

2018

NATIONAL RICE R&D HIGHLIGHTS



CROP BIOTECHNOLOGY CENTER



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CROP BIOTECHNOLOGY

Center Director: Roel R. Suralta

EXECUTIVE SUMMARY

The Crop Biotechnology Center was established through Administrative Order 21 to implement a rationalized, effective, and efficient AgBiotech R&D agenda for the DA. The Center further aims to generate improved agricultural technologies, productivity, and enhanced commercial potential value and activities for crops. Fundamental and applied research in the areas of genome biology, computational biology, genetics and microbial systems, and molecular biology will also be conducted at the Center.

The Center conducts one project on rice research dealing with molecular biotechnology techniques and approaches. The project on gene discovery and marker development for agronomically important traits focuses on examining 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 output will include QTLs/genes/molecular 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.

GENE DISCOVERY AND DEVELOPMENT OF MOLECULAR MARKER FOR RICE AND OTHER BENEFICIAL ORGANISMS

AA Dela Cruz

This project capitalized on use of recent advances in molecular biology techniques and plant genome to carry out basic researches that will help in understanding the molecular basis of rice traits. To help accelerate the genetic dissection of simple or complex traits that will be helpful in facilitating the advancement of rice breeding, this project implemented seven studies that examined various genetic compositions for traits such as rice tolerance to drought (deep-rooting, root plasticity and stay green traits) and salinity, and resistance to pests (green leaf hopper) and diseases (bacterial blight and tungro). Outputs of these studies will support rice breeding by expanding the genetic base of the rice germplasm through development of rice donor lines with improved traits. This project also supported the development of biotechnology products made or derived from organisms that can produce plant growth hormones and serve as biocontrol agents. This project also implemented a study on DNA fingerprinting of beneficial organisms, which can be used as reference in verifying identity of organisms used in bioinoculant development to ensure formulation of high-quality agricultural products.

The first of the three studies that work on drought tolerance is “Validation and fine-mapping of root plasticity QTLs on lateral root development in response to water stress in Rice”. This year, 100 back cross (BC) lines of IR64*2/CSSL47, 238 BC lines of IR62266*2/DHL96, and 160 BC lines of Nipponbare*2/CSSL47 were characterized. Three markers located on chromosome 2 were used in genotyping 238 recombinant inbred lines (RILs) of IR62266/DHL96 while 12 markers (1 marker on chromosome 11 and 11 markers on chromosome 12) were used for 99 RILs of IR64/CSSL47. Moreover, 10 markers (1 marker on chromosome 11 and 9 markers on chromosome 12) were used to genotype 160 RILs of Nipponbare/CSSL47. The second study, “QTL mapping analysis with emphasis on root plasticity traits under soil moisture fluctuation stress,” is expected to develop rice lines with deep rooting (>20cm) trait, which will be helpful in the development of rice with drought tolerance. The third study, “Detection and validation of QTL for functional stay green traits for rainfed lowland environment in rice,” derived 50 lines from NSIC Rc 160/Kutsiyam cross. These were characterized using 11 SSR markers (4 markers located on chromosome 1, 5 markers on chromosome 2, 2 markers on chromosome 3 and 1 marker on chromosome 12). Recombinant inbred lines with the highest functional stay-green characteristics are currently being identified.

Under the study “Evaluation of Drought Tolerance QTL Effect in Adapted Genetic Background,” four advanced lines (Rc 222 and Rc 160 background) with three QTLs for drought tolerance are being developed. Specifically, 50 rice lines in PR50578-DT026 (background of Rc 222) and 102 lines in PR50579-DT028 (background of Rc 160) were evaluated for reactions to drought. These materials were pyramided with three QTLs for drought tolerance through marker assisted selection.

The study, “Association of GLH and RTSV resistance to Glh14, tsv1 and eIF4G SNP type in selected popular Philippine traditional rice varieties (PTRVs) and mapping of novel GLH and RTSV resistance genes,” identified 10 candidate PTRVs with apparent resistance against

GLH or RTSV. Initially, F1 plants and resulting F2 seeds were generated from three PTRVs. Polymorphic SSR markers were also identified, which will be needed in the eventual mapping of new resistance.

