

2016 National Rice R&D Highlights

CROP BIOTECH CENTER



Department of Agriculture

Philippine Rice Research Institute

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Crop Biotech Center

Center Director: Roel R. Suralta

Executive Summary

The world experiences rapid increase in population coupled with climate change, a situation not favorable for rice growing setting, which may leave the next century to witness serious global rice shortage problems. Hence, there is an urgent need for a sustainable strategy to increase rice productivity. This calls for breeding rice varieties that can tolerate abiotic stresses such as higher temperatures, drought, submergence, and anaerobic germination; resist biotic stresses such as blast, tungro and bacterial blight; and contain other important traits such as functional stay-green, low phytic acid, crack resistance and good grain quality traits. Many important complex traits in rice are controlled by quantitative trait loci (QTLs) derived from natural variations. Recent studies have succeeded in isolating and characterizing genes and QTLs involved in the mechanisms of tolerance to biotic and abiotic stresses. Advances in molecular and biotechnological tools and techniques offer new opportunities in developing rice varieties with built-in resistance/tolerance to these stresses. Nevertheless, genetic systems responsible for tolerance remain largely unknown.

Germplasm is also an essential component of crop breeding programs. Hence, molecular characterization and diversity analysis are important to be able to design effective breeding strategies and obtain yield advantage particularly under biotic- and abiotic-stressed environments.

DNA fingerprinting using molecular tools is important to establish the genetic identity of crop germplasm. Traditionally, agromorphological characterization based on distinctness, uniformity and stability (DUS) is being done to establish the genetic characteristics of a cultivar. With the evolution of methods, DNA fingerprinting gains significant importance particularly in cases of intellectual property rights or ownership including plant variety protection (PVP). In rice, sequence tandem repeat (STR)-based DNA profiling system is being developed following the guidelines of the Scientific Working Group on DNA Analysis Method (SWGDM).

Genetic characterization is an important aspect of discovering novel genes and traits that are relevant in rice breeding for biotic and abiotic stress tolerance, excellent grain qualities and superior yielding ability. However, in the past years, conventional breeding alone has been proven to be time consuming especially in discovering new genes controlling agronomically important traits. Induced mutations such as chemical or gamma radiation allows fast generation of new traits. Field testing and disease screening are important tools in understanding which of these lines contain the desirable traits. Molecular characterization of the mutant lines is equally important

to investigate the genes responsible for the traits of interest. The advent of molecular marker technology allowed a fast and cost-effective approach in discovering variation, gene location, and their mode of inheritance.

I. Molecular Characterization, Diversity Analysis and Utilization of Rice Germplasm

Project Leader: VG Dalusong

Germplasm is an essential component of crop breeding programs. Hence, molecular characterization and diversity analysis are important to design effective breeding strategies and obtain yield advantage particularly under biotic and abiotic stressed environments.

DNA fingerprinting using molecular tools is important to establish the genetic identity of crop germplasm. Traditionally, agromorphological characterization based on distinctness, uniformity and stability (DUS) is being done to establish the genetic characteristics of a cultivar. With the evolution of methods, DNA fingerprinting has become significant particularly in cases of intellectual property rights or ownership including plant variety protection (PVP). In rice, sequence tandem repeat (STR)-based DNA profiling system is being developed following the guidelines of the Scientific Working Group on DNA Analysis Method (SWGDM). The ultimate goal of this study is to identify a set of STR markers for rice identification and develop allele ladders that will be used in standard profiling of cultivars.

The utilization of germplasm including wild rice species is necessary to enhance genetic diversity and broaden the genetic base of future cultivars that will be developed. This is important to provide a wide genepool for selection by breeders particularly in developing rice varieties tolerant to drought and other abiotic stresses. Wide-cross derived lines from *O. glaberrima* are being evaluated for drought tolerance and identification of possible breeding lines with tolerance to vegetative drought stress is a relevant output for the development of rice varieties that are able to mitigate effects of climate change.

Establishment of rice STR DNA profiling system

VG Dalusong, LH Santos, LM Perez

DNA-based cultivar identification has a great potential in resolving issues on plant variety protection or breeder's right. This method provides unique DNA profile that can serve as genetic identity of the cultivar in question. Numerous studies on rice DNA fingerprinting and analysis have been done using different types of DNA markers. However, none

of these studies has focused on establishing a DNA-based method of cultivar identification based on the Scientific Working Group on DNA Analysis Method (SWGDM) guidelines (SWGDM, 2004). SWGDM is the body that proposes and recommends guidelines for working forensic DNA laboratories. With the advent of intellectual property rights (IPR) and protection of ownership, there is a need to establish methods for the efficient identification of rice varieties using parameters or procedures that can be used as legal evidences in court proceedings.

This study was conducted to develop a Sequence Tandem Repeat (STR) DNA profiling system for rice based on SWGDM guidelines and to create genotype database that will be used as the reference population for comparative purposes. STR or simple sequence repeats (SSR) DNA markers have been used in forensic DNA investigation including humans. The study also aims that this system of cultivar identification can be routinely and reliably used for varietal identification, authentication, purity test and acquisition of property or breeder's right.

Activities:

- Completion of allele scores - Allele scores of the reference rice varieties were corrected using the constructed allele ladder.
- Optimization of PCR reaction to allow use of 2 to 3 STR markers in one reaction to maximize the time and use of laboratory consumables.
- Revision of prepared manuscript for publication to a journal.

Results:

- Allele ladder for 1 STR markers (total of 33) were completed and ready for use in the alignment activity. Optimization of the multiple allele PCR reaction was still on-going.
- Allele alignment of 186 reference DNA using STR-M41 and STR-M35 allele ladders is still on-going.
- A paper entitled "STR markers for identification of rice (*Oryza sativa* L.) Varieties" was revised for publication to Philippine Agricultural Scientist (PAS).
- DNA extraction protocols we previously developed, modified and established for rice DNA were used to analyze a rice sample from a private company. The objective was to conduct a similarity test through molecular analysis to identify whether the given sample will match or will not match to the 2 rice varieties from GRD that have the same or almost the same

grain morphology. Results are on-hold and waiting for advise to release.

Genetic and molecular characterization of rice mutants and introgression lines

RL Ordonio, RT Miranda and XGI Caguiat

Rice mutants have been regarded as powerful tools for genetic research. This is because their phenotypes can be used to explain the function of their mutated gene (functional genomics). This gene is usually discovered through fine mapping but can also be identified through genome sequencing approaches. Mutants have long served as donors for breeding varieties with abiotic and biotic stress tolerance, excellent grain and superior yield qualities. Mutation breeding, thus, provides useful genes/alleles that can be introgressed into existing varieties through conventional or marker-assisted breeding. In this study, we characterize rice mutants produced through chemical or physical mutagenesis using genetic and molecular tools in conjunction with field testing and disease screening. Specifically, this study aims to characterize the genetic and molecular profile of elite rice mutants and their introgression lines, analyze the sequence and expression of their mutant alleles, and identify the molecular functions of genes behind some novel mutants.

Activities:

- Primer design for the analysis of the rice red pericarp gene and rice actin.
- DNA extraction from MSL37, MSL40, NSIC Rc144, IRBB4, IRBB5, IRBB7, IRBB21, IRBB24 and TN1 leaf samples for PCR analysis.
- PCR analysis using Xa21 primers for MSL37, MSL40, NSIC Rc144 and IRBB62 and xa5 primers for MSL37, MSL40, NSIC Rc144, IRBB5 and TN1.
- Leaf sample collection from MSL37, MSL40, NSIC Rc144, IRBB4, IRBB5, IRBB7, IRBB21, IRBB24, and TN1 plants 24hrs, 48hrs and 1 week after inoculation with *Xanthomonas oryzae* race 3 strain 79.
- RNA extraction from MSL40, IRBB5, IRBB21 and NSIC Rc144.

Results:

- Primers were designed to analyze the entire OS07G0211500 (Japonica) or BGIOSGA025388 (Indica) (<http://ensembl.gramene.org/>) which codes for the red pericarp (pigmented)

trait of rice. Primers for rice actin gene expression analysis was also made (Table 1).

- PCR analysis using Xa21 primers revealed that Xa21 is deleted in MSL40 and that MSL37 and NSIC Rc 144 may have some kind of incomplete or pseudo Xa21 gene (Figure 1) based on the absence of bands corresponding to some parts of the gene. The case of MSL40 is consistent with the previous result of the next generation sequencing in which no reads from MSL40 were obtained at the expected position of the gene. On the other hand, PCR analysis using xa5F2R2 primers revealed that MSL40 might have a mutated xa5 gene (Figure 2). To further verify these results, the expression of the Xa21 and xa5 genes in the plants will still have to be analyzed.
- Initial RNA extraction was done on a few collected leaf samples inoculated with *Xanthomonas oryzae* using RNA extraction kit and this will be repeated again with more samples once trizol reagent is available.

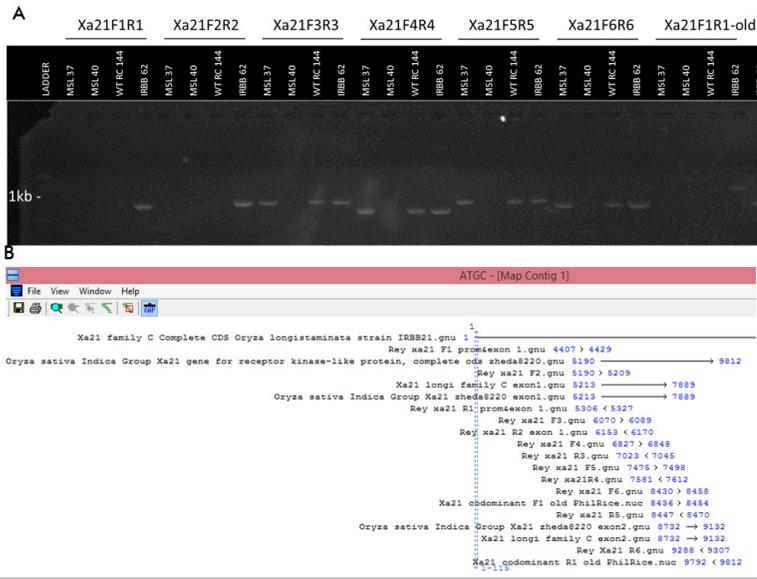


Figure 1. PCR analysis of the mutants MSL37 and MSL40 alongside with the controls NSIC Rc 144 (-) and IRBB62 (+). Seven sets of primers were used to check the entire Xa21 gene in rice. B. Contig assembly showing the positions of primers used in A. A pair of primers (e.g. xxF1-xxR1) will amplify a specific segment of the Xa21 gene (longest and second longest lines) which will appear on gel in A if the sequence/segment is intact. The result shows that Xa21 is absent in MSL40 and that MSL37 and NSIC Rc 144 may have some kind of incomplete or pseudo Xa21 gene. This is consistent with initial results that Rc144 does not have Xa21 based on disease reaction and PCR screening using SSRs.

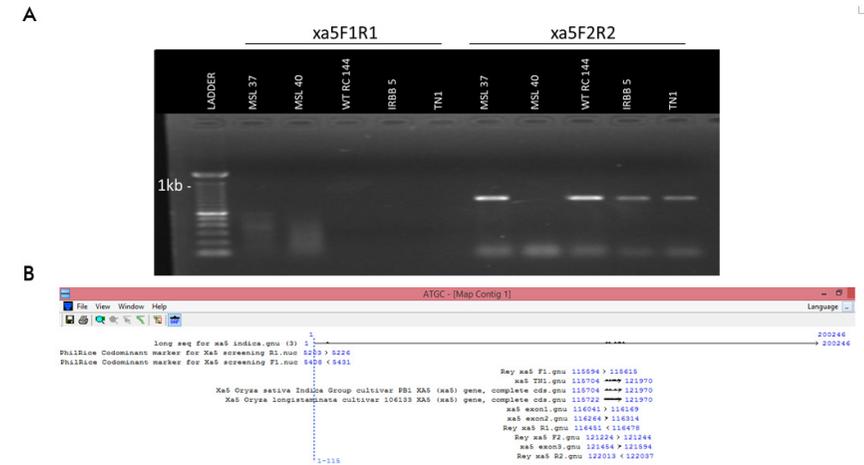


Figure 2. PCR analysis of the mutants MSL37 and MSL40 alongside with the controls NSIC Rc 144 (-), TN1 (-) and IRBB5 (+). Two sets of primers were used to check the entire xa5 gene in rice but only xa5F2R2 seems to be working. B. Contig assembly showing the positions of primers used in A. A pair of primers (e.g. xxF1-xxR1) will amplify a specific segment of the xa5 gene (longest line) which will appear on gel in A if the sequence/segment is intact. The result shows that xa5 is absent in MSL40. This result will still have to be confirmed especially that Rc144 is known not to have xa5.

