2020
PhilRice R&D Highlights

Crop Biotechnology Center

Contents

Crop Biotechnology Center

Center Director: Dr. Roel R. Suralta

EXECUTIVE SUMMARY

Established through Department of Agriculture (DA) Administrative Order No. 21, series of 2005, the Crop Biotechnology Center implements a rationalized, effective, and efficient AgBiotech R&D agenda for the DA to generate improved agricultural technologies, productivity and enhanced commercial potential value, and activities for crops. The Center focuses on applying advanced technologies combined with experimental and computational methods to discover, quantify, and validate important traits that contribute to yield increase, improve quality of rice (value-adding), and enhance varietal development for grain quality and climate change resiliency. The genes controlling the traits are also dissected to improve screening (phenotyping) systems and selection efficiency in crop breeding systems. It also performs fundamental and applied research in the areas of genome biology, computational biology, genetics and microbial systems, and molecular biology.

For rice-related research, the Center currently has a lone project with several studies dealing with molecular biotechnology techniques and approaches to improve research processes and methodologies. This project on gene discovery and marker development for agronomically important traits examined the genetic composition of rice to accelerate genetic dissection of simple or complex traits, such as agronomic, quality, or resistance to biotic or abiotic stresses, and other beneficial organisms to assess species identity. The final outputs of this project include QTLs/genes/markers for traits useful for different breeding objectives, advanced lines carrying pyramided QTLs/genes, trait-specific phenotyping protocol, modified and/or improved genetic information, and genetic database. These outputs address the Institute's strategic outcomes number 3, 5, 6 and 7.

PROJECT 1

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

Arlen A. Dela Cruz

This project aimed to generate genomic and molecular information useful to rice breeding toward development of promising lines with improved performance by identifying the position of major QTL/gene conferring the desired traits, developing or identification of molecular markers flanking the QTL or gene-based molecular markers that will eventually be useful for marker-assisted introgression of QTL/genes into a rice plant, and also pre-breeding rice plants that carry the important QTL/gene that will be useful as trait sources for rice breeding. This is necessary in expanding the genetic base of the rice germplasm by developing or identifying more sources of astronomically important traits. It was composed of four component studies that specifically focused on QTL mapping for deep-rooting and stay-green traits, grain crack resistance, and tungro resistance. The outputs of this project contribute to the PhilRice Strategic Plan Outcome 5, advanced rice science and technology as continuing sources of growth. This will yield a thorough understanding of genes/QTL that leads to development of effective molecular markers for marker-assisted selection, which has been widely used in breeding to select for traits that are difficult or expensive to measure, exhibit low heritability and/or are expressed late in development.

The first two studies strengthened the understanding of genetic mechanisms underlying root system development in response to drought and soil moisture fluctuation stresses. The first study, “Validation and fine-mapping of root plasticity QTL on lateral root development in response to water stress in Rice”, assessed the effect of lateral root plasticity in relation to shoot biomass using six recombinant inbred lines (RILs) with introgressed “Kasalath allele”. Demonstrating high root plasticity responses to fluctuating soil moisture stress, line-105 maintained its shoot biomass appearing to be the best candidate to be recommended for use in breeding of rice for drought tolerance. The second study aimed to identify RIL with the highest functional stay-green characteristics and identify the associated quantitative trait loci (QTL). Eleven lines were identified with stay greenness of an average duration of 28 days after flowering both in continuously waterlogged and progressive drought conditions, which is 10-14 days longer than their parents. Two QTLs were detected in SPAD on the regions of Chromosome 3 (*qSPAD3*) and Chromosome 12 (*qSPAD12),* with the latter being contributed from Kutsiyam allele known to have the stay-green trait.

As the head rice recovery after milling is affected by several factors such as chalkiness, moisture content, and cracking or fissuring of rice grain, improving the rice genotype by introgression of the gene for crack resistance can help increase the head rice recovery. The third study fine-mapped the major QTL controlling crack resistance into a 517 Kb region of the rice chromosome 3, flanked by SSR markers RM15291 and RM1588. Three of the 26 annotated loci identified along the region of the QTL including *wrky* transcription factor genes, ATP-binding cassette (ABC) transporter genes, and some domain of unknown function (DUF) family proteins have literature showing their possible roles in the mechanism of resistance. Further analyses, however, must be done to confirm the exact function of these genes and the mechanism of resistance.

