2015 National Rice R&D Highlights

Crop Biotechnology Center

Department of Agriculture
PHILRICE
PHILIPPINE RICE RESEARCH INSTITUTE

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CROP BIOTECHNOLOGY CENTER

Center Director: RR Suralta

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The world experiences rapid increase in population coupled with climate change, a situation which is not optimum for rice growing setting which may leave the next century to witness serious global rice shortage problems. There is an urgent need hence 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.

The Crops Biotechnology Center implemented 14 studies under two projects: 1) Molecular Characterization, Diversity Analysis and Utilization of Crop Germplasm; and 2) Gene Discovery and Marker Development for Agronomically Important Traits shall utilize advanced molecular and biotechnological tools and techniques to provide new opportunities in developing rice varieties with built-in resistance/tolerance to theses stresses.

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

Project Leader: VG. Dalusong

Germplasm is 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 stress 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 (SWGDAM). The ultimate goal of this project 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 gene pool 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 and 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, and 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 (SWGDAM) guidelines (SWGDAM, 2004). SWGDAM 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 SWGDAM 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.

Highlights:

- Allele ladder for 8 STR markers (total of 32) were completed and ready for use in the alignment activity. Sequence structure based on the number of repeats present in each allele was characterized. Optimization of the multiple allele PCR reaction

was still on-going for the remaining STR markers.

- Allele alignment of 186 reference DNA using STR-M7, STR-M28 and STR-M37 allele ladders is still on-going. Initial result shows the appearance of rare alleles, that is, the frequency of the appearance of that allele is less than or equal to 5% of the total samples. The number of repeat motif present on these alleles will be verified by sequencing.

- A paper entitled "STR markers for identification of rice (Oryza sativa L.) varieties" was submitted for publication to and reviewed by Journal of Forensic Sciences. Verification study was conducted on our submitted paper to address the comments and recommendations of the reviewer of the journal.

- Fragment sizes of DNA samples from cultivated rice, 13 wild rice species, corn, 4 weed species, rice field rat and human saliva on 53 STR markers were detected and analyzed using ABI3500 Genetic Analyzer and Gene Mapper® ID-X software. Fragment sizes were compared to establish the species specificity of the STR markers for rice identification purposes. Results revealed that there was no overlapping of allele sizes between cultivated rice and other non-rice DNA sources. But there was over-lapping between cultivated rice and wild rice varieties. This implies that the STR markers tested were specific to Oryza species. The results of fragment analysis were also compared to the result on conventional genotyping method.

- The efficiency of DNA extraction protocols we previously developed, modified and established for rice DNA were validated by an analysis we conducted on alleged fake rice. The objective of the analysis is to detect whether the alleged fake rice samples brought by NFA is truly a rice or a fake one. Due to some limitations of our study, we were only able to detect if the samples brought to us have rice DNA or do not have. We were not able to detect whether the samples are pure or mixture. Using our protocols, we were successful in obtaining DNA from cooked samples of rice grain, rice flour, corn, cornstarch, potato, sweet potato, cassava, cassava flour and wheat flour. To test whether the DNA we isolated were of rice, all samples were subjected to PCR using the set of STR markers we validated. Results shows PCR amplifications of the control rice DNA and alleged fake rice samples on 46 STR markers used. Comparison of DNA bands between rice and other crop samples were not possible due to insufficient data

on the DNA of other crops tested. The conclusion of this study is that on the three samples given to us for analysis, "presence of rice DNA cannot be eliminated".

Genetic and molecular characterization of rice mutants and introgression lines

AA Alfonso, RT Miranda, CFS Te, NR Sevilla, XGI Caguiat, and RL Ordonio

Genetic characterization has been an important aspect of discovering novel genes and traits that are relevant to rice breeding such as disease and pest resistance, abiotic and biotic stress tolerance, excellent grain and superior yield qualities. However, in the past years, conventional breeding alone has been proved to be time consuming especially in discovering new genes. Induced mutation such as chemical or gamma radiation allows fast generation of new traits. This would allow breeders to introgress desirable traits into new or existing superior varieties. Field testing and disease screening are important tools in understanding which of these lines contain the desirable traits. In the same manner, the genotype of these mutant lines is equally important since they would give the exact genetic basis that confers the desirable trait of interest. The advent of molecular marker technology facilitates fast, reliable and cost-effective approach in discovering variation, gene location and their mode of inheritance.