Table 1. Primers for PCR analysis of colored pericarp mutants and for expression analysis of the rice Actin gene.

	Primers	Sequences
1	RedPerChr7F1_973_59.3	aaa acc gtg ctg acgt cga
2	RedPerChr7R1_973_59.3	tat acc cga tgc cgg gag g
3	RedPerChr7F2_549_59.1	gga gct cta cga ctg gct gc
4	RedPerChr7R2_549_59.1	acc tct gat gta gtt gga tcg gag
5	RedPerChr7F4_856_59.3	tga agt gat gtg ccg ctt agc
6	RedPerChr7R4_856_59.3	tcc ttg caa atg ccc ttc ca
7	RedPerChr7F5_955_59.7	aat tgc cct tgc cca tgc a
8	RedPerChr7R5_955_59.7	ggg ttg gat gtt gag tgc tgt gag
9	RedPerChr7F6_953_59.5	tga tga atg gca gag tgc tgg
10	RedPerChr7R6_953_59.5	cct cgc aga gaa aat gcc aag agt g
11	RedPerChr7F7_847_59.2	tgc aac tag gta tca cca gcc aa
12	RedPerChr7R7_847_59.2	agg tgt ttc tgc tcc cct g
13	RedPerChr7F8_830_59.7	tac aac acg ctg cga cag caa
14	RedPerChr7R8_830_59.7	tta ccg gtg gat acg ggt agg a
15	RedPerChr7F9_983_60.9	cca tcc aag gtg att tca gtg cca acc
16	RedPerChr7R9_983_60.9	att gcg gcc att gct gct gct c
17	RedPerChr7F10_657_59.2	gca tac aag agc tcg agt cgt c
18	RedPerChr7R10_657_59.2	ggg tga tat cga tga gga gga gag gac g
19	RiceActinF1_164_57.4	caa ccc caa ggc caa tcg tg
20	RiceActinF1_164_57.4	gat cac gcc cag caa ggt c

II. CBC-003: Gene Discovery and Marker Development for Agronomically Important Traits

Project Leader: OE Manangkil

A need for an increase in rice grain yield is one of the concerns to keep up with the demand in consumptions. One of the key is to improve the rice yield in the stress prone growing environments. Many important complex traits of rice in tolerance to biotic and abiotic stresses are controlled by QTLs derived from natural variations. Recent studies have succeeded in isolating and characterizing genes involved in important traits for rice stress tolerance to biotic and abiotic stresses.

Thus, there is a need to study and breed rice varieties that can tolerate higher temperatures, drought, submergence, anaerobic germination with resistance to blast and tungro and other important traits (crack resistance, low phytic acid, etc.). Gene discovery and marker development of these agronomically important traits are the keys to improve yield in stress prone environments. With the anticipated global climate change that will affect rice important traits, rice production and food security is at stake.

Recent advances in molecular biology techniques and plant genome mapping offer new opportunities in breeding rice for biotic and abiotic stress tolerance. Several genes are known to be involved in the tolerance to these stresses although the genetic systems responsible for tolerance remain largely unknown yet.

Molecular Genetics of Grain Quality Traits in Support to Rice Quality Improvement

APP Tuaño, TE Mananghaya, ATDC Ardanas, RP Mallari, RC de Leon, LM Perez, and BO Juliano

Several rice breeding programs (e.g., USDA, IRRI, Australia, Japan, China, etc.) have used molecular markers technology in their selection for desired traits including grain quality. Functional markers have been developed and adopted in selection at early breeding stages without the need to wait for the harvested seeds for chemical analyses. PhilRice breeding program has employed molecular markers to complement conventional screening methods for pest and disease resistance, abiotic stress tolerance and other traits, but to date; grain quality screening is still based on physicochemical and sensory evaluation protocols requiring relatively large sample size and at least two months of ageing after harvest. Considering the availability of grain quality markers and PhilRice's capability in molecular genetics, a molecular platform composed of functional markers associated with important grain quality parameters, should be established, validated and

adopted in the grain quality screening at the early breeding stages, thus this study.

Activities:

- Optimization of PCR and separation protocols for the starch synthase IIa (SSIIa) single nucleotide polymorphism (SNP) markers and waxy gene SNP markers.
- Determination of fragrance and SSIIa alleles of farmers' specialty rices along with check varieties.
- Continuation of physicochemical properties determination and association studies.

Results:

- SNP markers for SSIIa indicative of gelatinization temperature (GT) type have been optimized and adopted in characterizing the 97 farmers' specialty rices collected by PhilRice Los Baños – 41 were classified as low GT while 56 were high/intermediate GT. One limitation of this platform is that it cannot distinguish between intermediate- and high-GT.
- Fragrance (*fgr*) gene genotyping via the optimized Bradbury method resulted in 35% of the farmers' specialty rices containing the *fgr* allele, 55% without the *fgr* allele and 10% heterozygous to the allele. Optimization of additional markers (*Shi* and *Shao* markers) is in progress.
- Waxy gene alleles determination of the same set of samples using optimized intron 1 G/T SNP marker is on-going. Genotyping using exon 6 A/C SNP marker will follow upon completion of optimization activities.
- Grain quality characterization results were published in the Philippine Agricultural Scientist December 2015 issue and a poster on the SSIIa alleles presented at the 2016 Crop Science Society of the Philippines Conference.

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

JM Niones, MCJ Cabral, RR Suralta, AB Aguelo, LM Perez, NB Lucob

Root plasticity plays significant roles for crop adaptation under drought even in mild water deficit and fluctuating soil moisture. Series of studies shown that promoted lateral root (LR) development as key traits for maintaining root system, dry matter production and yield under soil moisture fluctuations and progressive drought stresses. Many analyses of QTLs for root morphological traits such as QTL associated with LR production regardless of the types of LR, root mass and penetrated root length have been reported. The ultimate application of this QTL approach is the development of rice cultivars suitable for soil moisture fluctuating and drought-prone environments via marker-assisted selection (MAS). This study therefore, aims to localize the candidate genomic region of QTLs associated with L-type lateral root development through fine-mapping. Specifically, to develop recombinant inbred line (RIL) mapping population of DHL96 backcrossed with IR62266 and CSSL47 introgressed into IR64 (F3 and BC1F2), and to develop RIL mapping population of DHL96 and CSSL47 crossed with NSIC Rc160 as background.

Activities:

- Development of mapping populations was conducted. Field evaluation of the different target crosses (IR62266*2/DHL96, NSIC Rc160/DHL96, IR64*2/CSSL47, and Nipponbare*2 / CSSL47) were conducted during the dry and wet season of 2016.
- Root phenotyping of 238 lines (BC1F4) IR62266*2/DHL96 were carried out in a root box experiment under three water treatments on 2016WS: CWL (Continuously waterlogged); SMF (soil moisture fluctuation, transient waterlogged to drought); and PDR (progressive drought).
- 125 lines (F5) of NSIC Rc160/DHL96 were evaluated using the raised bed system on 2016WS.
- Additional of polymorphic markers through polymorphism survey was conducted for all the target crosses.

Results:

- In 2016DS, 238 lines (BC1F4) IR62266*2/DHL96 and 249 lines (F5) NSIC Rc160/DHL96 were selected through marker aided selection. These lines were used in genotyping and phenotyping in 2016WS.

- IR64*2/CSSL47 (BC1F3) with 100 individuals and Nipponbare*2 /CSSL47 with 160 lines (BC1F4) were evaluated in the field.
- Phenotyping of the 238 lines (BC1F4) IR62266*2/DHL96 showed three lines which had increased by 7.8% - 24.1% in shoot biomass production relative to IR62266 under SMF condition (Figure 3). On-going root data processing and analysis.
- Shoot and root data collection for the 125 lines (F5) of NSIC Rc160/DHL96 evaluated using the raised bed system is still on-going.
- Analyzed genotypic data of 5 polymorphic SSR markers on 249 F5 populations (NSIC Rc160/DHL96) and 9 polymorphic SSR markers on 160 lines BC1F4 (Nipponbare*2/CSSL47).
- Additional polymorphic markers were identified for each respective cross: 125 SSR markers for IR62266/DHL96; 158 SSR markers IR64/CSSL47; and 84 SSR markers for Nipponbare/CSSL47 (Figure 4). Table 2 shows the distribution of identified polymorphic markers across the 12 chromosomes.

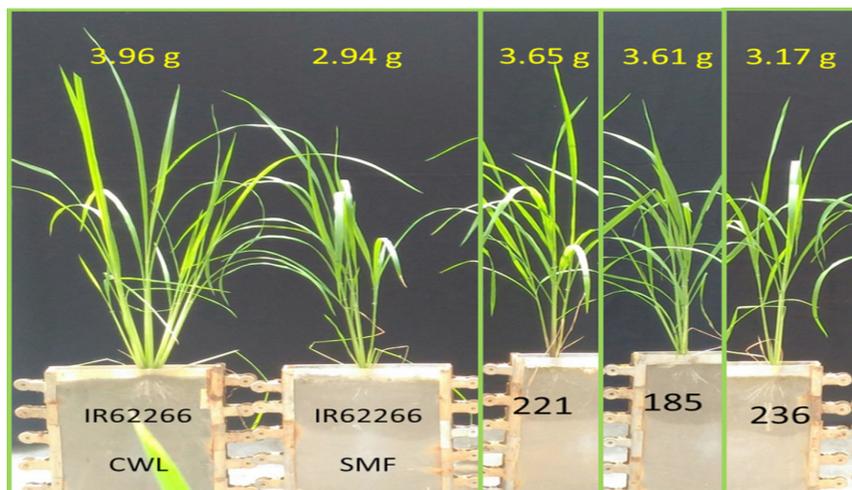


Figure 3. Individuals with increased shoot biomass relative to IR62266 under soil moisture fluctuation stress.

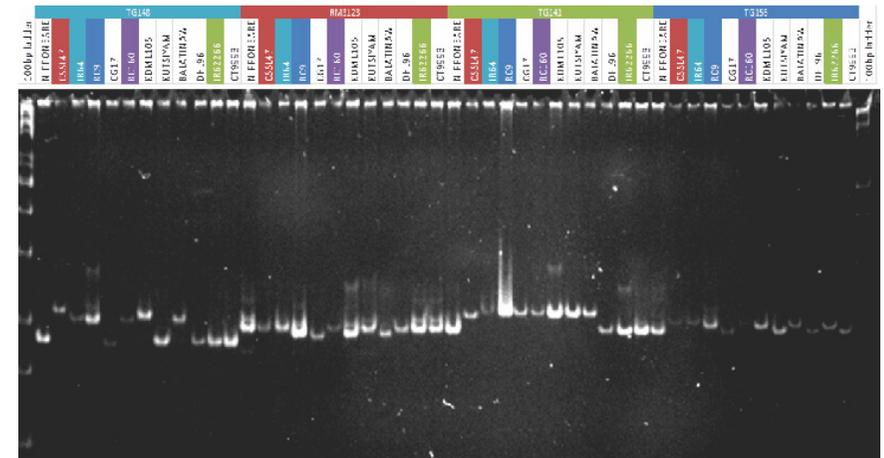


Figure 4. Representative of Gel Image of the polymorphic markers using EST and SSR markers.

Table 2. Distribution of polymorphic markers along 12 chromosomes.