Under the study, “Novel Gene Identification for Rice Grain Crack Resistance,” presence of QTL in chromosome 3 with the same region found in previous study were located. The region of QTL on chromosome 3 was further narrowed down from 28.97 Mb to 7.31 Mb. Analysis is ongoing for the fine map of the QTL located in chromosome 2. Quantitative trait loci controlling the cracking of rice grains will be identified to benefit the agricultural industry in breeding rice varieties with increased head rice recovery through molecular breeding.

The study, “Establishment of marker system in developing thermo-sensitive genic male sterile (TGMS) lines with bacterial leaf blight (BLB) and tungro resistance,” will validate a marker system for development of TGMS rice with BLB and tungro resistance.

“DNA fingerprinting of beneficial organisms for identification and product quality assessment” confirmed the true of a bacterial isolate previously identified as *Bacillus cibi*. Based on biochemical characterization, this bacterial isolate is identified *Alcaligenes faecalis* (with 99% likelihood based on BLAST). Using the fungi-specific 28S rRNA primers, the two biopesticidal PhilRice-Agusan fungal isolates were shown to be 99% similar with *B. bassiana* and *M. anisopliae*. Results of this study will ensure that only effective organisms of true identity will get incorporated in bioinoculant products.

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

JM Niones, AS Cruz, MC Cabral, and RR Suralta

Soil moisture fluctuations and progressive drought at varying degrees are abiotic stresses that limit crop growth and productivity in rainfed rice systems particularly lowlands. Root plasticity plays significant roles for crop adaptation under drought even in mild water deficit and fluctuating soil moisture. Recent studies on plastic root system development responses showed that plastic developmental response of root system to transient waterlogged to droughted soil conditions was contributed greatly by the promotion of L-type lateral roots, which consequently contributed to greater water uptake and biomass. This study aimed to fine map the genomic regions associated with L-type lateral root development using recombinant inbred lines (RIL) mapping populations of IR62266*/DHL96, Nipponbare*2/CSSL47 and IR64*2/CSSL47. RILs of IR64*2/CSSL47 (100 lines), 238 lines IR62266*2/DHL96, and 160 Nipponbare*2/CSSL47 developed from DS 2014-2015 were subjected to root phenotyping, together with the parental lines, using rootbox method. For the control, parental lines were grown to continuously waterlogged (CWL) and soil moisture fluctuation (SMF) or progressive drought (PDR) treatments while the progenies were subjected to either SMF or PDR treatment only. There were wide and variable frequency distribution of lines in terms of total root length (TRL). Three lines of IR62266*2/DHL96 (Line 221, 185, and 236) showed an increased in shoot biomass ranging 7.82-24.15% relative to IR62266 under SMF condition while none of the lines surpassed SDW of IR62266 under CWL. In Nipponbare*2/CSSL47, 45 lines (28%) have comparable shoot dry matter production relative to their parental lines CSSL47 (SMF) and Nipponbare (CWL). Line 63 was comparable to Nipponbare in terms of shoot biomass production and greater RSD under CWL; CSSL47 under SMF conditions. While 9 lines from IR64*2/CSSL47 showed higher shoot dry matter production and greater TRL than its parents subjected to PDR treatment only.

Detection and validation of QTL for functional stay green traits for rainfed lowland environment in rice

RR Suralta, AS Cruz, MC Cabral, and JM Niones

Stay green is the capacity of plant to postpone senescence during post-anthesis. Under drought, functional stay green is important as it has been correlated with higher grain filling, which results from delay in the onset of leaf senescence. Slower decrease of chlorophyll content and photosynthetic activity can also extend the assimilatory capacity of the canopy and might contribute to higher grain yields. Plants with the stay green trait are more photosynthetically active than genotypes without this trait. This study aimed to identify a recombinant inbred lines (RIL) with the highest functional stay-green characteristic and to identify quantitative trait loci (QTLs) associated with functional stay-green characteristics. Fifty of 275 lines were subjected to pot experiment to determine the stay green capacity of each line under two water treatments: continuously waterlogged (CWL) and progressive drought (PDR). At 50% heading, SPAD and stomatal conductance were measured and water was withheld for PDR treatment. Data collation, summarization, and root scanning are on-going. One hundred fifty-four SSR markers polymorphic to Rc 160/KUTSIYAM were established. Eleven SSR markers – 4 markers located on Chromosome 1, 5 markers on Chromosome 2, 2 markers on Chromosome 3, and 1 marker on Chromosome 12 were used on genotyping. Genotyping of additional markers on the RILs of Rc 160/Kutsiyam is still on-going to be used to detect QTL/s associated with stay-green trait.