There are several rice mutants developed with resistance to diseases like blast (Han et al., 2004), bacterial blight, and tungro (Wu et al., 2005). At PhilRice CES, there are several promising rice mutants that are agronomicallly and phenotypically tested such as BLB resistant mutant lines with NSIC Rc144 background, some putative lesion mimic mutants ("ratik-ratik"), mutant with various traits such as double grains, extra glumes, extra reproductive organs, homoetic mutations (open hull), red pericarp and others. Some of these mutants are being used as donor parents and as possible sources of novel alleles in the current rice breeding program. Most screening was done based on agromorphological and disease resistance screening to facilitate line selection for succeeding breeding activities. However, phenotype-based evaluation has been proved to be partially affected by the environment; thus results could be less accurate. Therefore, it is ideal that breeders also evaluate promising mutant lines using genetic markers known to be linked to the trait as determined by previous gene mapping studies. Such a breeding program is geared to develop new varieties with relative ease. In the case of mutants with yet unknown causal genes, forward genetics could be conducted to determine the chromosomal location, molecular function and sequence of the novel gene. Ultimately, these mutants can be utilized in breeding programs to develop better commercial varieties that are high-yielding, disease/pest-resistant and biotic and abiotic stress-tolerant.

This research is conducted to characterize rice mutants and their introgression lines using genetics and molecular tools. Specifically, it aims to:

1. Characterize the genetic and molecular profile of elite rice mutants and their introgression lines;
2. Analyze the nature of mutant alleles present in these elite rice mutants and their introgression lines; and
3. Identify molecular functions of genes behind some novel mutants

Highlights:

- Whole genome sequencing results of NSIC Rc144 wild type and its bacterial blight-resistant mutant, MSL 40 were obtained from the Philippine Genome Center. Basic information data for the sequencing results are shown in Table 1 and 2. Assembly of the sequencing data for analysis and interpretation is on-going. Preliminary analysis showed that sequence data reads of wild type and mutant are of high quality as obtained through paired-end sequencing using MiSeq (Illumina, USA). Table 1 shows that there was no poor quality sequence detected and that the total volume of sequences is 4 Mb for wild type (Rc144) and 7Mb for the mutant (MSL40). The reference sequence used for both samples was Oryza sativa L. cv. Nipponbare (http://plants.ensembl.org/). In Table 2, variant calls or single nucleotide polymorphisms per chromosome are listed showing variation between wild type and mutant samples. Trend shows that the wild type has more SNPs compared to the mutant line in all chromosomes which suggests that the wild type has higher variation against the reference genome.

- Generated 23 sets of primers (Table 3) specific to the four different Xa genes (Xa4, Xa5, Xa7, Xa21) to be used in the molecular characterization of MSL 37, MSL 40 and other rice mutants and introgression lines thru TILLING, a reverse genetics screening technique.

- 23 F1 crosses between nine mutants and their wild types for genetic analysis were seed-increased for generation advance. All mutant traits were confirmed to have a recessive gene action as all F1 plants did not express the traits of the mutant parent.

- Conducted tungro, BLB and drought screening of different generations of lesion mimic introgression lines derived from NSIC Rc144 X lesion mimic mutant. Figure 1 shows the genotyping result of the lesion mimic mutant along with that

of ARC11554 (positive check) and TN1 (negative check) using simple sequence repeat markers RM5495 and RM8213 linked to tsv1 loci for tungro resistance and glh14 loci for GLH resistance, repectively. Results show that out of 30 plants subjected to genotyping, one plant is heterozygous for tsv1 (RM5495) and homozygous for glh14 (RM8213). Further screening and genotyping of its progenies will be carried out for more in-depth characterization of the selected lesion mimic mutants.

Table 1. Summary of quality check information from FastQC.

MEASURES	VALUE/ENTRY	
Sample	Rc144 (wild)	MSL40 (mutant)
Total Sequences	4758741	7241936
Sequences flagged as poor quality	0	0
Sequence length	35-251	35-251
%GC	42	42

Table 2. Summary of SNP counts for the wild type NSIC Rc144 and the mutant MSL-40 as derived from the whole genome sequence analysis.

Chromosome No./Description	NSIC RC144 (WT)	MSL-40 (MUTANT)
Chr1	148,508	129,989
Chr2	122,574	111,111
Chr3	116,271	106,044
Chr4	111,007	105,291
Chr5	89,702	75,424
Chr6	106,151	95,963
Chr7	106,444	93,592
Chr8	95,431	89,307
Chr9	79,045	74,710
Chr10	95,733	83,843
Chr11	124,676	105,122
Chr12	106,193	91,691
ChrSy	1,663	1,680
ChrUn	2,979	2,633
Total	1,306,377	1,166,400

Table 3. List of primers designed for TILLING.