Target Cross	Chromosome											
	Chr 1	Chr 2	Chr 3	Chr 4	Chr 5	Chr 6	Chr 7	Chr 8	Chr 9	Chr 10	Chr 11	Chr 12
IR62266 x DHL96	11	16	12	5	2	12	13	9	10	4	7	19
IR64 x CSSL47	16	21	15	11	9	15	10	7	12	9	11	16
Nipponbare x CSSL47	10	6	10	7	4	5	2	6	2	5	7	15

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

RR Suralta, MCJ Cabral, JM Niones, NB Lucob, LM Perez, AB Aguelo and NV Desamero

Drought refers to shortage of water in the root zone that affects plants, which led to reduction of yield. Post flowering drought response is associated with stay green which is basically the retention of green leaf area at maturity (GLAM). Maintenance of stay green trait during grain-filling stage under soil moisture-deficit constitutes an important component of drought tolerance. Functional stay green can be of huge importance for it has been correlated with higher grain filling and increased yield under post anthesis drought. Our study aims to develop doubled-haploid mapping population from the cross CG17 x NSIC Rc9. Specifically, to identify a DHL with the highest stay-green characteristic and identify QTL associated with functional stay-green trait.

Activities:

- Development of mapping population. The F2 and F3 lines derived from NSIC Rc160/Kutsiyam evaluation were conducted during the 2016DS and 2016WS respectively.
- Leaf sample collection and DNA extraction for 275 F3 lines of NSIC Rc160/Kutsiyam were conducted on 2016WS.
- Generation of F1 seeds from the cross IR64/NSIC Rc9 on 2016WS.
- Identification of polymorphic markers through polymorphism survey was conducted for the target crosses Ballatinaw/NSIC Rc160 and NSIC Rc160/Kutsiyam.

Results:

- There were 275 F3 lines derived from NSIC Rc160/Kutsiyam evaluated in the field and collected with leaf samples for DNA extraction and analysis. These lines will be advanced in 2017DS for genotyping and phenotyping.
- In 2016WS, 20 F1 seeds were generated from the cross IR64/NSIC Rc9. These will be evaluated and seed increase in 2017DS. While re-crossing of Ballatinaw/NSIC Rc160 will be done in 2017DS.
- In polymorphism survey result (Figure 5), additional 105 polymorphic SSR markers for Ballatinaw/NSIC Rc160 and 133 SSR markers NSIC Rc160/Kutsiyam were identified. Table

3 shows the distribution of identified polymorphic markers across the 12 chromosomes.

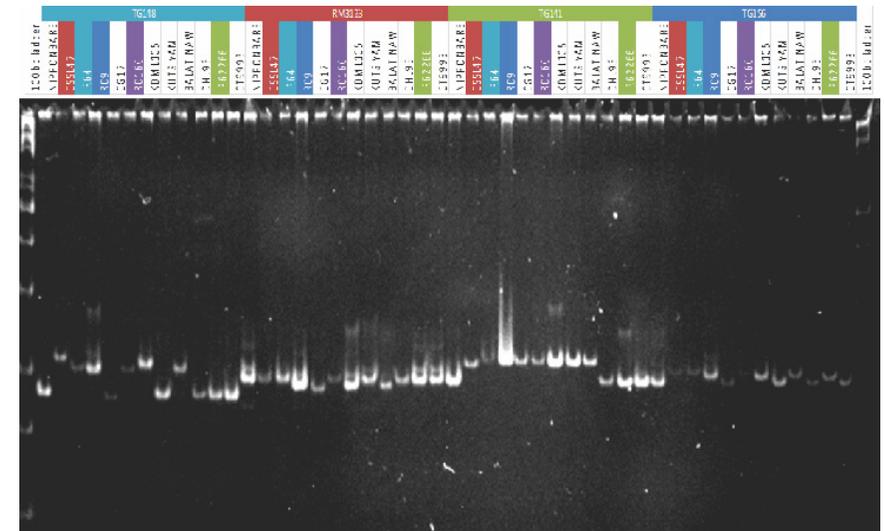


Figure 5. Result of polymorphism survey on different EST and SSR markers.

Table 3. Distribution of polymorphic markers across 12 chromosomes in rice.

Target Cross	Chromosome											
	1	2	3	4	5	6	7	8	9	10	11	12
NSIC Rc160 x Kutsiyam	18	17	14	8	11	13	9	10	10	8	11	15
Balatinaw x NSIC Rc160	13	16	10	3	8	6	6	9	5	6	9	11

Evaluation of drought tolerance QTL effects in adapted genetic background

JD Caguiat, FP Waing, AA Palanog, JOS Enriquez, JIIC Santiago, RA Millas, KAA Garcia, and XGI Caguiat

Drought is a recurrent and one of the most devastating phenomenon that constraints rice production in Asia where at least 23 million hectares or 20% of the total rice area is affected in varying intensities. A fast and suitable breeding scheme should be implemented in order to cope up with the onset of drought (Pandey and Bhandari, 2009). Several introgression lines with single quantitative trait loci (QTL) conferring resistance to drought are available and could be utilized to develop drought tolerant lines through QTL pyramiding. This could decrease the period of plant selection compared with conventional phenotypic selection wherein individual plants are screened for all traits of interest.

This study therefore aims to pyramid two and three QTLs for grain yield under drought stress in NSIC Rc160 and NSIC Rc222 backgrounds; identify and evaluate individual effects of these QTLs and evaluate field performance of potential drought tolerant lines that will be developed.

Activities:

- Pyramiding three QTL from donor parent to high yielding varieties via the marker-aided breeding approach
- Genotyping which includes DNA extraction and amplification of PCR products.
- Generation of F1 crosses, backcross progenies and advance breeding lines.
- Screening of identified potential breeding lines for drought tolerance.

Results:

- In dry season, a total of seven BC1F2 populations (200 plants/population) were established in the field. Leaf samples from these populations were collected at 14 days after transplanting for DNA extraction and marker genotyping. Two flanking markers (RM250 and RM573) for qDTY2.3 were used in genotyping and plant selection. Out of 1,400 plants that were genotyped, 37 plants were found to have alleles that were homozygous to the resistant donor in both markers. Molecular genotyping of F2 lines is shown in Figure 6 and samples of line selection were shown in Figure 7.

- A number of cross combinations generated to pyramid drought QTLs in dry season were established in wet season. The crosses generated were: two crosses for qDTY2.3+qDTY2.2+qDTY4.1; five crosses for qDTY2.3+qDTY12.1; and 7 crosses for qDTY12.1+qDTY2.2+qDTY4.1. Leaf samples were collected from each individual F1 plants and used for marker genotyping. Identified true F1 plants were harvested into bulked which comprised the F2 populations. These populations will be established in 2017 dry season.
- Evaluation of the field performance of selected backcross lines was done in dry season. A total of 116 BC4F5 near-isogenic lines (NILs) and five checks (Kali Aus, Vandana NIL, IR64 NIL, NSIC Rc160 and Rc222) were established in an experimental field in PhilRice Negros. The NIL populations were comprised of 85 lines derived from the cross NSIC Rc222*3/Vandana (PR47201-A102A) and 31 lines derived from the cross NSIC Rc160*3/IR64NIL (PR47202-A103). These NILs with QTL for drought tolerance were screened for grain yield (GY) under lowland drought reproductive stress (DRS) and non-stress (NS) condition. The experiment was laid out in an alpha lattice design with two replications. In NS experiment condition (Figure 8A), standing water was maintained from transplanting to 10 days before maturity by providing water through rain or by supplementary irrigation through water pump and when required. The DRS experiment condition (Figure 8B) was irrigated like the NS condition by keeping standing water up to 30 days after transplanting. Thereafter, the DRS experimental field was drained and irrigation was withheld to impose drought stress at the reproductive stage. Stress was continued until severe leaf rolling (LR) was observed in at least 75% of the population and water table depth remained below 100 cm for more than two weeks. Life-saving irrigation was provided thereafter through flash flooding and drained after 24 hours to impose a second cycle of drought stress. The second cycle of stress continued up to maturity. Water table depth was measured through a 1.1-meter PVC (polyvinyl carbonate) pipe installed strategically in experimental field. Depletion in the water table was measured daily through a meter scale after the onset of the stress.
- Drought stress was successfully imposed during the reproductive stage in stressed field plots while no water stress condition was observed in non-stress condition based on the parched water table records (Figure 9). Average grain

yield performance of entries evaluated under stressed set-up was 0.922 t/ha with yield ranging from 0.111 to 3.057t/ha. PR47201-A102A-29-14-1 (introgressed qDTY12.1) with yield 3.057t/ha out-yielded all other entries with 50% yield advantage (YA) over the tolerant check (Vandana) and 155% YA over NSIC Rc222 with 1.197t/ha yield. In contrast, PR47201-A102A-29-51-1 obtained the lowest yield. For non-stress or irrigated lowland condition, average grain yield was 4.228t/ha where yield ranges from 1.245 to 7.404t/ha. From which, PR47201-A102A-28-123-B obtained the highest yield with 21% yield advantage over the high-yielding check NSIC Rc222 with 5.839t/ha. This was followed by PR47201-A102A-29-14-1 with 6.963t/ha yield and 19.25% YA over NSIC Rc222. Comparing the mean yield between the two conditions, a yield reduction of 78% or 3.306t/ha was obtained when lines are subjected to reproductive drought stress. Productive tillers were reduced by 2, plant height was reduced by 26cm, and flowering was delayed by almost 1 month when lines are subjected to drought stress (Figure 10). Finally, PR47201-A102A-29-14-1 was identified as consistently high-yielding in both stress and non-stress conditions. These lines will be established for further evaluation under reproductive drought stress screening and non-stress set-up in selected PhilRice branch station (Isabela and Negros Occidental) in 2017 DS.

- Field performance of the 116 BC4F5 near-isogenic lines (NILs) and five checks (Kali Aus, Vandana NIL, IR64 NIL, NSIC Rc160 and Rc222) were also evaluated in experimental field in PhilRice CES. Unreplicated yield trial set-up was established under irrigated condition. The grain yield of the lines derived from the cross NSIC Rc222*3/Vandana (PR47201-A102A) ranged from 4.98 to 12.65 t/ha as compared to 7.68 t/ha grain yield of NSIC Rc222. In addition, the grain yield of the lines derived from the cross NSIC Rc160*3/IR64NIL (PR47202-A103) ranged from 4.51 to 10.35 t/ha as compared to 7.16 t/ha grain yield of NSIC Rc160. Moreover, marker genotyping was done to confirm the presence of the introgressed QTLs in NIL population i.e., qDTY12.1 for PR47201-A102A, and qDTY2.2 and qDTY4.1 for PR47202-A103. A total of 73 BC4F5 NILs were confirmed to have qDTY12.1, eight NILs with qDTY2.2 + qDTY4.1, 15 with qDTY4.1 alone. To further evaluate the performance of these lines under irrigated condition, a total of 66 lines in PR47201-A102A, and 22 lines in PR47202-A103 were selected and evaluated in replicated yield trial in 2016WS. However, the

set-up was severely damaged by two consecutive typhoons (Karen and Lawin) during the onset of flowering and grain filling. Thus, data for grain yield was not gathered.

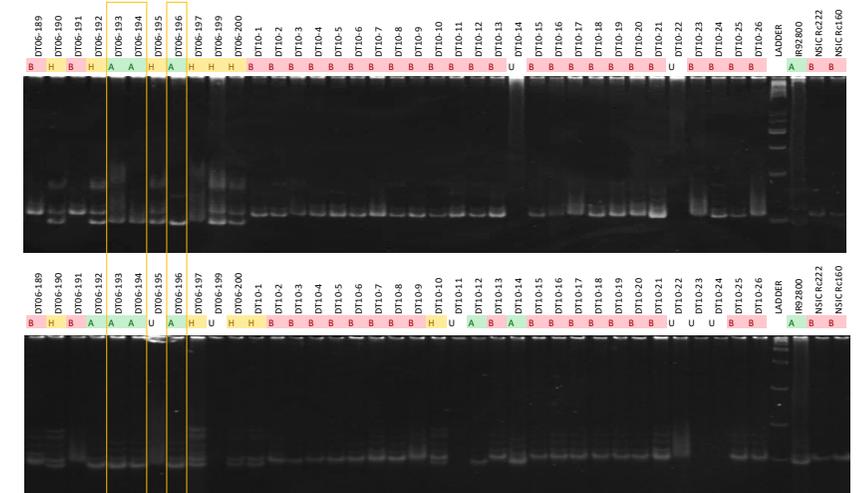


Figure 6. DNA banding pattern of F2 lines using RM250 and RM573 generated from the introgression of qDTY2.3 derived from IR92800 (Kali Aus/2*IR64 NILs) in NSIC Rc160 and NSIC Rc222 background. Selected F2 lines are distinguished. A- donor type allele, B- non-donor type allele, and H- heterozygous.