Meanwhile, among major rice diseases, tungro is considered the most serious viral disease in terms of yield losses. The breeding for tungro resistance remains a great challenge due to the complexity of the disease and the limited availability of resistance donors. The fourth study, "Association of GLH and RTSV resistance to *Glh14*, *tsv1* and *eIF4G* single nucleotide polymorphism (SNP) type in selected popular Philippine traditional rice varieties and mapping of novel GLH and RTSV resistance genes", promoted the use of Philippine traditional rice varieties (PTRVs) as potential sources of novel disease resistance genes. After systematic evaluation for reactions to GLH and tungro viruses, the cultivar Lead exhibited the most notable GLH resistance and was considered as the most promising PTRV for mapping of novel GLH resistance QTL. On the other hand, the GLH antibiosis results fitted the 1 resistant : 3 susceptible ratio, implying that the gene controlling the GLH resistance of the PTRV Lead may be a single recessive gene. The initial QTL and linkage analysis showed relative peaks on rice chromosomes 1, 2, 6, and 9.

Validation and fine-mapping of root plasticity QTL on lateral root development in response to water stress in Rice

Jonathan M. Niones, Roel R. Suralta, Teodora E. Mananghaya, Antoinette S. Cruz, Rachelle Mallari, Ricky Jay M. Gonzaga, and Via Ann C. Marcelo

Soil moisture stresses at varying degrees of stresses limit crop productivity in rainfed lowland rice systems. Lateral root (LR) plasticity plays a significant role for rice adaptation in response to soil moisture stresses. LR significantly enhances water uptake and nutrients that leads to the maintenance of plant biomass. This study aimed to narrow down the identified QTL associated with lateral root development using Nipponbare*2/CSSL47 mapping population. Four identified QTLs on chromosome 12 regions (qTLRN12.1, qTLRN12.2, qTLRN12.3, and qLLRN12.1) were associated with lateral root plasticity under soil moisture fluctuation stress. Moreover, the qLLRN12.1 location was narrowed down the distance approximately to 302,500bp on the target gene from 3,850,000bp. Six recombinant inbred lines (RILs) with introgressed "Kasalath allele" were further assessed for the effect of lateral root plasticity in relation to shoot biomass. Among RILs, line-105 demonstrated high root plasticity responses to fluctuating soil moisture stress that led to maintaining its shoot biomass. Line-105 would be the best candidate to be utilized for drought tolerance breeding program.

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

Roel R. Suralta, Chief SRS, Jonathan M. Niones, Antoinette S. Cruz,
and Ricky Jay M. Gonzaga

Stay-green is the capacity of plants to postpone senescence during post-anthesis. Under drought, functional stay-green plays a significant role in the efficient filling of spikelets. Stay- green results in a slower decrease of chlorophyll content, extending the photosynthetic activity and assimilatory capacity of the plant leading to higher grain yields. This study aimed to identify RIL with the highest functional stay-green characteristic and identify the associated quantitative trait loci (QTLs). One hundred twenty-one lines derived from the NSIC Rc160/Kutsiyam crosses were subjected to continuously waterlogged (CWL) and progressive drought (PDR) using bucket method system. Eleven lines were identified, which greeness stayed an average duration of 28 days after flowering both in CWL and PDR conditions. Moreover, these lines stayed their leaf greenness 10 to 14 days longer than their parents in CWL and PDR, respectively. Also, RIL-140 under PDR and RIL-196 under CWL surpassed both parents' SPAD value after heading. Two QTLs were detected in SPAD on the regions of Chromosome 3 (*qSPAD3*) and Chromosome 12 (*qSPAD12)* between markers RM411 (21,430,757bp) and RM16238 (36,276,109bp), and markers RM28166 (17,613,713bp) and RM519 (19,932,321bp), respectively. The *qSPAD12* was contributed from Kutsiyam allele, which is known to have the stay-green trait. Saturation and addition of markers on the regions where QTLs detected will be the next step to narrow down the distance of the target functional stay-green QTLs.