For Xa4: Xa4_F5_923_63 ctgcacatgtatcggactttgg Xa4_R5_923_63 acatgtcttaatgtccagtgtttgc Xa4_F4_949_60.7 ccatggtcagataccaaaaggggtgta Xa4_R4_949_60.7 cgtgacgcttttccaagagcacca Xa4_F3_847_59.2 tttcactggaaacctcccagac Xa4_R3_847_59.2 ggaagaggttgtttggcatgatgg Xa4_F2_850_59.3 tacctggttgcattggctcc Xa4_R2_850_59.3 gctagcaccatgaggcca Xa4_F1b_849_60.0 tattcctgccgtgccaatgca Xa4_F1a_1057_61.0 ggtcttggttagtcacaagtgggaaaggga Xa4_R1b gggaggttgaaacttgcgttgcca	**For Xa7:** Xa7_F10_675_59.3 gcactgtactgctcactcccac Xa7_R10_675_59.3 tgcgatttctgcaacgcga Xa7_F9_494_60.0 acccatcaaagcataatcgcatgcagc Xa7_R9_494_60.0 tgggcattggaaaagggaggacaa Xa7_F8_927_60.7 tacagacgggctctacctccgcacg Xa7_R8_927_60.7 acgaagtgcaacaacaacgctcctc Xa7_F7_68_60.2 agggccttttgctgacgtgg Xa7_R7_68_60.2 ttttcgtgcggaggtagagcccgt Xa7_F6_899_60.2 gcatgagctgtgattaccttcgagc Xa7_R6_899_60.2 acattgtgacgagcagagcggta Xa7_F5_874_60.5 ggtgtccaaagttgttgccatctgc Xa7_R5_874_60.5 acaagtgccagtaatatgcacgagggtg Xa7_F4_814_59.4 atgagcctagcgggcatgg Xa7_R4_814_59.4 cagagttccacctaggctgc Xa7_F3_917_60.4 gtgcgatctgttaatggctgcacac Xa7_R3_917_60.4 tgcagatgagaaacaacttttggcctcgtg Xa7_F2_896_60.4 ccagtgtaggaaatttagctcgccaatacc Xa7_R2_896_60.4 gatccccagcataacgaaggaccagc Xa7_F1_992_59.3 agtggaaatgcagcccaagaa Xa7_R1_992_59.3 tgcatgaatcgaacttctcgctgttgc
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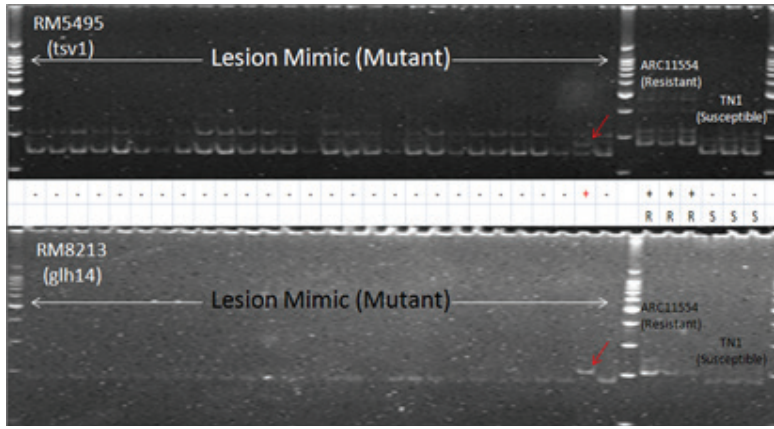

Figure 1. Genotyping of lesion mimic mutants together with resistant (ARC11554) and susceptible (TN1) check for rice tungro virus using SSR markers, RM5495 (tsv1 for tungro resistance) and RM8213 (glh14 for glh resistance).

II. Gene Discovery and Marker Development for Agronomically Important Traits

Project Leader: OE Manangkil

Shortages in food production coupled with plateaued rice yield necessitates the use of modern technologies in improving our rice varieties. Yield of modern varieties is affected by stresses, both biotic an abiotic due to their intolerance to several stresses including the new emerging climate change induced stresses. Improvement of rice may be left behind due to the fast phase of changing world towards situations not favorable to rice. The need to fast tract the improvement of rice through the use of different molecular genetics approaches is indeed necessary and timely.

Moreover, the world has a rapid increase in population with changing climate not optimum to rice growing setting. These consequences have made the next century may witness serious global rice shortage problems. Consequently, there is a need for an increase in rice grain yield. One of the key is the rice tolerance to biotic and abiotic stresses. Many important complex traits of rice in tolerance to biotic and abiotic tolerance are controlled by QTLs derived from natural variations. Recent studies have succeeded in isolating and characterizing genes involved in the rice tolerance to many important traits.

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 the solution in global rice shortage in the coming centuries. 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 to these stresses. Nevertheless, genetic systems responsible for tolerance remain largely unknown yet.

The goal of this project is to study the molecular genetics of rice important traits. Specifically, the objectives are to study/determine rice genes/loci responsible to heat tolerance, crack resistance, low phytic acid (lpa), grain quality traits, functional stay-green traits, root plasticity, grain quality, blast resistance, insect resistance, tungro resistance, salinity tolerance, bacterial leaf blight, drought tolerance, and root plasticity in response to drought and under soil moisture fluctuation stresses. This project is being conducted to identify, clone and map important tolerant genes through

different molecular genetics approaches on Oryza sativa L., commonly known as rice.