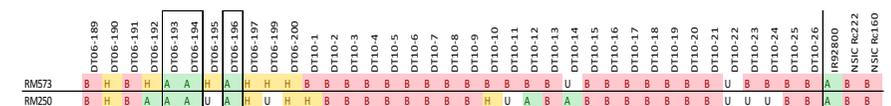


Figure 7. Selected entries based on the result of the molecular genotyping of F2 lines generated in the introgression of qDTY2.3 derived from IR92800 (Kali Aus/2*IR64 NILs) in NSIC Rc160 and NSIC Rc222 background. Selected true F2 lines are distinguished. A- donor type allele, B- non-donor type allele, and H- heterozygous.



Figure 8. Experimental field set-up in non-stress condition (A) drought stress (field after re-watering) (B) at PhilRice Negros.

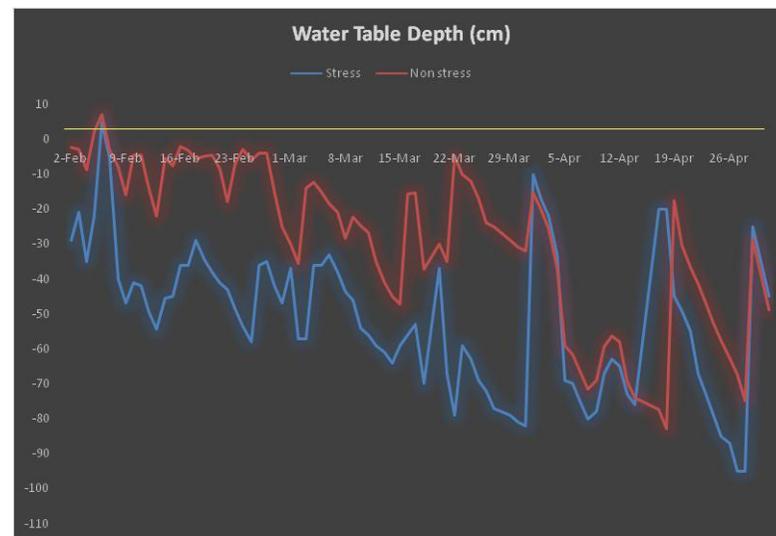


Figure 9. Parched water table indicating drought stress condition experienced during reproductive stress in stress field condition while no significant water stress was observed in non-stress field condition.

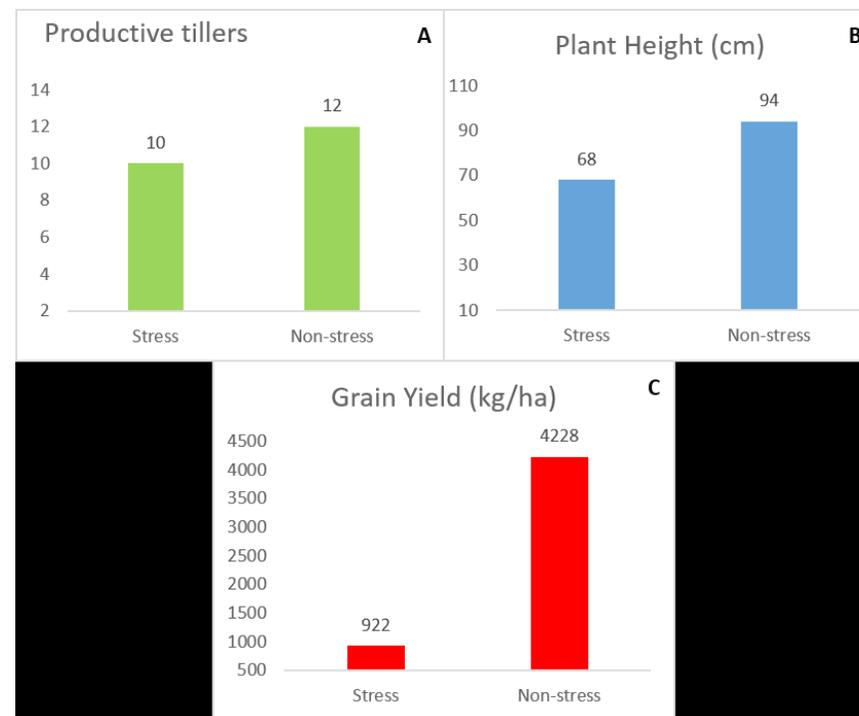


Figure 10. Productive tillers (a), plant height (b), and grain yield (c) performances of NILs under drought stress and non-stress field conditions, 2016 DS at PhilRice Negros.

Novel gene identification for rice crack resistance

VG Dalusong, AP Tũaño, LM Perez, TE Mananghaya, JBM Alvarino, RP Mallari, and BO Juliano

High quality milled rice is a factor that dictates the marketability of the rice varieties with the profit of millers, traders and farmers. As rice self-sufficiency is one of the targets of most rice-growing countries, they also aim to provide rice with a good grain quality. In trade and commerce, grain quality in rice is more emphasize than other cereals. Oftentimes, people tend to look for rice varieties with translucent, long, slender and less broken grains (less chalkiness). Grain cracking (chalkiness), imbalance of pressure during water penetration, hull tightness and bran diffusivity are some factors contributing to fissuring/cracking. Determination of the mechanism behind cracking of the grain is just a step in the development of rice crack resistant variety.

PSB Rc38 and PSB Rc52 are the potential rice varieties depicting the crack resistant trait. PSB Rc38 has a % head rice recovery with 61 and 58 unstressed and stressed, respectively, depicting no significant reduction of head rice recovery due to stress (BO Juliano et al 2009, unpublished) compared to other rice varieties. Molecular characterization of these 2 varieties (PSB Rc38 and PSB Rc52) was used to improved head rice recovery in Philippine rice varieties through marker assisted breeding.

Activities:

- Additional SSR markers located across the 12 chromosomes were screened.
- Generated 3 F2 mapping populations and a total of 1047 F2 seeds were planted to constitute the mapping population.
- DNA of F2 plants were extracted and quantified.
- Genomic DNA of 2217 F2 plants DNA samples were extracted and quantified.
- Seeds of 1269 F2:3 entries were generated and prepared.

Results:

Molecular Analysis

- QTLs identified on PSB Rc38xNSIC Rc160 F2 mapping population were reanalyzed using softwares ICIMapping v 4.0., WinQTL Cartographer v.2.5., and QGene v4.3.10. All data analysis was analyzed by the method of Composite

Interval Mapping where threshold for identifying QTLs was set to 2.0.

- Using Composite Interval Mapping by ICIMapping v.4.0, 2 putative QTLs were identified. These QTLs were located each in chromosome 3 with flanking markers RM545 and RM15187 (LOD=2.59, R²=46.99) and chromosome 11 in between RM286 and RM5926 (LOD=2.54, R²=56.23). Using WinQTL Cart v.2.51, 2 QTLs were also identified in chromosome 4 (RM335 and RM17116) and chromosome 11 (RM286 and RM5926) using interval mapping with 1000 permutation. Using QGene v.4.3.10, only peaks have been identified. These 4 peaks have been revealed by Composite Interval Mapping located in chromosome 1 (LOD=2.183) in between RM3412 and RM10711, chromosome 3 (LOD=1.59) between RM545 and RM15187, chromosome 5 (LOD=1.081) in between RM18193 and RM592, and chromosome 8 (LOD=1.495) between markers RM7057 and RM342A. Using QGene v.4.3.10, 1 QTL was identified located in chromosome 1 with LOD=2.18 and R²=5.3 (Table 4).

Fine-mapping of QTLs identified

- Established 7 F2 mapping populations from the following crosses: NSIC Rc160/PSB Rc52-4, NSIC Rc160/PSB Rc52-3, PSB Rc52/NSIC Rc160-13, PSB Rc52/NSIC Rc160-18, NSIC Rc160/PSB Rc38-5, NSIC Rc160/PSB Rc38-6, and PSB Rc38/NSIC Rc160-1.
- A total of 2,293 F2 plants from 7 mapping populations were properly tagged and collected leaf samples for DNA extraction and genotyping of the rice crack resistant trait. F3:2 seeds were harvested, properly cleaned and labeled. Ten grams of cleaned seeds per entry were prepared for phenotyping activity to be conducted in PhilRice-Los Baños. Analysis of the phenotype data gathered are still on-going.
- The remaining seeds were planted to established F3 population. Cleaning of harvested F3 seeds for planting to advance generation are on-going.
- Genomic DNA extraction and quality check of 2,293 DNA samples from seven mapping populations were done. However, due to budget constraints, we were only able to cater genotyping of two mapping populations with a total of 684 plants on 21 polymorphic SSR markers.

- Polymorphism survey of parentals PSB Rc38, NSIC Rc160, and PSB Rc52 using 231 SSR markers was also conducted to increase the markers genome coverage on the target region for fine mapping. Out of 231 markers screened, 43 polymorphic markers were identified between PSB Rc38 and NSIC Rc160 and 47 polymorphic markers between PSB Rc52 and NSIC Rc160. Table 5 shows the summary of chromosome positions of identified polymorphic SSRs. Figures 11 and 12 shows the graphical genotype of all polymorphic SSR markers identified between the parentals used in the mapping populations.

Generation of new mapping population for validation of QTLs identified

- Generated 4 F2 mapping population with a total of 1502 F2 plants from crosses BPI Ri10/NSIC Rc154, BPI Ri10/NSIC Rc152, NSIC Rc154/BPI Ri10 and NSIC Rc152/BPI Ri10. DNA extraction and quality check were already done and waiting for the result of polymorphism survey between the parents to continue the work to genotyping.
- Conducted polymorphism survey of BPI Ri10, NSIC Rc152, and NSIC Rc154 using 320 SSR markers. Identification on the number of polymorphic markers per parental cross are still on-going.

Table 4. Quantitative trait locus (QTL) identified on PSB Rc38 Rc38xNSIC Rc160 F2 mapping population.

Mapping software	QTL identified	Chromosome Location	Flanking markers	LOD score	%PVE or R ²
ICIMAPPING v4.0	qtl1	3	RM545 and RM15187	2.59	46.99
	qtl2	11	RM286 and RM5926	2.54	56.23
WinQTL Cartographer v2.51	qtl1	4	RM335 and RM17116	11.88	1.68
	qtl2	11	RM286 and RM5926	12.07	0.35
QGene v4.3.10	qtl1	1	RM3412 and RM10711	2.18	5.3

Table 5. Additional polymorphic SSR markers identified for use in the fine-mapping activity.

Chromosome	PSB Rc38 x PSB R160	PSB Rc52 x PSB R160
1	2	4
2	16	16
3	10	9
4	1	2
5	2	3
6	3	4
7	3	4
8	2	1
9	2	2
10	1	0
11	1	1
12	0	1
Total	43	47

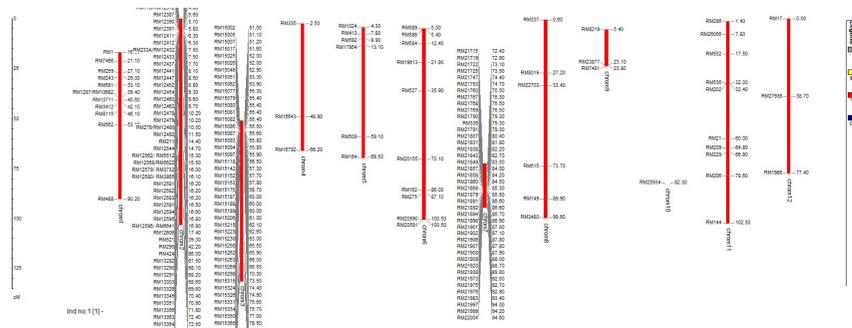


Figure 11. Graphical Genotype of the 230 identified polymorphic markers from the cross PSB Rc38 x NSIC Rc160 using Graphical Genotyping version 2.5.