Novel Gene Identification for Rice Grain Crack Resistance

Verna G. Dalusong, Rachelle P. Mallari, Teodora E. Mananghaya, Arvin Paul P. Tuaño, Loida M. Perez, Bienvenido O. Juliano

Head rice recovery after milling is affected by several factors such as chalkiness, moisture content, and cracking or fissuring of rice grain. Proper post-harvest practices in drying, storage, and milling can prevent losses due to broken grains. Increasing the resistance to grain cracking by introgression of the gene that controls the trait will also increase the head rice recovery. Major QTL found on chromosome 3 was fine-mapped to identify and locate the precise position of gene(s) involved in crack resistance. Results of analysis mapped the QTL on chromosome 3 in the interval between RM15291 and RM1588. The region of QTL on chromosome 3 was narrowed down from 7.31 Mb to 517 Kb. Twenty-six annotated loci were identified along the region of the QTL. Three of these annotated loci including wrky transcription factor genes, ATP-binding cassette (ABC) transporter genes, and some domain of unknown function (DUF) family proteins have literature showing their possible roles in the mechanism of resistance. Further analyses should be done to confirm the exact function of these genes and the mechanism of resistance. Understanding the function of these identified candidate gene loci is very essential in successful breeding activities. Furthermore, it will benefit the agricultural industry in breeding rice varieties with increased head rice recovery through molecular breeding.

Association of GLH and RTSV resistance to Glh14, tsv1 and elF4G SNP type in selected popular Philippine traditional rice varieties and mapping of novel GLH and RTSV resistance gene

Arlen A. Dela Cruz, Verna G. Dalusong, Ma. Johna C. Duque,
and Mary Joyce G. Yapchiongco

Tungro is considered the most serious rice viral disease in terms of yield losses, and the breeding for resistance remains a great challenge due to the complexity of the disease and the limited availability of resistance donors. The green leafhopper (GLH) *Nephotettix virescens* is the most efficient vector in the semi-persistent transmission of the tungro viruses. In the current PhilRice breeding initiatives, the Glh14 resistance locus from ARC11554 cultivar has been widely used in improving the resistance of rice breeding lines. With the aim to further promote the utilization of the Philippine traditional rice varieties (PTRV), which were reported as potential sources of useful traits including novel resistance genes, this study aimed to examine selected PTRVs systematically by evaluating reactions to GLH and tungro viruses separately. In the series of screening for reactions to GLH, the PTRV Lead exhibited the most notable resistance; hence, an F2 mapping population was generated from it with TN1 as its susceptible partner.

For the GLH antibiosis test, 18-day-old seedlings were individually added with five 2nd-3rd instar nymphs in a test tube, and the nymph mortality was evaluated daily for five consecutive days. Results showed that 57 plants exhibited moderate resistance to resistant reactions to GLH while 195 plants appeared susceptible. The population phenotypic segregation fitted the 1 resistant is to 3 susceptible ratio, which apparently leads to the hypothesis that the gene controlling the GLH resistance of the PTRV Lead is a single recessive gene. The initial QTL and linkage analysis, based on ICIMapping v4.2 and Win QTL cartographer v2.5, showed relative peaks on rice chromosomes 1, 2, 6, and 9. At least 60 evenly-spaced SSR markers will be used in the final analysis to increase further of the LOD values to 2.5, which is the ideal threshold for identifying QTL.