The expected outputs of this project includes expression of candidate genes in the NILs, clones of the resistance QTL from the genomic library of ARC11554, transfer of the resistance QTL to susceptible high-yielding varieties, inheritance of the low phytic acid (lpa) gene, gene expression of seedling vigor candidate genes, salinity genes identified from Nypa tree, and good grain quality traits in modern rice cultivars. Studies involving the development of disease resistant cultivars includes outputs such as DNA fingerprints and profiles of target blast resistance donor parents and recipients established using new and effective functional blast resistance markers, new information on functional blast resistance gene markers including primer sequences, diagnostic allele sizes and chromosome locations obtained, disease reaction of popular and high yielding rice varieties as well as elite and advanced breeding lines against virulent blast isolates, and durable blast resistant elite breeding lines developed with pyramids of blast resistance genes through marker assisted breeding. Other outputs expected in this project involves working on BLB and RTD marker system in developing thermo-sensitive genic male sterile (TGMS) lines, DHLs with improved grain yield under progressive drought phenotypically characterized for root traits relevant for dehydration avoidance under greenhouse and field conditions, QTLs controlling root traits relevant for dehydration avoidance and yield under progressive drought, tungro resistance, seedling vigor and heat tolerance, and DNA marker system for low phytic acid (lpa) gene validated and established for marker assisted breeding approach.

Molecular genetics and breeding of rice heat tolerance: QTL analysis of trait and line development

AL Baniqued, OE Manangkil, WV Barroga, and PN Marcelo

Rice grows optimally between 20 to 35°C and becomes increasingly sensitive to increasing temperatures especially during flowering which can eventually reduce yields (Redona, et al 2007). Matthews et al. (1994 a, b) reported that the severe losses in South, Southeast and East Asia for rice was due to a threshold temperature effect that caused spikelet sterility but that genetic variation with regard to the threshold can provide significant opportunity to switch varieties as temperature rose. Therefore the development of rice varieties for high temperature tolerance is very important in addressing climate change scenarios in rice growing areas where 90-95% of the population depends on rice. There is a need to breed rice varieties that can tolerate higher temperatures or can avoid exposure to high temperatures by having shorter growing seasons or flowering that occurs during cooler periods of the day (Redona et al 2007). With the anticipated global climate change,

rice production and food security is at stake in these countries. Thus, there is a need to develop more productive and sustainable crop production technologies adaptable to respective regions.

This study aimed to: (a) map QTLs in rice cultivars associated to heat tolerance to understand the genetic and physiological systems responsible for the heat tolerance in rice plants, and (b) to generate advance lines with high pollen fertility despite high temperature during the flowering stage of the crop.

Highlights:

- There were 40 entries including parental checks (Nagina22 and PSB Rc82) and one susceptible check (IR52) established in the field. Rice plants were planted late to coincide flowering with the hottest period of the year (between April-May). 21-day old seedlings were transplanted in 1m x 5m, 25 hills per row. Entries were laid out in a randomized complete block design (RCBD) with three replications. Standard crop management practices such as water, fertilizer and weeding at PhilRice were followed.

- Phenotypic data such as days to flowering, plant height, number of productive tillers, grain yield, pest and diseases were gathered.

- Heat tolerant check varieties PSB Rc82 and Nagina22 yielded 2,305.61 and 146.60 kg/ha, respectively while and susceptible check IR52 yielded 2,512.83 kg/ha. Yield ranged from 58.48-2,512.83 kg/ha (Figure 2). Only one line, PR40714-376-2-1-1-1-B-B was found to be superior to checks with 1.78 yield advantage over PSB Rc82. Plant height ranged from 76-105.8 cm and productive tiller numbers ranged from 7.2-13.8.

- Stem borer, brown plant hopper, sheath blight, bacterial leaf blight, and tungro were observed.

- High infestation of stem borer was observed in the area across the three replicates due to late planting (Figure 2). Also, when the entries started to flower it was affected by heavy rainfall which contributed to the low yield of all of the entries including the parental checks. Thus, screening for heat tolerance during dry season was not attained.