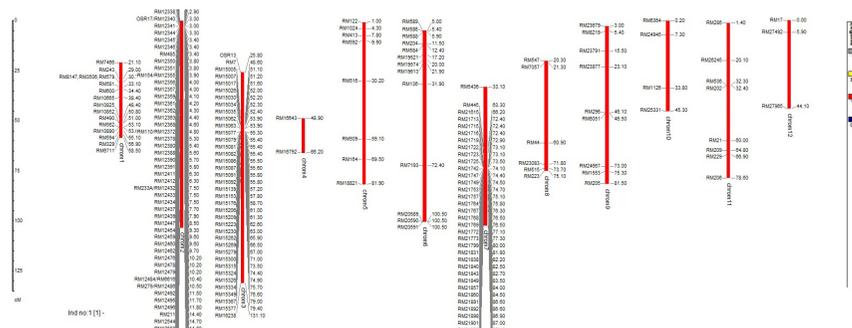


Figure 12. Graphical Genotype of the 248 identified polymorphic markers from the cross PSB Rc52 x NSIC Rc160 using Graphical Genotyping version 2.

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

JM Niones, MCJ Cabral, RR Suralta, LM Perez, AB Aguelo, and VA Marcelo

Fluctuation of soil moisture and progressive drought at varying degrees are regularly recurring stresses in rainfed lowland areas that limits rice growth and production. Rainfed lowlands fields are usually exposed to this continuous cycle of soil moisture fluctuation due to erratic rainfall pattern. Several studies have shown that variability in soil moisture condition adversely affects shoot and root growth and functions on rice crop. Root plasticity plays a key role in the adaptation of rice plants to drought and soil moisture fluctuation (SMF) through promotion of lateral root (LR) thereby maintaining dry matter production and yield. This study aims to develop recombinant inbred lines (RILs) with root plasticity traits in rice under progressive drought and fluctuating soil moisture conditions (transient waterlogged to drought and vice versa).

Activities:

- Field evaluation of the mapping populations CSSL47/KDML 105, CSSL47/NSIC RC160, and NSIC Rc160/KDML 105 on 2016DS and 2016WS.
- Root phenotyping of CSSL47 and KDML 105 was conducting using rootbox method under 3 water treatments on 2016WS.
- Genotyping using 9 polymorphic markers on 115 F4 mapping population of CSSL47/KDML 105.
- Identification of additional polymorphic markers through polymorphism survey on the target crosses.

Results:

- Advanced generation of mapping populations of the following: 115 F4 lines derived from CSSL47/KDML 105, 209 F3 lines derived from CSSL47/NSIC RC160, and 83 F1:2 derived from NSIC Rc160/KDML 105 were characterized in the field.
- The mapping population parents CSSL47 and KDML 105 were root phenotyped using rootbox method under 3 water treatments: CWL (continuously waterlogged), SMF (soil moisture fluctuation, transient waterlogged to drought) and PDR (progressive drought, 10%SMC). SMF and PDR significantly reduced shoot biomass about 50% in CSSL47 and 31% in KDML 105 (Figure 13). Root data revealed CSSL47 had decreased in TRL, TNRL, and number of nodal roots under

SMF and PDR, however, increased in TLRL specifically L-type LR under both water stresses. On the other hand, KDML105 showed increased in major root traits on both SMF and PDR (Figure 14).

- Analyzed 9 identified polymorphic SSR markers on 115 F4 mapping population of CSSL47/KDML 105.
- Identified 187 and 186 polymorphic SSR markers for CSSL47/KDML 105 and CSSL47/NSIC Rc160, respectively (Table 6).

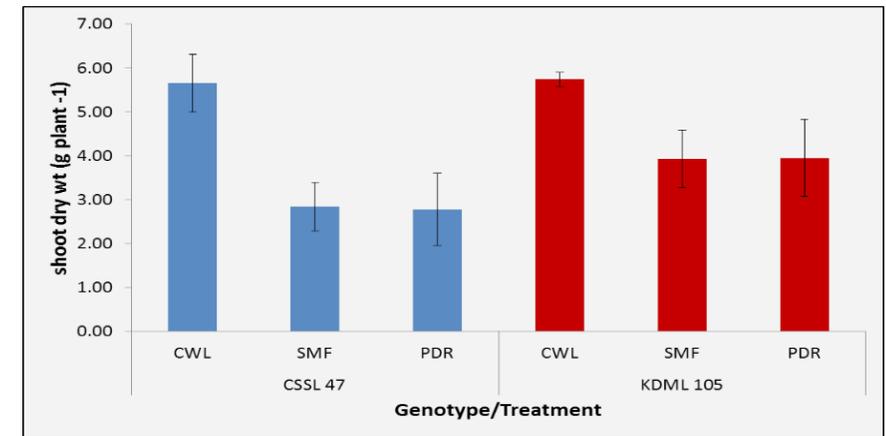


Figure 13. Shoot dry matter production of CSSL47 and KDML 105 under different water stresses.

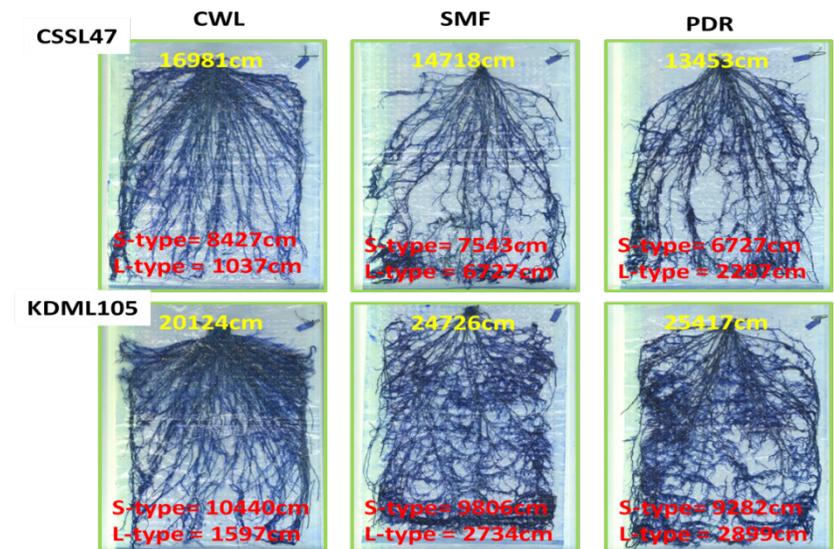


Figure 14. Root system development of CSSL47 and KDML 105 under different water treatments, 2016WS.

Table 6. Distribution of polymorphic markers across 12 chromosomes.

Target Cross	Chromosome											
	1	2	3	4	5	6	7	8	9	10	11	12
CSSL47 x KDML105	18	24	17	13	11	17	13	12	10	9	11	24
CSSL47 x NSIC Rc160	21	25	18	8	11	15	15	14	7	10	9	25
NSIC Rc160 x KDML105	11	13	9	6	2	9	5	9	7	7	9	9

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

AADelaCruz, MMRosario, MJCDuque

Nephotettix virescens (green leafhopper, GLH) is the most efficient insect vector in the semi-persistent transmission of its causal viruses, the rice tungro bacilliform virus (RTBV) and rice tungro spherical virus (RTSV). Various Philippine Traditional Rice Varieties (PTRV) were observed with tungro resistance however breeders hesitate to use them because the corresponding resistance genes were yet identified. This study aims to diversify the sources of tungro resistance genes for rice varietal improvement through finding novel sources of GLH, RTSV and/or RTBV resistance genes among PRTVs. The presence or absence of Glh14 and tsv1 among selected PTRVs are verified prior to screening for reactions to GLH. For the examination of reactions to tungro viruses, GLH-susceptible plants are used since no vector resistance will likely interfere in the virus inoculation. The involvement of a gene coding for a translation initiation factor (eIF4G) in plants carrying tsv1 will also be associated later to the RTSV resistance of plants. Eventually, the novel tungro resistance genes present in the promising PTRVs will be identified through gene mapping.

Activities:

- Screening of selected PTRVs for reactions to GLH through series of tests (preference, antibiosis and fecundity).
- Screening of selected PTRVs for reactions to tungro viruses using forced tube virus inoculation and enzyme linked immunosorbent assay (ELISA) at 14 and 21 dpi.

- Genotyping of PTRVS for presence/absence of Glh14 and/or tsv1
- PCR amplification and sequencing of eIF4G gene sequences in selected PTRVs carrying tsv1.

Results:

- 16 out of the 72 PTRVs evaluated for reactions to GLH were found resistant while 44 were susceptible. The putative GLH-resistant PTRVs were further evaluated for reactions to tungro viruses through forced-tube inoculation using 3 and 5 GLH. Table 7 shows the 5 most promising GLH-resistant PTRVs that likewise yielded resistant reactions to RTSV and/or RTBV at 21 dpi based on ELISA.
- At 14 and 21 dpi, t-test showed that the %HR in PTRVs Surigao, Sinan Miguel, Binangkuro and ARC11554 (inoculated with tungro viruses using 3 GLH) had the least significant differences. The %HR in the 3 PTRVs were lower than in ARC11554. In terms of percent leaves with discoloration (%LD), the least significant difference was observed between Binangkuro and ARC11554.
- Some of the GLH-resistant PTRVs were able to recover from/ tolerate the stress brought about by tungro when lesser population of GLH (3 per rice plant) is present. In fact, Agricultura and few more PTRVs (not shown) had relatively smaller %HR at 21 dpi. However, the severity of tungro symptoms continued to escalate from 14 to 21 dpi when the vector population was increased to 5 GLH per rice plant.
- Table 8 shows the GLH-susceptible PTRVs that yielded very low RTBV and RTSV accumulation, at 21 dpi, based on enzyme-linked immunosorbent assay (ELISA). These were Caidol, Candenavia, Kayanga and Ginatuday. The absence of Glh14 on the selected PTRVs were verified. These 4 promising GLH-susceptible-but-tungro virus-resistant PTRVs lack tsv1 hence can be explored as potential sources of novel tungro virus resistance genes.
- The percent leaves with discoloration (%LD) observed in the promising tungro virus-resistant PTRVs were significantly higher than in ARC11554, which can be attributed to presence of tsv1 alone.
- 48 additional PTRVs are currently being tested for reactions

to GLH and RTSV. Mass screening, conducted to test for GLH preference, revealed that Ita and Maconting were the least preferred varieties with number of alighted GLH comparable to that observed in ARC11554. Based on ELISA, on the other hand, 13 PTRV were observed with low RTSV and/or RTBV accumulation at 21 dpi based on ELISA. Antibiosis and fecundity tests will also be conducted to verify the initial observations.

- The PTRVs are promising sources of resistance genes. Though the tungro resistance observed in the selected PTRVs were not as strong as in ARC1554, PTRVs with single or combined GLH, RTSV and/or RTBV resistance were identified. My mapping these resistance genes, and pyramiding them into rice plants by marker-assisted selection, rice varieties with durable tungro resistance can eventually be developed for deployment to tungro endemic areas.
- The elf4G gene sequence in PTRVs carrying tsv1 will be analyzed for association to reactions of plants to RTSV.

Table 7. Promising GLH-resistant Philippine Traditional Rice Varieties.