QTL mapping analysis with emphasis on root plasticity traits under soil moisture fluctuation stress

JM Niones, AS Cruz, MC Cabral, and RR Suralta

Rice fields, particularly in the rainfed lowlands fields, are usually exposed to the continuous cycle of soil moisture fluctuation due to erratic rainfall pattern. Studies show that variability in soil moisture condition adversely affects shoot and root growth and functions on rice crop. This study aimed to develop RIL populations using the high root plasticity genotypes CSSL47 x KDML105 and Rc 160 x KDML105 with specific component of root plasticity traits. Mapping populations developed from CSSL47/KDML105 in 2015 WS, which generated 115 recombinant inbred lines (RILs) were evaluated for root system development using rootbox method. The progenies were subjected to SMF treatment while the parental lines to CWL and SMF. Most of the lines were plant type CSSL47. As to plant stature, 67 lines (58.3%) were moderately taller (71-80cm) than CSSL47 subjected to SMF condition. A total of 195 and 189 SSR markers polymorphic to CSSL47/KDML105 and CSSL47/ NSIC Rc 160, respectively, were established. Forty-three and 16 markers were genotyped on 115 RILs of CSSL47/KDML 105 and 209 RILs of CSSL47/Rc 160, respectively. Genotyping of additional SSR markers is still on-going that will to be used to identify QTL/s with root plasticity development traits under soil moisture fluctuation stress.

Novel gene identification for rice grain crack resistance

VG Dalusong, R Mallari, TE Mananghaya, AP Tũaño, LM Perez, and BO Juliano

Head rice recovery after milling was affected by several factors such as chalkiness, moisture content, and cracking or fissuring of rice grain. Proper post-harvest practices such as 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 head rice

recovery. QTLs found on chromosomes 2, 3, and 7 are being fine-mapped to identify the possible gene(s) involved in crack resistance. Genotype-phenotype linkage analysis of NSIC Rc 152/BPI Ri10 and BPI Ri10/Rc 154 F2 plants to verify the QTLs in multiple populations are being conducted. Initial results showed that QTL in chromosome 3 is located in similar region found in previous study. The region of QTL on chromosome 3 was narrowed down from 28.97 Mb to 7.31 Mb, analysis is on-going for chromosome 2.

Association of GLH and RTSV resistance to *Glh14*, *tsv1* and *eIF4G* SNP type in selected popular Philippine traditional rice varieties and mapping of novel GLH and RTSV resistance genes

AA Dela Cruz, MJC Duque, and R Aguilar

Traditional rice varieties are more aromatic, tasty, and nutritious than modern rice varieties. Some Philippine traditional rice varieties (PTRVs) pre-observed with tungro resistance; however, breeders hesitate to use them in breeding because the useful genes were yet to be determined. Thus, this study aimed to identify potential sources of GLH, RTSV, and/or RTBV resistance genes among PRTVs.

PhilRice breeding programs for rice tungro disease has been limited on use of either ARC11554 or Utri Merah as donors of resistance. In this study, selected PTRVs were systematically examined to dissect the mechanisms involved in the pre-observed resistant/tolerant reactions to tungro. As a strategy, varieties were systematically screened for presence of known tungro resistance locus/gene by polymerase chain reaction (PCR), which identified PTRVs that can serve as alternative sources of *Glh14* and *tsv1*. Results showed that though the tungro resistance observed among the 120 selected PTRV were not as strong as in ARC1554, there were accessions that displayed apparent resistance either to GLH, RTSV, or RTBV.

Some PTRVs carrying the *tsv1* resistant allele, however, exhibited susceptible reactions to RTSV. To elucidate the ambiguous relationship observed between the molecular marker RM5495 and expression of the desired RTSV resistance, the involvement of another gene coding for a translation initiation factor (*eIF4G*) is further being examined among selected varieties. RTSV-susceptible plants showed similarities to the SNP type in the tungro-susceptible cultivar TN1. RM5495, though had successfully co-segregated RTSV-resistant and RTSV-susceptible plants derived from Indian rice cultivar ARC11554, is not working as well in some PTRVs. Thus, finding molecular markers appropriate for PTRVs is deemed necessary thru detailed examination of gene/s involved and interactions of genes.