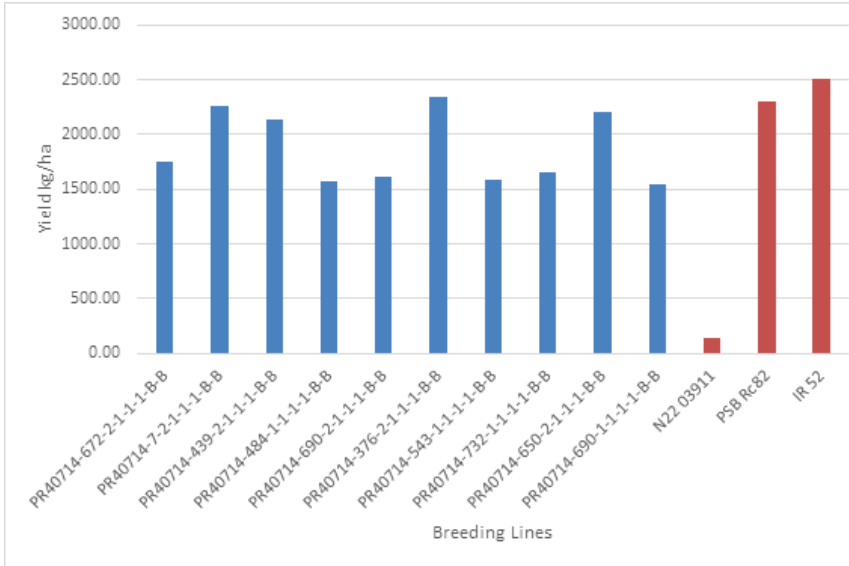

Figure 2. Grain yield performance of top 10 heat tolerant lines evaluated during 2015DS.

Establishment of marker system and marker assisted selection (MAS) for blast resistance in irrigated lowland rice cultivars

TE Mananghaya and LM Perez

In the Philippines, and perhaps in all rice farming areas around the globe, rice blast is a disease which poses great constraint to rice production. While there are several options for control strategy, the use of resistant variety is the most effective way. Because of this, identification of blast resistance genes (R genes) has gained so much attention. Through the years, many blast resistance genes have been discovered and numerous QTLs have been identified. Utilization of this genetic information can be exploited to develop durable resistance to the disease in rice. The combining or pyramiding of different blast resistance genes through marker aided selection probably represents the best strategy to achieve durable resistance.

Highlights:

- Four advanced lines of PR34172-10-1-1-3-2*2/SHZ-2 were introduced to Multi- Environment Testing (MET 0) in 2015 dry season and three out of four advanced breeding lines exhibited resistance to rice blast disease in PhilRice blast nursery in 2015 dry season (Table 4). The two breeding lines generated using three- way cross (NSIC Rc9/SHZ-2//IRBLzFu, PSBRc10/IRBLsh-Ku//IRBLzFu) possibly harboring two blast R genes were introduced to observational nursery for evaluation

of agronomic and morphological traits and advance to yield trial in 2016 dry season. These lines were also evaluated for blast resistance and conferred resistant reaction to the pathogen (Table 4). Pyramiding of blast R genes using marker assisted selection (MAS) into identified rice genotypes will provide a broad spectrum resistance to different blast isolates.

- Another three advance lines were introduced to MET0 in 2016 dry season, NSIC Rc9/IRBLz5-CA, NSIC Rc9/SHZ-2//IRBLzFu and NSIC Rc128/IR65482-4-136-2-2. Two cross combinations were found resistant to rice blast disease while NSIC Rc128/ IR65482-4-136-2-2 showed intermediate reaction to rice blast pathogen present in blast nursery (Table 4).

- Out of 213 F3 segregating population established, 54 lines were selected and introduced to Observational Nursery (ON) for morpho-agronomic characterization and 28 advanced lines for yield trial. A total of 23 new cross combinations were generated using NSIC Rc224, NSIC Rc238, NSIC Rc298,NSIC Rc300,NSIC Rc304 as recipient parent and SHZ-2 (QTL), DACCA 6, IR65482-4-136-2 (Pi40 gene), IRBL9-W (Pi9) IRBLz5-CA(Piz5) IRBLsh-S(Pish) IRBLzFu (Piz) as source of blast resistant genes. These identified effective blast resistance genes can be used for genetic crop improvement of promising and high yielding rice varieties in the Philippines.

- Polymorphism survey of 12 recurrent parents used in introgression of blast R genes were conducted using z565962 (Piz) and pBBA14 (Pi9). All background genotypes showed negative allele to both markers, while F1 progenies generated and differential varieties showed positive allele to corresponding diagnostic marker.

- A total of 490 F2 test entries were evaluated for rice blast disease resistance in Crop Protection Division blast nursery in 2015 wet season. Out of 490 materials, 235 were found resistant, 124 for intermediate and 131 test entries showed susceptible reaction to rice blast pathogen present in blast nursery (Table 6). Out of 11 F2 cross combinations introduced, 4 F2 segregating populations (NSIC Rc160/IRBLsh-S (CO), NSICRc224/SHZ-2 9, NSIC Rc 240/ Dacca 6-1-18, NSIC Rc 298/ SHZ-2-11) showed resistant reaction to rice blast disease with a range of 69-81% from the total entries of each cross introduced for blast resistance evaluation (Table 6). Two resistant checks (IR65482-4-136-2-2 and SHZ-2) and two susceptible checks (US 2 and CO39) were used in rice blast

screening, National Cooperative Testing (NCT) manual was used in scoring of leaf blast lesion. Test entries found resistant were transplanted for advanced generation and harvested seeds will be introduced again in blast resistance evaluation in 2016 dry season.