Rice Varieties	GENOTYPE		ANTIBIOSIS		FECUNDITY		FORCED-TUBE VIRUS INOCULATION						
	tsv1	Glh14	Day3	Day5	Week 4		% height reduction (HR)		% leaves with discoloration		ELISA (A ₄₀₅)		
					No. of GLH		14dpi	21dpi	14dpi	21dpi	RTSV	RTBV	
1 SINAN MIGUEL	-	-	S	MR	Adult	0	3GLH	6 ^e	8 ^e	22 ^{abcd}	29 ^{bcd}	I	I
					Nymph	0	5GLH	14 ^{bcd}	15 ^{cd}	22 ^{ef}	22 ^{def}	I	I
2 SURIGAO	-	-	S	R	Adult	2	3GLH	8 ^e	11 ^{cd}	23 ^{abc}	19 ^{de}	H	I
					Nymph	3	5GLH	18 ^{abc}	22 ^{abc}	33 ^{cde}	29 ^{cde}	H	I
3 BINANGKURO	-	-	S	R	Adult	2	3GLH	6 ^e	10 ^{de}	15 ^{cd}	21 ^{cde}	H	I
					Nymph	1	5GLH	17 ^{abc}	23 ^{abc}	39 ^{cde}	55 ^b	I	I
4 PANGASINAN	-	-	S	R	Adult	1	3GLH	11 ^{de}	15 ^{cd}	22 ^{abcd}	26 ^{bcd}	H	H
					Nymph	1	5GLH	22 ^a	28 ^a	9 ^{fg}	19 ^{ef}	I	I
5 AGRICULTURA	-	-	S	R	Adult	0	3GLH	23 ^{abcd}	13 ^{cd}	18 ^{bcd}	28 ^{bcd}	I	H
					Nymph	0	5GLH	15 ^{bcd}	17 ^{cd}	33 ^{cde}	42 ^{bcd}	I	I
6 TN1	-	-	S	S	Adult	many	3GLH	24 ^{abc}	20 ^{bcd}	32 ^{abc}	57 ^a	I	I
					Nymph	many	5GLH	11 ^{cd}	11 ^{de}	57 ^{ab}	43 ^{bc}	I	I
7 ARC11554	+	+	R	R	Adult	0	3GLH	13 ^{cde}	12 ^{cde}	0 ^d	0 ^e	H	H
					Nymph	0	5GLH	8 ^d	5 ^e	0 ^d	2 ^f	H	H

(-) absence, (+) presence; S (susceptible, with % nymph mortality = 80 - 100%), MR (moderately resistant, with % nymph mortality = 21 - 79%), R (resistant, with % nymph mortality = 0 - 20 %); dpi (days post inoculation): H (healthy, A₄₀₅ < 1.0); I (infected A₄₀₅ ≥ 1.0); comparison of means based on t-test (5% level of significance)

Table 8. Promising tungro virus-resistant Philippine Traditional Rice Varieties.

RICE VARIETIES	GENOTYPE		ANTIBIOSIS		FORCED-TUBE INOCULATION			
	tsv1	Glh14	Day3	Day5	% leaves infected		ELISA (21dpi)	
					14 dpi	21 dpi	RTSV	RTBV
1 CAIDOL	-	-	S	S	7	12	H	H
2 CANDENAVIA	-	-	S	S	10	26	H	H
3 KAYANGA	-	-	S	S	18	34	H	H
4 GINATUDAY	-	-	S	S	27	45	H	H
5 ARC11554	+	+	R	R	2	3	H	H
6 TN1	-	-	S	S	30	45	I	I

(-) absence; (+) presence; S (susceptible, with % nymph mortality = 80 - 100%); R (resistant, with % nymph mortality = 0 - 20 %); dpi (days post inoculation): H (healthy, A₄₀₅ < 1.0); I (infected A₄₀₅ ≥ 1.0); comparison of means based on t-test (5% level of significance)

Field and screen house evaluation of individual and combined influences of Glh14 and tsv1 in ARC11554-derived lines infected with RTBV+RTSV

AADelaCruz, MMRosario, MJCDuque, DKMDonayre, and CUSeville

Nephotettix virescens (green leafhopper, GLH) is the most efficient insect vector in the semi-persistent transmission of the causal rice tungro viruses, the rice tungro bacilliform virus (RTBV) and rice tungro spherical virus (RTSV). RTSV alone does not cause any distinctive symptoms except mild stunting in some rice cultivars but it plays the role of a helper virus for vector transmission of RTBV. ARC11554 is a tungro-resistant Indian rice cultivar. Its GLH and RTSV resistance were associated to Glh14 and tsv1. Specific marker-assisted selection (MAS)-bred rice lines were generated for demonstration purposes and these will be examined for reactions to RTD under controlled and field conditions to determine the influence of Glh14 and/or tsv1 in rice plants infected with RTBV+RTSV. Once Glh14 and tsv1 are proven effective, these will be very useful in marker-assisted breeding for tungro-resistant rice.

Activities:

- Evaluation of reactions to tungro viruses of selected rice lines by forced-tube virus inoculation.
- assessment of tungro symptoms at 14 and 21 days post inoculation (dpi).
- Enzyme-linked immunosorbent assay (ELISA) at 21 dpi.
- Verification of presence/absence of Glh14 and tsv1 among rice lines.

Results:

- Table 9 shows the individual and combined influences of Glh14 and/or tsv1 in MAS-bred rice lines infected with tungro through forced-tube virus inoculation. RL1, RL4 and RL5 are F7 lines derived from a TN1 x ARC11554 cross, which were primarily developed for demonstration purposes. On the other hand, rice lines RL2, RL3 and SL are BC3F6 were selected from the materials initially developed by the group of Dr. Gabriel Romero.
- RL2, which carries both Glh14 and tsv1, exhibited severe percent height reduction (%HR = 62%) at 14 dpi but was able to recover very fast and achieved the lowest percent height reduction (% HR = 18) in 21 dpi. This remarkable tungro resistance/tolerance was further emphasized when not even a single leaf of RL2 plants displayed any signs of discoloration.

- RL1, which also carries both Glh14 and tsv1, likewise manifested remarkable tungro resistance/tolerance as it consistently exhibited relatively very small % HR and percent leaves with discoloration (% LD) at both 14 and 21 dpi.
- Interestingly, under controlled condition, the combined effect of Glh14 and tsv1 in RL1 and RL2 resulted to no clear expression of disease symptoms and restrained RTSV multiplication.
- Field evaluation of MAS-bred rice lines introgressed with Glh14 and/or tsv1 are currently being evaluated for reactions to tungro under natural field condition in a hot spot area in PhilRice Negros Experiment Station. The same with the observation of rice lines infected with tungro under screen house conditions, rice lines carrying Glh14 +tsv1 exhibited strong resistance/tolerance against tungro in the field.

Table 9. Influence of Glh14 and/or tsv1 in MAS-bred rice lines infected with tungro under controlled screen house condition.

Elite lines/ Varieties	GENOTYPE		FORCED TUBE VIRUS INOCULATION					
	Glh14	tsv1	%HR		%LD		ELISA (A ₄₀₅)	
			14dpi	21dpi	14dpi	21dpi	RTBV	RTSV
1 RL1	+	+	13 ^d	9 ^d	4 ^{bc}	1 ^{cd}	0.16	0.16
2 RL2	+	+	62 ^a	18 ^c	0 ^c	0 ^d	0.18	0.16
3 RL3	+	-	33 ^c	32 ^b	24 ^a	29 ^a	0.49	0.40
4 RL4	+	-	12 ^d	18 ^c	12 ^d	10 ^{bc}	0.79	0.16
5 RL5	-	+	27 ^c	24 ^c	27 ^c	12 ^b	0.47	0.14
6 SL	-	-	32 ^c	24 ^c	28 ^a	27 ^a	0.60	1.68
7 ARC11554	+	+	3 ^e	5 ^d	0 ^c	0 ^d	0.15	0.14
8 TN1	-	-	42 ^b	41 ^a	24 ^a	29 ^a	1.08	1.14

comparison of means (based on t-test, 5% level of significance); means with the same letter are not significantly different (-) absence; (+) presence; ELISA A₄₀₅ < 1.0 (Healthy), A₄₀₅ ≥ 1.0 (Infected); dpi (days post inoculation); percent height reduction (%HR); percent leaves with discoloration (%LD)

SALT Gene Sequence and Expression Levels in Selected Philippine Traditional and Modern Varieties

JOS Enriquez, FP Waing, RA Millas, and JD Caguiat

The Oryzata or the *Oryza sativa* agglutinin is a mannose-binding jacalin-related lectin found in salt-stressed rice. Oryzata corresponds to the product of the 1.5 kb salt-stress-induced salt gene (LOC_Os01g24710) which is located at the short arm of the 1st chromosome at around the 13.9 Mb region within the SalTol region. It was found to have insecticidal activities against biting-chewing and piercing-sucking insects (Al Atalah et al., 2014). GLH and BPH, which also feed by sucking the plant sap, were also inhibited by mannose-binding lectins (Powell et al., 1995; Rao et al., 1998; Saha et al., 2006).

There is a possible association of the salt gene with the salt tolerance and insect resistance due to the location of the salt gene within SalTol. This study aims to analyze variation in DNA sequence and gene expression levels of the salt gene in a panel of Philippine traditional and modern varieties.

Activities:

- Gene-specific primers were re-designed, spanning the ~1.5 kb salt gene.
- PCR amplification of the salt gene was performed using the designed primers for the assembled panel. High quality PCR products were purified and sent for DNA sequencing.
- The assembled rice panel were evaluated for their insect resistance through test tube antibiosis based on the modified protocol of Sebastian et al. (1996). For GLH resistance, TN1 (S) and ARC11554 (R) were used as checks. IR117285 (R) and TN1 (S) were used as checks for BPH resistance.
- The assembled panel was screened for their tolerance to salinity stress at seedling stage. The screening process was performed based on the protocol of Gregorio et al.'s (1997) with modifications. FL478 (T) and IR29 (S) were used as checks.
- salt gene sequence was amplified in 60 entries composed of traditional and modern varieties.
- Contiguous sequences were assembled and the assembled salt gene sequence among the assembled panel was analyzed.

Results:

- The new primers, saltJE_N1 and saltJE_N2 were optimized and the salt gene sequence from the assembled rice panel were PCR-amplified. Figure 15 shows the PCR products obtained using saltJE_N1 (A) and saltJE_N2 (B). PCR products obtained were purified and sent for sequencing. Sequence data will be analyzed once the sequence results are obtained.
- Insect resistance of the assembled panel was screened based on Sebastian et al.'s (1996) protocol for test tube antibiosis with modifications. Seven to ten day seedlings from each variety were used. Five 2nd or 3rd instar nymphs were introduced from each test tube containing a single seedling. Each entry tested has three replicates with five plants per replicate. Test plants were incubated at room temperature under fluorescent light during day time and without light at night. For GLH resistance, TN1 (S) and ARC11554 (R) were used as checks. IR117285 (R) and TN1 (S) were used as checks for BPH resistance. After the introduction of the insects, the surviving nymphs in each test tube were counted for 3 days. The weighted mean of nymph survival rate was calculated using this formula:

$$\text{Antibiosis score} = \frac{(A_1 * 1) + (A_2 * 2) + (A_3 * 3)}{1 + 2 + 3}$$

- For GLH resistance, antibiosis scores ranged from 20.86% (SERIES-PRRI 004825) to 100% (Dinorado Barako- PRRI 006826) nymphal survival. From the assembled panel, only NSIC Rc330 (antibiosis score: 52.88% nymph survival) showed a moderately resistant score aside from SERIES-PRRI 004825. Table 10 shows the antibiosis scores of the assembled traditional and modern varieties.
- For BPH resistance, nymph survival percentages ranged from 32.55% (PINILI-PRRI 005285) to 97.80% (TN1). Within the panel, aside from PINILI, PSB Rc2 displayed resistance against BPH nymphs with 37.11% nymph survival. Additionally, five varieties are moderately resistant against BPH: KATUHAN-PRRI 00016 (40.40%), MANTICA BAGUIAN-PRRI 000017 (45.49%), PSB Rc8 (46.98%), NSIC Rc294 (51.43%), and IMMANANCA-PRRI 000289 (51.58%). Results for the BPH antibiosis is presented in Table 11. Additionally, a seed box preference test will be performed for GLH and BPH

resistance in order to gather more data on the resistance of the assembled panel against these prevalent pests.