Establishment of Marker System in Developing Thermo-Sensitive Genic Male Sterile Lines With Bacterial Leaf Blight and Tungro Resistance

RT Miranda and I Besas

Hybrid rice can increase production even in less land as it can yield 15-30% higher than inbred varieties. Two-line system or the environment sensitive genetic male sterility (EGMS) is simpler and more efficient hybrid rice seed production and thermo-sensitive genic male Sterility (TGMS) is one system that is being used in hybrid rice production. This technology, however, is faced with the two major rice diseases in the irrigated lowland environment, which may cause significant yield loss: bacterial leaf blight (BLB) and tungro. Resistance genes to these diseases have been known to be linked to molecular markers (such as *Xa4*, *Xa7* and *Xa21* for BLB, and RM5495 and RM8213 for tungro), which can facilitate easier and faster

introgression to susceptible varieties through marker-assisted breeding. In 2017, 11 reported markers linked to tms gene, which confers sterility using the two existing S-line of Mestiso 19 and 20 (PRUP 101 S and PRUP 102 S, were validated. However, none of these markers were found useful. This study was conducted to establish a marker system in developing new or improved TGMS lines with resistance to BLB and tungro. Four molecular markers namely RM 71, RM21, RM174 and C365-1 was validated across tungro and BLB resistant breeding lines with PRUP 101 S and PRUP 102 S background. Initial results showed that RM71 produced more distinct PCR-amplified products with PRUP 101 S, and RM174 with PRUP 102 S, compared with the resistant donor parent used and their corresponding P lines. The usefulness of these molecular markers for marker-assisted introgression of TGMS genes will be further validated across progenies of the 92 BC1F1 disease-resistant plants.

DNA fingerprinting of beneficial organisms for identification and product quality assessment

TC Fernando, AA dela Cruz, R Aguilar, MJC Duque, RL Ordonio, JA Cruz, B Tabudlong, and TH Xuan

PhilRice conducts studies on the efficacy of beneficial organisms as bioinoculants to enhance soil and plant health and manage rice pests and diseases (fungi). Accessions of *Nostoc commune*, an edible and nitrogen-fixing blue-green algae common to rice paddies, are also being cultured as biofertilizer and/or alternative protein source for small farm animals. However, determining true identity of *N. commune* strain is needed to produce a protocol that will guarantee safe use of *N. commune* strains as agricultural bioinoculants.

DNA extraction protocols, gene amplification, and DNA sequencing were successfully optimized for the available specimens of bacteria, cyanobacteria, and fungi. Universal 16S rDNA primer combinations, primer 1492R, and 8F were used to perform PCR analysis for one biofertilizer, one biocontrol, and three lignin-degrading bacterial isolates. For the amplification of 16S rDNA in cyanobacteria, primer combination 27E, and 1492R were used to determine the DNA sequences of the seven *N. commune* accessions. Results showed that 28S primer combinations specific for fungi gave high fidelity results in two *B. bassiana* and four *M. anisoplea* isolates. All DNA sequences obtained were listed and analyzed using Basic Local Alignment Search Tool (BLAST) for confirmation and verification of their identity. The optimized protocol can now be used in decision-making, especially in identifying the most suitable strain of *N. commune* for large-scale production, and in ensuring the potency and quality of bioinoculant products through strict use of pure strains of microorganisms.

We are a government corporate entity (Classification E) under the Department of Agriculture. We were created through Executive Order 1061 on 5 November 1985 (as amended) to help develop high-yielding and cost-reducing technologies so farmers can produce enough rice for all Filipinos.

With a "Rice-Secure Philippines" vision, we want the Filipino rice farmers and the Philippine rice industry to be competitive through research for development in our central and seven branch stations, coordinating with a network that comprises 59 agencies strategically located nationwide.

We have the following certifications: ISO 9001:2008 (Quality Management), ISO 14001:2004 (Environmental Management), and OHSAS 18001:2007 (Occupational Health and Safety Assessment Series).

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