Table 4. Advanced lines with blast resistance genes and their reaction to rice blast disease.

Parentage	Pedigree Number	Generation	Gene	Reaction	Remarks
PR 34712-10-1-1-3-2*2/SHZ-2	PR39543-5-1-1	F8	*QTL*	R	MET 0, 2015 DS
PR 34712-10-1-1-3-2*2/SHZ-2	PR39543-26-1-1	F8	*QTL*	R	MET 0, 2015 DS
PR 34712-10-1-1-3-2*2/SHZ-2	PR39543-40-2-1	F8	*QTL*	I	MET 0, 2015 DS
PR 34712-10-1-1-3-2*2/SHZ-2	PR39543-41-2-2	F8	*QTL*	R	MET 0, 2015 DS
NSIC Rc9/IRBLz5-CA	PR47382	BC3F4	*Piz5*	R	ON, 2015 WS, MET 0 2016 DS
NSIC Rc194/SHZ-2	PR47381	BC3F4	*QTL*	R	ON, 2015 WS, Yield Trial 2016 DS
PSB Rc82/SHZ-2	PR42235	BC3F4	*QTL*	I	ON, 2015 WS, Yield Trial 2016 DS
NSIC Rc9/SHZ-2//IRBLzFu	PR42304	F8	*QTL,Piz*	R	ON, 2015 WS, MET 0 2016 DS
NSIC Rc9/SHZ-2//IRBLzFu	PR42304	F8	*QTL,Piz*	R	ON, 2015 WS, Yield Trial 2016 DS
NSIC Rc9/IRBLzFu//SHZ-2	PR42304	F8	*QTL,Piz*	R	ON, 2015 WS, Yield Trial 2016 DS
NSIC Rc9/IRBLzFu//SHZ-2	PR42304	F8	*QTL,Piz*	R	ON, 2015 WS, Yield Trial 2016 DS
PSB Rc10/IRBLsh-Ku//IRBLz-Fu	PR42262	F8	*Pish,Piz*	R	ON, 2015 WS, Yield Trial 2016 DS
NSIC Rc128/IR65482-4-136-2-2	PR47394-74-21-2-6-B-B	BC2F4	Pi40,Pizt	I	MET 0, 2016DS

Legend: R- Resistant I- Intermediate

Table 5. DNA genotyping of recurrent parents, differential varieties and F1 progenies using z565962 and pBA14 molecular markers.

	Piz	*Pi9*									
Recurrent Parent	z565962	pBA14	1st Donor	z565962	pBA14	2nd Donor	z565962	pBA14	Three-way cross	z565962	pBA14
NSIC Rc128	-	-	SHZ-2	-	-	IRBLz-Fu	+	-	BLF1-2(zFu)	+	-
NSIC Rc148	-	-	IRBL9-W	-	+	SHZ-2	-	-	BLF1-47(SHZ2)	+	-
NSIC Rc152	-	-	SHZ-2	-	-	IRBL9-W	-	+	BLF1-3(9W)	-	+
NSIC Rc154	-	-	SHZ-2	-	-	IRBL9-W	-	+	BLF1-15(9W)	-	+
NSIC Rc154	-	-	IRBLz-Fu	+	-	IRBL9-W	-	+	BLF1-32(9W)	-	+ (faint)
NSIC Rc158	-	-	SHZ-2	-	-	IRBLz-Fu	+	-	BLF1-5 (zFu)	+	-
NSIC Rc184	-	-	IRBLz5-CA	-	-	IRBL9-W	-	+	BLF1-34 (9W)	+	+
NSIC Rc194	-	-	IRBLz-Fu	+	-	SHZ-2	-	-	BLF1-23(SHZ2)	-	+ (faint)
NSIC Rc226	-	-	IRBLz-Fu	+	-	SHZ-2	-	-	BLF1-21 (SHZ2)	+	-
NSIC Rc9	-	-	SHZ-2	-	-	IRBLz-Fu	+	-	BLF1-1 (zFu)	-	-
NSIC Rc9	-	-	IRBLz-Fu	+	-	SHZ-2	-	-	BLF1-18 (SHZ2)	-	+
PSB Rc10	-	-	SHZ-2	-	-	IRBL9-W	-	+	BLF1-9(9W)	-	+
PSB Rc10	-	-	IRBLi-F5	-	-	IRBL9-W	-	+	BLF1-24(9W)	-	+
PSB Rc10	-	-	IRBLsh-Ku	+	-	IRBLz-Fu	+	-	BLF1-37(zFU)	-	+
PSB Rc10	-	-	IRBLz5-CA	-	-	IRBL9-W	-	+	BLF1-30(9W)	-	+
PSB Rc82	-	-	IRBLz-Fu	+	-	SHZ-2	-	-	BLF1-22(SHZ2)	+	-
PSB Rc82	-	-	IRBLi-F5	-	-	IRBL9-W	-	+	BLF1-26 (9W)	-	+
PSB Rc82	-	-	IRBLi-F5	-	-	IRBL9-W	-	+	BLF1-25 (9W)	-	+
PSB Rc18	-	-	PSB Rc14	-	-	IRBL9-W	-	+	BLF1-27(9W)	-	+