- At seedling stage, the assembled panel was evaluated for their salinity tolerance using Gregorio et al.'s (1997) protocol with modifications. The reaction of the assembled rice panel to salinity stress was evaluated for two times at EC=16. Scoring was based on a modified version of the Standard Evaluation System (SES; IRRI, 2002). Thirty-seven varieties were already screened. Results for the salinity screening at seedling stage is shown in Table 12. Only five out of the 36 varieties exhibited tolerance to saline stress at seedling, having a score of 3 or lower at the second scoring. These lines are: PRRI 004825 (Tolerant), PRRI 006558 (Tolerant), NSIC Rc184 (Tolerant), FL478 (Highly Tolerant), and Pokkali (Highly Tolerant). The remaining rice varieties from the assembled panel will undergo salinity screening at seedling stage once the chemicals are available.
- salt gene sequence was amplified in 60 entries composed of traditional and modern varieties. Contiguous sequences were assembled and the assembled salt gene sequence among the assembled panel was analyzed. DNA sequence of the salt among selected entries is shown in Figure 16. DNA sequencing results indicate high diversity among the assembled panel as signified by the numerous polymorphisms along the gene. Significance of the association of these DNA polymorphisms with insect resistance and salinity tolerance will be determined once the phenotyping of the entries is accomplished.

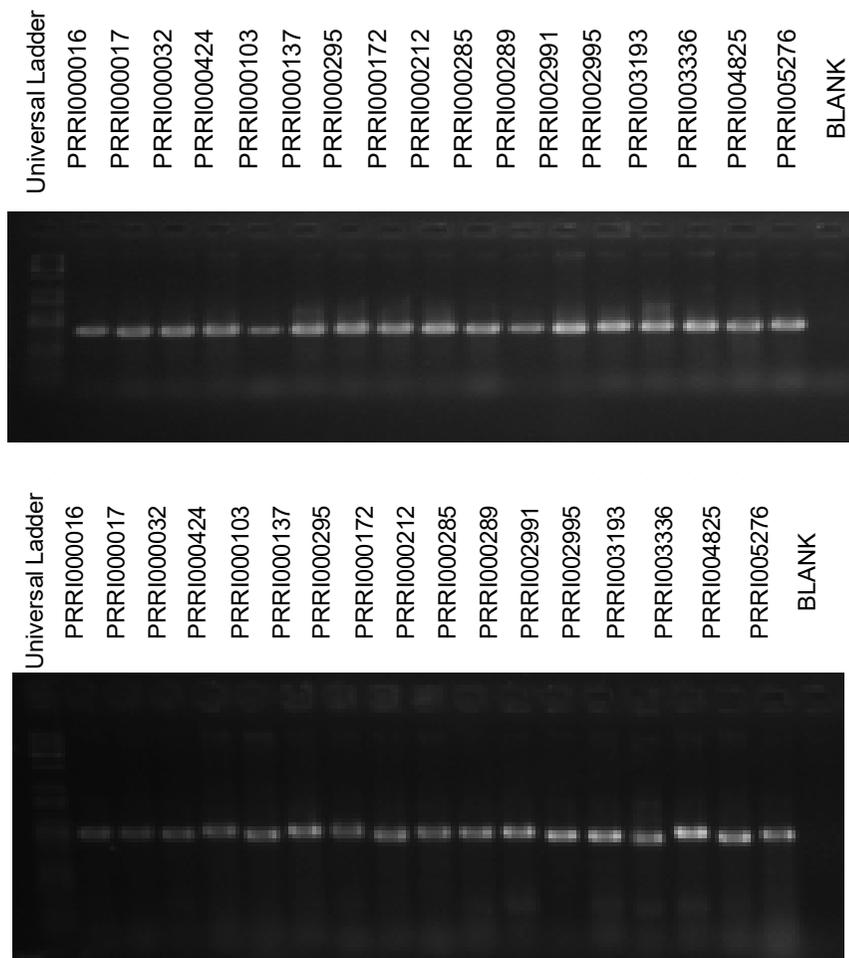


Figure 15. Optimized PCR conditions of (A) saltTJE_N1 (~1000 bp) and (B) saltTJE_N2 (~1,200 bp) on some of the varieties included in the assembled panel. PCR products shown were used for purification and DNA sequencing.

Table 10. GLH nymph survival among the modern and traditional varieties obtained through test-tube antibiosis.

Entry	Antibiosis Score (% Survival)	Category	Entry	Antibiosis Score (% Survival)	Category
PRRI00016	88.78	VS	NSIC Rc184	99.33	VS
PRRI00017	96.22	VS	NSIC Rc190	97.33	VS
PRRI00032	98.60	VS	PRRI006783	93.33	VS
PRRI000424	98.67	VS	PRRI006826	100.00	VS
PRRI000103	93.96	VS	NSIC Rc328	93.29	VS
PRRI000137	89.64	VS	NSIC Rc330	52.88	MR
PRRI000295	97.21	VS	NSIC Rc334	82.83	VS
PRRI000172	89.22	VS	PRRI000386	100.00	VS
PRRI000212	95.89	VS	PRRI000303	99.00	VS
PRRI000285	88.67	VS	PRRI006558	98.89	VS
PRRI000289	96.33	VS	PRRI001182	97.78	VS
PRRI002991	88.50	VS	PRRI000510	79.28	S
PRRI002995	97.33	VS	PRRI000538	69.28	S
PRRI003193	91.33	VS	PRRI000548	78.05	S
PRRI003336	98.22	VS	PRRI000376	90.78	VS
PRRI004825	20.86	R	PSB Rc2	92.22	VS
PRRI005276	80.00	S	PSB Rc4	97.75	VS
PRRI005285	98.00	VS	PSB Rc6	99.17	VS
PRRI005397	98.33	VS	PSB Rc8	99.00	VS
PRRI005614	97.56	VS	PSB Rc10	94.83	VS
PRRI006311	80.61	VS	PSB Rc20	99.44	VS
PRRI006313	90.67	VS	PSB Rc32	98.36	VS
NSIC Rc82	92.89	VS	PRRI 006560	99.33	VS
NSIC Rc186	97.67	VS	TN1	99.67	VS
NSIC Rc188	98.89	VS	ARC11554	39.61	R
NSIC Rc290	91.17	VS	PRRI 001775	88.50	VS
NSIC Rc292	94.48	VS	FL478	98.89	VS
NSIC Rc294	97.71	VS	POKKALI	95.00	VS
NSIC Rc340	80.15	VS	IR29	95.19	VS
PRRI000422	96.50	VS	NSIC Rc222	98.15	VS

Categorization of insect resistance: <20%: Highly resistant, 20-40%: Resistant, 40.1-60%: Moderately Resistant, 60.1-80%: Susceptible, 80.1-100%: Highly Susceptible.

Table 11. BPH nymph survival among the modern and traditional varieties obtained through test-tube antibiosis.

Entry	Antibiosis Score (% Survival)	Category	Entry	Antibiosis Score (% Survival)	Category
PRRI00016	40.40	MR	NSIC Rc184	85.44	HS
PRRI00017	45.49	MR	NSIC Rc190	85.11	HS
PRRI00032	85.69	HS	PRRI006783	89.59	HS
PRRI000424	96.78	HS	PRRI006826	93.28	HS
PRRI000103	90.07	HS	NSIC Rc328	81.17	HS
PRRI000137	87.09	HS	NSIC Rc330	60.64	S
PRRI000295	62.17	S	NSIC Rc334	88.67	HS
PRRI000172	78.25	S	PRRI000386	88.56	HS
PRRI000212	77.03	S	PRRI000303	92.76	HS
PRRI000285	88.22	HS	PRRI006558	88.82	HS
PRRI000289	51.48	MR	PRRI001182	90.22	HS
PRRI002991	84.55	HS	PRRI000510	95.33	HS
PRRI002995	91.17	HS	PRRI000538	88.89	HS
PRRI003193	92.89	HS	PRRI000548	92.20	HS
PRRI003336	86.83	HS	PRRI000376	93.00	HS
PRRI004825	93.39	HS	PSB Rc2	37.11	R
PRRI005276	78.62	S	PSB Rc4	84.00	HS
PRRI005285	32.55	R	PSB Rc6	79.47	S
PRRI005397	72.05	S	PSB Rc8	46.98	MR
PRRI005614	79.72	S	PSB Rc10	63.34	S
PRRI006311	83.72	HS	PSB Rc20	74.54	S
PRRI006313	89.94	HS	PSB Rc32	64.42	S
NSIC Rc82	82.12	HS	PRRI 006560	94.92	HS
NSIC Rc186	94.50	HS	TN1	97.80	HS
NSIC Rc188	76.22	S	ARC11554	77.04	S
NSIC Rc290	89.45	HS	PRRI 001775	85.61	HS
NSIC Rc292	85.19	HS	FL478	92.22	HS
NSIC Rc294	51.43	MR	POKKALI	94.44	HS
NSIC Rc340	81.22	HS	IR29	97.78	HS
PRRI000422	84.78	HS	117285	46.17	MR

Categorization of insect resistance: <20%: Highly resistant, 20-40%: Resistant, 40.1-60%: Moderately Resistant, 60.1-80%: Susceptible, 80.1-100%: Highly Susceptible.

Establishment of Marker System in Developing Thermo-Sensitive Genic Male Sterile (TGMS) Lines with BLB and RTD Resistance

RT Miranda, CFS Te, NRL Sevilla and IL Besas

Heterosis or hybrid vigor has been extensively used to further increase rice production in the Philippines. Since 1994, the National Seed Industry Council (NSIC) has approved 44 hybrid rice varieties which mostly are cytoplasmic male sterile (CMS) or three-line hybrids. There are popular three-line public hybrids like Mestizo 1 and Mestizo 29 because of their good eating quality and high yielding trait, respectively. However, the three-line system has a lot of disadvantages as compared to the two-line system. Two-line system or the environment sensitive genetic male sterility (EGMS) is simpler and more efficient method of hybrid rice seed production. EGMS includes photo period-sensitive genetic male sterility (PGMS) and thermosensitive genetic male sterility (TGMS) systems. In the TGMS system, when temperature is high (24 °C - 32 °C) plants will become sterile and hybrid seed production can be done. On the other hand, when the temperature is low (18 °C - 24 °C) plants revert to fertile and seed multiplication can take place. This novel trait in rice opens the door to new opportunities as we face different challenges caused by global warming as high temperature may ensure high purity of two-line F1 hybrid seeds. In 2011, NSIC approved two rice varieties which were products of the two-line hybrid system namely NSIC Rc202H popularly known as Mestizo 19 and Rc204H known as Mestizo 20 for commercial cultivation.

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, PhilRice has successfully developed several lines possessing resistance to both BLB and tungro disease which can readily be used as donors in its rice improvement breeding programs. Several markers have also been reported to be linked to TGMS genes but are yet to be validated.

This study was conducted to (1) validate markers for reported TGMS loci using populations derived from PRUP-TG101 and PRUP-TG102; (2) introgress TGMS, tungro and BLB resistance genes in the maintainer parents of Mestizo 1 and Mestizo 48 (IR58025B), Mestizo 29 (IR68897B) and other selected female parents through MAS; and (3) evaluate the sterility, resistance to BLB and tungro (RTV), and performance of the developed lines under male sterile growing condition.

Activities:

- Conducted resistance screening of F1 and F2 materials for BLB using PXO 99 (Race 6) and tungro for F2 materials.
- Performed DNA extraction and molecular screening of F1 materials for the detection of resistance genes using Xa21 and xa5 primers for BLB resistance and RM8315 and RM95 for tungro and glh resistance
- Seed increased of breeding lines for generation advanced.

Results:

- For DS, we have generated 18 F1 crosses from cross combination, M19 x LINE 27/IR58025B; M19 x NSIC Rc120/IR58025B; M19 x NSIC Rc120/IR68897B; M19 x LINE 27/IR68897B; M20 x LINE 27/IR68897B and M20 x LINE 27/IR58025B and 24 F1 crosses from four cross combination during WS
- Extracted DNA from 217 individual plants from 18 F1 crosses, genotyped using xa5 and Xa21 for BLB resistance, RM8315 for Glh14 for GLH resistance and RM5495 tsv1 for tungro resistance.
- For genotyping F1 plants using Xa21 primers, there were 32 heterozygous, two plants have same bonding pattern with positive check (IRBB21), 42 have same pattern with susceptible check (TN1 and IR24) while the 2n1 donor parent, Line 27, has the same banding pattern with IRBB21. Five other parental donor used (NSIC Rc120, IR58025B, IR68897B, PRUP 101 and PRUP 102) have similar banding pattern with susceptible check, TN1 and IR24 (Figure 17).
- For RM8213, there were 47 heterozygous, 11 plants with same bonding pattern as Line 27 (parental donor check) and 24 plants with banding pattern similar to TN1 and IR64. (Figure 18) Further analysis is being done to verify the result.
- In screening result, we have selected 152 individual F2 plants with resistant to intermediate reaction to RTV (Figure 19), of these 152, 74 were rated resistant and five with moderately resistant reaction to BLB using PXO 99. For BLB screening result of F1, there were 13 resistant and 32 moderately resistant plants from three cross combination as shown in Table 13.