Nutritionally Enhanced Rice: Finishing and Delivering Golden and High-Iron and Zinc Rice Varieties

Reynante L. Ordonio, Marissa V. Romero, Jonathan M. Niones, Trinidad C. Fernando, Ronalyn T. Miranda, Luilene A. Miranda, Mark Philip B. Castillo, Rhona Jane Fabros, Rodel M. Bulatao, Gerome Corpuz, and Maricar Castillo

The Healthier Rice Project, also known as the Golden Rice Project, is composed of three teams of different specializations, namely: Research, Deployment, and Communication and Stakeholder Engagement teams that complement each other to release the micronutrient-dense Golden Rice in the Philippines. The Research team involves Crop Biotechnology Center staff working on conventional and marker-assisted breeding and Rice Chemistry and Food Chemistry Division staff handling the grain quality and sensory evaluations of the rice grains. Golden Rice in different genetic backgrounds is developed and carefully selected by breeders in the team and the grains are passed on for analysis (e.g., total carotenoids and beta carotene content). To ensure that Golden Rice will be successfully deployed to the target areas, the Deployment team from the Socioeconomic and Business Development Divisions will facilitate survey activities at the farm, market, and consumer levels to receive feedback from different stakeholders as well as to craft the pilot-scale deployment plan for Golden Rice. They will also lead the implementation and monitoring of the pilot-scale production and consumption of Golden Rice. The Communication and Stakeholder Engagement team from the Development Communication Division develops and implements activities in support to both the Research and Deployment teams. The team helps to establish a favorable political and regulatory environment for Golden Rice, leading to regulatory approvals and wide public acceptance of Golden Rice as a complementary intervention to combat vitamin A deficiency.

The Research Team had three major accomplishments. First was the securing of permit for the direct use as Food, Feed, or for Processing of Golden Rice issued on December 10, 2019 by the DA-BPI. This permit allowed for the initial testing of sensory properties of Golden Rice and will also give way to a series of nutritional studies. Second was the certificate of completion for the field trial of Golden Rice Event E issued on August 26, 2020, which indicated that Golden Rice had been assessed to be safe not only to health but also to the environment. The application for the commercial propagation of Golden Rice had been submitted to the DA-BPI on October 6, 2020 for their review and approval.

Development of provitamin A-enriched and disease-resistant rice lines through marker-assisted breeding

Reynante L. Ordonio, Trinidad C. Fernando, Ronalyn T. Miranda, Luilene A. Miranda, Mark Philip B. Castillo, Rhona Jane B. Fabros, and Marissa V. Romero

By building on the upcoming Golden Rice variety, this project addresses the persistent and pervasive problem of Vitamin A deficiency in the country and, at the same time, makes Golden Rice more profitable to farmers by adding resistance against two of the most important diseases in the Philippines today — tungro and bacterial leaf blight (BLB). Tungro, a condition brought about by the co-infection of plants by the rice tungro spherical virus (RTSV) and rice tungro bacilliform virus (RTBV) from green leafhoppers (GLH; *Nephotettix virescens*), is characterized by severe stunting and yellowing of the rice plant, resulting in 20-100% crop yield loss. On the other hand, BLB can cause about 74 - 81% yield loss, or may even result in total crop failure in more severe cases. The pathogen, *Xanthomonas oryzae* pv. *oryzae* (*Xoo*), infects the xylem, causing lesions that may lead to plant death. To control and manage both tungro and BLB, farmers usually resort to chemical sprays and biocontrol agents as the methods of choice. However, these do not always assure plant protection/recovery and chemical sprays may even pose a threat to the environment and human health. Hence, a safer and long-term approach would be to introduce inherent disease resistance to the rice plant instead.

The Golden Rice trait, characterized by having high beta carotene content in the grain that is naturally converted into Vitamin A by the body as needed, was first introduced into a US temperate variety, Kaybonnet, and has now made its way to our very own local variety PSB Rc82 through conventional breeding. This was done to ensure adaptability of Golden Rice to specific locations in the world; hence, promoting better Vitamin A nutrition in priority areas especially among poor women and children who are more prone to Vitamin A deficiency by complementing their existing diets. To increase the chance of Golden Rice being readily adopted and more available in the Philippines, we hereby focus on introducing disease resistance to it. Using the best Golden Rice candidates developed by IRRI and PhilRice for breeding in combination with tungro and BLB-resistant varieties, we intend to produce a 3-in-1 variety with excellent agronomic traits that will benefit Filipinos.