Legend: + (positive to diagnostic marker) - (negative to diagnostic marker)

Table 6. Summary of test entries evaluated for rice blast resistance evaluation in 2015 wet season in PhilRice-CES blast nursery.

		Reaction to leaf blast		
Test Entries	**No. of Entries**	**Resistant**	**Intermediate**	**Susceptible**
F_2 population	486	235	123	128
Improved rice cultivar	4	0	1	3
Total	**490**	**235**	**124**	**131**

Molecular genetics of grain quality traits in support to rice quality improvement

APP Tuaño, TE Mananghaya , ATP Dela Cruz, RP Mallari, LM Perez and BO Juliano

Several rice breeding programs (e.g., USDA, IRRI, Yanco Agricultural Institute, Australia, National Institute of Crop Sciences, Japan, Zhejiang University, China, etc.) have used molecular markers technology to assist in their selection for various traits including grain quality. Functional markers have been developed and adopted in marker-aided selection at early breeding stages and without the need to wait for the harvested seeds to be analyzed using chemical methods before breeders can select which lines to discard or advance to the next stage/season. This technology is useful in determining disease and pest resistance, yield-related parameters and eating quality traits using DNA markers associated with major genes responsible for the expression of the desired traits, provided environmental effect on the trait is minimal.

PhilRice breeding program has employed molecular markers to complement conventional screening activities towards disease and pest resistance, tolerance to abiotic stresses, desirable agronomic properties and other traits of the breeders' interest but to date grain quality screening is still based on physicochemical and sensory evaluation protocols requiring seeds at harvest, relatively large sample size (i.e. 125-250 g rough rice) and at least 2 months of ageing to stabilize some grain quality properties. These requirements hamper breeders' selection for good grain quality at early breeding stages and they place grain quality screening at a later and more advanced stages where large sample size can be acquired from their field trials. The downside is that they might have lost some good materials in terms of grain quality due to the lack of a rapid, simple but reliable screening platform in the earlier stages. Considering the availability of molecular markers for grain quality and PhilRice's enhanced capability in molecular genetics and DNA-related research, a molecular platform composed of functional markers associated with grain physicochemical properties related to eating quality, should be established and will be helpful in addressing

this problem of grain quality screening at the early breeding stages, thus this study proposal.

Highlights:

- Molecular analysis of the PhilRice crossing block, Philippine released modern varieties, farmers' specialty rices, selected PhilRice germplasm accessions and DA export varieties, using the optimized protocols for grain quality markers, is still ongoing.

- Farmers' specialty rices collected from various regions of the country have been characterized for grain quality properties (Table 7). Specialty lowland and upland rices (n = 229) collected from Filipino farmers in 2009 to 2012 were long-, medium- and short-sized grains. Of these, 108 had intermediate apparent amylose content (AC), 80 had low AC, 26 had high AC and 15 were waxy (mean AC 17.7%). They had mainly intermediate–high gelatinization temperature (GT) by alkali spreading value. High GT was predominant among low-AC specialty rices, instead of low GT. However, the cultivars did not exhibit high milled rice translucency (only 10 had 0 to 2% chalky grains) and crack (fissure) resistance needed for high head rice recovery required of export quality rice.

- Only 50 of 225 rices had the 8-base pair deletion at exon 7 of the fragrance gene although many of the rices were aromatic as noted by the farmer donors.

- Three single nucleotide polymorphism (SNP) markers for Waxy gene of which combinations are highly associated with apparent amylose content (AC) type were optimized and adopted using selected farmers' specialty rices and Philippine modern varieties differing in AC.

