice tungro spherical virus (RTSV) in rice plants as a tool for tungro disease surveillance and detection. New primers targeting RTSV coat protein (CP) genes and optimized Polymerase Chain Reaction (PCR) conditions were also designed to overcome challenges in detecting Philippine tungro virus isolates using PCR. Significant contributions were also made in optimizing protocols for resistance and tolerance screening against green leaf hoppers (GLH) and BPH.

Gene editing along with classical biotechnology approaches were the features of another project that aimed to produce improved lines for abiotic stress tolerance. The team generated a strain of Agrobacterium transformed with gene for stress adaptability and productivity in rice.

Future researches will soon benefit from DA-CBC's microbial preservation facility with the progress in its establishment, including the Center's microbial resources wherein viable cultures with validated plant growth-promoting traits are now stored.

CBC-231-000

CBC Research and Analytical Laboratory Systems and Maintenance

Roel R. Suralta

The Center prioritized the safety of its laboratory personnel and efficient research, even with limited resources. To ensure safety, the Center has implemented protocols including orientation sessions for new staff, trainees, and student interns to familiarize them with laboratory facilities, equipment, and procedures. It also ensured proper waste disposal and maintained a meticulous inventory system for cost-effective use of chemicals and supplies.

In 2023, the Center actively shared its expertise by conducting training programs, namely: Basic Molecular Technique, which was attended by 37 participants. These sessions equipped participants from universities and government agencies, namely: Bulacan State University, Nueva Ecija University of Science and Technology, UP Los Baños, Philippine Carabao Center, and DA-Regional Field Offices 1 - 3 and 5 - 6. Further, the Center hosted 19 on-the-job training sessions for Visayas State University, Mariano Marcos State University, Mabalacat City College, and Central Luzon State University. These training opportunities provided valuable platforms for participants to enhance their skills and knowledge in molecular techniques, contributing to the advancement of molecular research and application across academic and partner-institutions.

CBC-232-000

Molecular Approaches for Improving Rice Pest and Disease Resistance

Arlen A. Dela Cruz

The project aimed to improve rice production and food security using molecular approaches to develop rice with durable resistance to pests and diseases. It comprised six studies, each focusing on different aspects of rice resistance development, gene discovery, marker development, and the development of diagnostic tools for improved detection of rice viral infections.

This year, nine rice varieties with potential resistance to RTBV were initially identified: Rc82, Rc238, Rc354, Rc358, Rc400, Rc416, Rc438, Rc442, and Rc18. These will serve as potential candidates for selecting recurrent parents in breeding rice with durable tungro resistance. Moreover, three candidate donor rice lines

were selected for use in rice breeding (MAS-205-3-3-2, F10-22, and F10-23). They possess moderate resistance or tolerance to tungro and bacterial leaf blight (BLB) and contain various combinations of bacterial blight resistance genes.

For the development of rice with enhanced resistance against the Brown Plant Hopper (BPH), 87 candidate donor lines for BPH resistance genes were screened, leading to the identification of six lines containing the target genes Bph3, Bph9, and Bph17. These lines will undergo further phenotyping to validate their resistance. Single crosses were also generated between BPH-resistant donor lines and NSIC Rc 402, generating 740 F1 seeds for future double cross-hybridization. Meanwhile, among the rice germplasm screened, 12 were recognized as potential sources of novel BPH resistance genes, and these will be further examined. In the development of molecular diagnosis and detection tools for enhanced tungro surveillance, monitoring, and breeding, significant progress was made in producing antisera for the immunological detection of RTBV and RTSV in rice plants. This includes the successful generation of two recombinant bacteria expressing antigenic RTBV CP and RTSV CP by gene synthesis for the recombinant protein-based antisera production. Also, 1kg each of RTSV-alone and RTBV-alone-infected rice plants were produced for the traditional purified virus-based antisera production. Modifications using RTBV- or RTSV-resistant rice breeding in virus mass production are being explored to facilitate the rapid multiplication of the desired tungro virus.

The successful generation of cloned tungro virus coat protein (CP) gene sequences for diversity analysis, overcoming challenges in cloning and transformation, resulted in establishing the diversity of tungro viruses maintained in the PhilRice Central Experiment Station (CES) screen house, which had been used in evaluating reactions to tungro in rice germplasm. In the analysis of 40 cloned RTBV-CES CP1 gene sequences, these showed at least 90% similarity with RTBV-G2, China isolates, the RTBV DNA-complete genome, RTBV Padang isolates, and RTBV ORF234 genes. Meanwhile, for RTSV-CES, the 25 CP2 gene sequences obtained were 94.9 - 100% similar to the RTSV CP gene, RTSV Vt6 strain, pe21iii CP2 gene, and pc03vi CP2 gene.