- Seven plants were identified to have 90-100% sterility, from which two plants with cross combination M19 x Line27/ IRB58025B were identified to be 100% sterile.

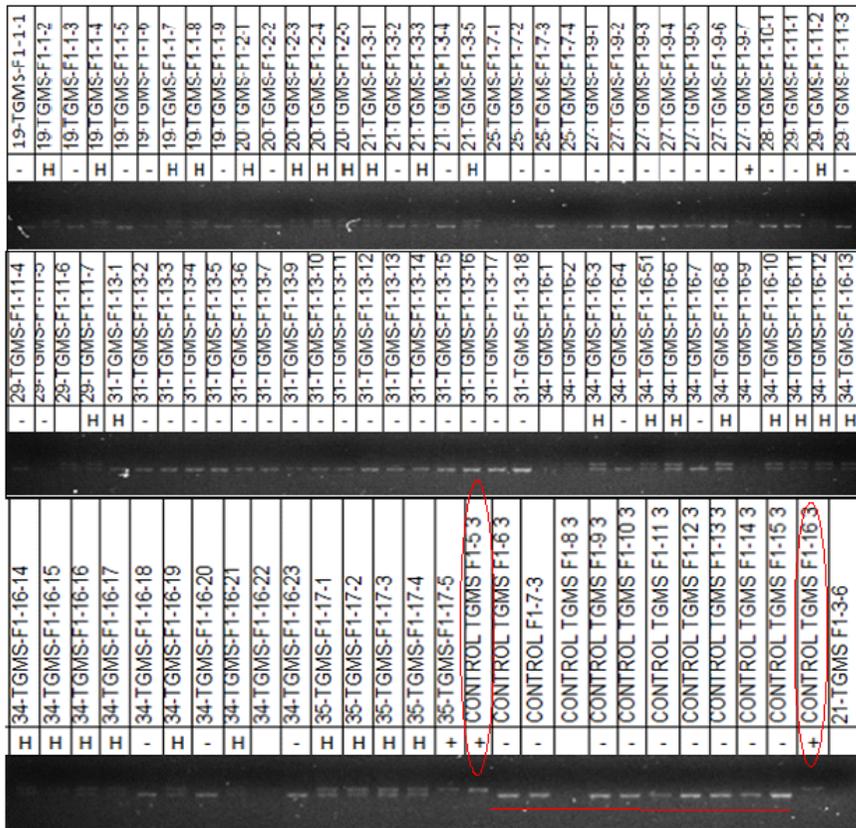


Figure 17. PCR amplification of F1 plants, six parental lines (LINE 27-Control-TGMS-5, NSIC Rc120 Control-TGMS-6, M19S, M20, IR58025B and IR68897B) together with control checks IR24, TN1, IRBB4, IRBB5, IRBB7, and IRBB21- Control-TGMS-16) for BLB using Xa21 marker.

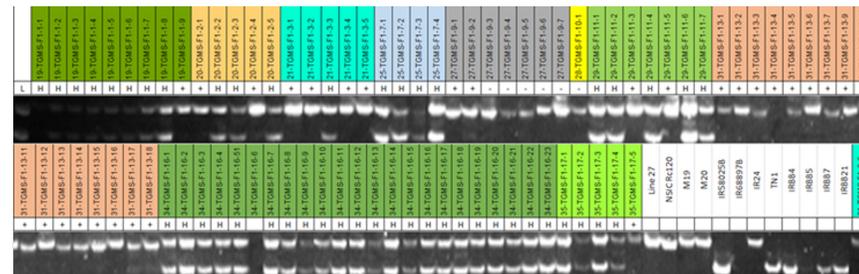


Figure 18. PCR amplification of F1 plants, six parental lines (LINE 27-Control-TGMS-5, NSIC Rc120 Control-TGMS-6, M19S, M20, IR58025B and IR68897B) together with control checks IR24, TN1, IRBB4, IRBB7, and IRBB21-Control-TGMS-16) for BLB using RM8213 marker.



Figure 19. Inoculation of selected tungro resistant using PXO 99 (Race 6) at 40 DAT.

Table 13. List of materials screened for Tungro and BLB resistance.

Entry Description	Generation	No of entries Screened	No of Selected Plants for Tungro	No. of Selected plants for BLB
M19 x LINE 27	F2	13	71	49 (R'), 2 (MR)
M19 x NSIC Rc120	F2	5	40	1 (R')
M20 x LINE 27	F2	8	41	24 (R'), 3 (MR)
M19 x LINE 27/R58025B	F1	8	*	5 (R'), 7 (MR)
M19 x NSIC Rc120/R58025B	F1	1	*	0
M19 x NSIC Rc120/R68897B	F1	3	*	0
M19 x LINE 27/R68897B	F1	1	*	0
M20 x LINE 27/R68897B	F1	2	*	2 (R'), 11 (MR)
M20 x LINE 27/R58025B	F1	3	*	6 (R'), 14 (MR)

*No tungro screening for F1 generation

Abbreviations and acronyms

ABA – Abscisic acid
 Ac – anther culture
 AC – amylose content
 AESA – Agro-ecosystems Analysis
 AEW – agricultural extension workers
 AG – anaerobic germination
 AIS – Agricultural Information System
 ANOVA – analysis of variance
 AON – advance observation nursery
 AT – agricultural technologist
 AYT – advanced yield trial
 BCA – biological control agent
 BLB – bacterial leaf blight
 BLS – bacterial leaf streak
 BPH – brown planthopper
 Bo - boron
 BR – brown rice
 BSWM – Bureau of Soils and Water Management
 Ca - Calcium
 CARP – Comprehensive Agrarian Reform Program
 cav – cavan, usually 50 kg
 CBFM – community-based forestry management
 CLSU – Central Luzon State University
 cm – centimeter
 CMS – cytoplasmic male sterile
 CP – protein content
 CRH – carbonized rice hull
 CTRHC – continuous-type rice hull carbonizer
 CT – conventional tillage
 Cu – copper
 DA – Department of Agriculture
 DA-RFU – Department of Agriculture-Regional Field Units
 DAE – days after emergence
 DAS – days after seeding
 DAT – days after transplanting
 DBMS – database management system
 DDTK – disease diagnostic tool kit
 DENR – Department of Environment and Natural Resources
 DH L– double haploid lines
 DRR – drought recovery rate
 DS – dry season
 DSA - diversity and stress adaptation
 DSR – direct seeded rice
 DUST – distinctness, uniformity and stability trial
 DWRSR – direct wet-seeded rice
 EGS – early generation screening
 EH – early heading

EMBI – effective microorganism-based inoculant
 EPI – early panicle initiation
 ET – early tillering
 FAO – Food and Agriculture Organization
 Fe – Iron
 FFA – free fatty acid
 FFP – farmer’s fertilizer practice
 FFS – farmers’ field school
 FGD – focus group discussion
 FI – farmer innovator
 FSSP – Food Staples Self-sufficiency Plan
 g – gram
 GAS – golden apple snail
 GC – gel consistency
 GIS – geographic information system
 GHG – greenhouse gas
 GLH – green leafhopper
 GPS – global positioning system
 GQ – grain quality
 GUI – graphical user interface
 GWS – genomwide selection
 GYT – general yield trial
 h – hour
 ha – hectare
 HIP - high inorganic phosphate
 HPL – hybrid parental line
 I - intermediate
 ICIS – International Crop Information System
 ICT – information and communication technology
 IMO – indigenous microorganism
 IF – inorganic fertilizer
 INGER - International Network for Genetic Evaluation of Rice
 IP – insect pest
 IPDTK – insect pest diagnostic tool kit
 IPM – Integrated Pest Management
 IRR – International Rice Research Institute
 IVC – in vitro culture
 IVM – in vitro mutagenesis
 IWM – integrated weed management
 JICA – Japan International Cooperation Agency
 K – potassium
 kg – kilogram
 KP – knowledge product
 KSL – knowledge sharing and learning
 LCC – leaf color chart
 LDIS – low-cost drip irrigation system
 LeD – leaf drying
 LeR – leaf rolling
 lpa – low phytic acid
 LGU – local government unit

LSTD – location specific technology development
 m – meter
 MAS – marker-assisted selection
 MAT – Multi-Adaption Trial
 MC – moisture content
 MDDST – modified dry direct seeding technique
 MET – multi-environment trial
 MFE – male fertile environment
 MLM – mixed-effects linear model
 Mg – magnesium
 Mn – Manganese
 MDDST – Modified Dry Direct Seeding Technique
 MOET – minus one element technique
 MR – moderately resistant
 MRT – Mobile Rice Teknoklinik
 MSE – male-sterile environment
 MT – minimum tillage
 mtha⁻¹ - metric ton per hectare
 MYT – multi-location yield trials
 N – nitrogen
 NAFC – National Agricultural and Fishery Council
 NBS – narrow brown spot
 NCT – National Cooperative Testing
 NFA – National Food Authority
 NGO – non-government organization
 NE – natural enemies
 NIL – near isogenic line
 NM – Nutrient Manager
 NOPT – Nutrient Omission Plot Technique
 NR – new reagent
 NSIC – National Seed Industry Council
 NSQCS – National Seed Quality Control Services
 OF – organic fertilizer
 OFT – on-farm trial
 OM – organic matter
 ON – observational nursery
 OPag – Office of Provincial Agriculturist
 OpAPA – Open Academy for Philippine Agriculture
 P – phosphorus
 PA – phytic acid
 PCR – Polymerase chain reaction
 PDW – plant dry weight
 PF – participating farmer
 PFS – PalayCheck field school
 PhilRice – Philippine Rice Research Institute
 PhilSCAT – Philippine-Sino Center for Agricultural Technology
 PHilMech – Philippine Center for Postharvest Development and Mechanization
 PCA – principal component analysis

PI – panicle initiation
 PN – pedigree nursery
 PRKB – Pinoy Rice Knowledge Bank
 PTD – participatory technology development
 PYT – preliminary yield trial
 QTL – quantitative trait loci
 R - resistant
 RBB – rice black bug
 RCBD – randomized complete block design
 RDI – regulated deficit irrigation
 RF – rainfed
 RP – resource person
 RPM – revolution per minute
 RQCS – Rice Quality Classification Software
 RS4D – Rice Science for Development
 RSO – rice sufficiency officer
 RFL – Rainfed lowland
 RTV – rice tungro virus
 RTWG – Rice Technical Working Group
 S – sulfur
 SACLOB – Sealed Storage Enclosure for Rice Seeds
 SALT – Sloping Agricultural Land Technology
 SB – sheath blight
 SFR – small farm reservoir
 SME – small-medium enterprise
 SMS – short message service
 SN – source nursery
 SSNM – site-specific nutrient management
 SSR – simple sequence repeat
 STK – soil test kit
 STR – sequence tandem repeat
 SV – seedling vigor
 t – ton
 TCN – testcross nursery
 TCP – technical cooperation project
 TGMS – thermo-sensitive genetic male sterile
 TN – testcross nursery
 TOT – training of trainers
 TPR – transplanted rice
 TRV – traditional variety
 TSS – total soluble solid
 UEM – ultra-early maturing
 UPLB – University of the Philippines Los Baños
 VSU – Visayas State University
 WBPH – white-backed planthopper
 WEPP – water erosion prediction project
 WHC – water holding capacity
 WHO – World Health Organization
 WS – wet season
 WT – weed tolerance
 YA – yield advantage
 Zn – zinc
 ZT – zero tillage

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Samar Satellite Station, UEP Campus, Catarman, 6400 Northern Samar; Mobile: 0948-800-5284