We intended to establish the whole genome sequences of these PhilRice CES RTBV and RTSV. However, a hurdle arose when the service provider required PCR-amplified DNA fragments for sequencing, as the need to amplify the viral genetic material was crucial, prompting a revision of the approach. Procuring RTBV and RTSV primers, optimizing PCR conditions, and locating service providers caused a deviation from the original timeline and budget. Consequently, the delivery of this output is postponed.

To overcome challenges in detecting Philippine tungro virus isolates using PCR, new primers targeting RTSV CP genes and optimized PCR conditions were designed. Collaborating with a CLSU BS Biology thesis student, 11 sets of primers

were developed based on the genome sequence of the RTSV-A strain, the first identified RTSV in the Philippines. Five of these primers successfully amplified PCR products specific to RTSV CP genes, verified by DNA sequencing. For the CP1 gene, we plan to design new primers based on the Vt6 strain, a highly virulent RTSV strain from Mindanao. Not only that, we initiated the optimization of a multiplex-PCR protocol for the detection ofRTBV and RTSV, aiming to enhance virus detection efficiency and accuracy.

The project is also making significant contributions to the optimization of protocols for insect resistance and tolerance screening against GLH and BPH by further optimizing the antibiosis protocol.

The project's practical application was demonstrated by extending support to partners in the Regional Crop Protection Center Region 3 and PhilRice Crop Protection Division in the diagnosis of diseased rice plants collected from the field with the diagnostic results being used for disease management recommendations.

CBC-233-000

Advanced Biotechnology Tools for Improving Rice Abiotic Stress Tolerance

Reynante L. Ordonio

Rice-growing areas are vulnerable to fluctuations in the environment, especially in terms of water availability, leading to plant stress and subsequent reduction in yield. The estimated yield losses due to drought are around 26% (Palaystat, 2020); thus, the annual production (4.7M tons) is lower than its favorable counterpart, the irrigated lowland areas (14.5M tons). Irrigated fields can experience reduced yields under drought conditions even with water-saving technologies. Additionally, water limitation in irrigated fields is expected due to the increasing atmospheric temperatures and unpredictable rainfall distribution that result in reduced water levels of dams. With climate change, the above abiotic stresses could become worse. It is therefore important to develop better rice varieties with improved tolerance traits that could adapt to these abiotic stresses. Toward this end, gene editing is a powerful and precise tool that can be used to modify abiotic stress tolerance genes in rice that are already characterized by different groups. Other

biotechnological tools like mutagenesis through tissue culture have been proven to produce interesting mutants related to abiotic stresses. Moreover, abiotic stress-related QTLs and/or genes (i.e., regulating promoted root system) that are already available in certain donors can be deployed into different varieties using marker-assisted introgression.

This project aimed to produce improved lines of popular released varieties that carry abiotic tolerance traits through gene editing and classical biotechnology approaches. Currently, one strain of Agrobacterium transformed with the weg1 for root elongation and branching was generated. The weg1 gene produces a high number of L-type (thick, long and branching) lateral roots, which are essential for stress adaptability and productivity in rice.

The K7 and plr1 mutation genes that promote a well-developed root system were likewise introgressed to Philippine genetic backgrounds. In particular, these genes identified through mutant analyses, produce a high number of L-type lateral roots and longer lengths, which are essential root traits for rice adaptation and productivity under drought conditions. The K7 was introgressed to four high-yielding and popular Philippine rice varieties, specifically NSIC Rc160, Rc240, Rc402, and Rc480 whereas the plr1 was introgressed to the same varieties except Rc160.

In this project, we utilized a water culture and a modified rapid generation advancement (RGA) technique as an efficient screening protocol at seedling stage. This is supported by our marker survey wherein the backgrounds don't possess the gene, and thus, the non-branching phenotypes observed in the lines. Currently, 1498, 171, 404, and 1423 F3 lines from NSIC Rc160, Rc240, Rc402, and Rc480, respectively, which are highly branched were selected. Corresponding backcross lines with the K7 mutation gene were also generated, specifically 25 BC1F1. On the other hand, 395, 7 and 103 F3 lines from NSIC Rc240, Rc402 and Rc480, in the same order, were selected. Genotyping of these lines is ongoing.

Furthermore, we validated the fine-mapped region of a QTL associated with root plasticity trait and found 19 candidate genes in the region. We are now progressing the expression studies of these genes to be able to identify the causal gene.

CBC-234-000

Conservation and Maintenance of Genetically Pure Microbial Cultures and other Beneficial Organisms

Arlen A. Dela Cruz

Microbial culture collections are important materials for future research interests. They are natural sources of novel desirable traits such as plant growth-promoting and environmental conservation abilities. Proper and careful characterization and preservation are therefore crucial to maintain their morphology, physiology, and purity, for future research and industrial applications. This project overall aimed to initiate the establishment of a microbial preservation facility in the DA-Crop Biotechnology Center, starting with the optimization of protocols for the characterization and validation of important traits of the center's microbial resources, specifically bacteria with plant growth-promoting traits.

Viability testing of 110 bacterial isolates from previous research projects was revived and stored. These were also identified through 16S rRNA gene sequence analysis and using the BLASTn tool (https://blast.ncbi.nlm.nih.gov/Blast.cgi). Plant growth-promoting traits were tested through five tests/assay, namely: Starch Hydrolysis Test, Phosphate Solubilization Assay, Nitrogen-fixation Test, Indole Acetic Acid Production Assay, and Simmons Citrate Test. These assays found 49 bacterial isolates with at least one plant growth-promoting trait. Thirty-six viable cultures of these isolates are stored with information such as growth media, temperature, and 16s sequence, and are available for utilization in future research. Two preservation methods used to determine duration of viability of isolates included agar slants at 4°C and glycerol stocks at -40°C. Storage in agar slants maintained the isolates for at least 3 months, with most isolates losing viability by 6 months. Storage by glycerol stocks need further testing of viability of the isolates after 4, 5, and 6 months of storage.

RTF-022-354

Communicating Crop Biotech Successes

Roel R. Suralta, Reynaldo F. Diocton IV, Liezel C. Peralta, Erlyn Jane B. Garcia, Rice Denise V. Alcantara, and Precious Mae C. Gabato

The project, carried out from January to July, focused on raising awareness and appreciation for biotechnology through various Information, Education, and Communication (IEC) activities including the National Biotech R4D Agenda Workshop, Regional Crop Biotechnology Symposium, Joint Seminar on Biotechnology, The Biotech Champions, National Best Thesis Competition, Biotech Trivia, Career Orientation for STEM Students, and Post-NBW Assessment aimed to inform the public about the benefits and advancements in biotechnology and its potential contributions to society and engage students, researchers, product developers, farmers, and the general public.

Throughout the 6-month project duration, the team organized and executed a series of IEC activities to heighten biotechnology awareness. By disseminating accurate information and engaging the public in interactive sessions, the project played a vital role in fostering a better understanding of biotechnology's significance.

An increased awareness and appreciation for biotechnology can pave the way for its responsible and innovative application in various sectors, including healthcare, agriculture, and environmental conservation.

The outputs generated, including the partnerships and collaboration, will be utilized by the Center for its continuous preparation. The effective biotech promotional activities and strategies will also be adopted by the Technology Commercialization and Management Service group of the Center for its future initiatives and endeavors.

RTF-022-344

Rationalizing Agricultural Crop Biotechnology R&D Using the Newly-established Crop Biotechnology Center and Facility

Roel R. Suralta, Reynaldo F. Diocton IV, Liezel C. Peralta, Erlyn Jane B. Garcia, Rice Denise V. Alcantara, and Precious Mae C. Gabato

This project was conducted to contribute to the updating of the National Agribiotechnology Agenda to develop several commodity research programs and proposals, and further strengthen the research capability of the Center through laboratory accreditation.

For the National Crop Biotechnology Research for Development and Extension (BR4DE) Agenda, the Center conducted an extensive desktop review to identify the major crop challenges in the DA commodity industry roadmaps. Several potential biotechnology interventions were proposed, and a series of consultation meetings with DA agencies and banner program implementers were conducted for the prioritization of the identified key issues. The assessment on their inputs led to the formulation of the National BR4DE Agenda with three subprograms: Disease and Health Management; Crop Genetics and Breeding; and Capacity and Capability Building. In support of this agenda, the previous agenda implementation was also reviewed that further strengthened the need for the updated goals, program-based proposals, and central information database. Further, these recommendations aim to move forward with the takeaways and suggestions specific for each commodity program.

Relevant to this, the Rice Biotechnology Program Proposal was developed with the help of PhilRice, unleashing the development of four components that were submitted to the National Rice Program and DA-BAR for funding. The program aims to increase productivity and cost-effectiveness of rice farming in a sustainable and environment-friendly manner through the development of high-yielding, disease-resistant, climate change-resilient, high fertilizer-use efficiency, and high-nutrition rice.

On the other hand, the banana and cassava research programs were crafted with the help of the Center's collaborators and experts. The component proposals are underway. To sustain the momentum, potential international collaborations are currently being formalized with the Institute for International Crop Improvement of the Donald Danforth Plant Science Center, Missouri USA for cassava research.

Finally, to further increase the capacity in conducting cutting-edge biotechnology research, the Center applied for and was granted the accreditation of the Biosafety Containment Level 2 Laboratory by the Department of Science and Technology. This milestone will strengthen the position of the Center as one of the agricultural research institutions in the country.