- Farmers' specialty rices collected previously characterized were subjected to molecular analysis and showed various Waxy SNPs combinations but were able to differentiate AC types. A representative set is shown in Table 8 where the determined alleles of the Waxy (Wx) gene included the: (1) number of cytosine-thymine repeats [(CT)n]; (2) G/T single nucleotide polymorphism (SNP) at intron 1; (3) A/C SNP at exon 6; and (4) C/T SNP at exon 10. Combination of the Wx alleles [*e.g.* 17TAC where: (CT)n is 17, intron 1 SNP is T, exon 6 SNP is A, and exon 10 SNP is C] differentiated the cultivars based on ammonium buffer colorimetric AC. Waxy and low-

AC rices were mainly 17TAC. Intermediate-AC rices showed 17GCC, 19GCC and 20GCC Wx alleles combination while 10GAT and 11GAT were mainly found among high-AC rices. A revision for lowering the AC ranges of apparent amylose types for classification of Philippine rice has been proposed based on ammonium buffer AC values verified by differential scanning calorimetry and combination of the Wx alleles: waxy 0.0 to 2.0%, low AC 10.1–17.0%, intermediate AC 17.1 to 22.0% and high AC > 22.0%. Only Kaltao from Isabela had high AC and GCC SNPs, and needs verification. Waxy alleles combinations supported the differentiation of rices in terms of AC brought by the recent lowering of AC ranges per type using the ammonium buffer AC assay.

- Two SNPs and one functional nucleotide polymorsphism (FNP) markers were optimized to predict gelatinization temperature (GT) type complementary to alkali spreading value (ASV) – a rapid and simple GT index employed in the breeding program. These starch synthase IIa (SSIIa) markers have been reported to associate well with GT. The combination of these SNPs and FNP only differentiated the samples into high and low GT types (Table 8). Only Improved Malagkit Sungsong 2 had AAGC (where: SNP2 is A, SNP3 A, FNP GC) while the rest of the low-GT samples had AGTT. High-GT and intermediate-GT rices had either GGGC or AGGC SNPs/FNP combinations.

- Detailed discussions on some data presented here are reported in the following papers:
  a. Tuaño APP, Aoki N, Fujita N, Oitome NF, Merca FE, Juliano BO. 2014. Grain and Starch Properties of Waxy and Low-Apparent Amylose Philippine Rices and of NSIC Rc222. Philipp Agric Scientist 97:329:339; and
  b. Tuaño APP, Padolina TF, Perez LM, Juliano BO. 2015. Survey of Grain Quality of Philippine Farmers' Specialty Rices. Philipp Agric Scientist 98:446:456.

Table 7. Range and mean physical and physicochemical properties of farmers' specialty rices collected from various provinces of the Philippines, 2009-2012.

Property	Samples (no.)	Range	Mean	
Brown rice (% of rough rice)	224	64 – 83	77	
Total milled rice (% of rough rice)	224	54 – 73	67	
Head rice (% of rough rice)	224	14 – 70	53	
Head rice of stressed grain (% of rough rice)	173	6 – 59	31	
Grain length (mm)	229	3.4 – 7.5	6.1	Medium
Grain shape (L/W)	229	1.4 – 3.7	2.6	Bold
Chalky grains (% of milled rice), nonwaxy	214	1 – 78	21	
Apparent amylose content (%), nonwaxy	214	11 – 26	18	Intermediate
Alkali spreading value, nonwaxy	214	3.2 – 7.0	5.0	Intermediate
Gel consistency (mm), nonwaxy	152	30 – 100	53	Medium
Instron cooked rice hardness (kg cm^{-2}), nonwaxy	210	1.4 – 4.0	2.2	
RVA peak viscosity (RVU), nonwaxy	109	142 – 426	235	
RVA breakdown (RVU), nonwaxy	109	26 – 223	88	
RVA setback (RVU), nonwaxy	109	–34 – 166	42	
RVA consistency (RVU), nonwaxy	109	75 – 288	130	
Amylograph peak viscosity (BU), nonwaxy	63	440 – 840	646	
Amylograph breakdown (BU), nonwaxy	63	95 – 290	217	
Amylograph setback (BU), nonwaxy	63	–125 – 865	278	
Amylograph consistency (BU), nonwaxy	63	40 – 990	495	
Chalky grains (% of milled rice), waxy	15	58 – 100	88	
Apparent amylose content (%), waxy	15	0 – 4	1	Waxy
Alkali spreading value, waxy	15	3.0 – 7.0	4.9	High
Gel consistency (mm), waxy	11	30 – 90	80	Soft
Instron cooked rice hardness (kg cm^{-2}), waxy	15	0.8 – 1.4	1.1	
RVA peak viscosity (RVU), waxy	9	122 – 208	168	
RVA breakdown (RVU), waxy	9	17 – 84	57	
RVA setback (RVU), waxy	9	–64 – 79	–15	
RVA consistency (RVU), waxy	9	24 – 137	42	
Amylograph peak viscosity (BU), waxy	7	125 – 780	477	
Amylograph breakdown (BU), waxy	7	5 – 240	130	
Amylograph setback (BU), waxy	7	–180 – 285	11	
Amylograph consistency (BU), waxy	7	45 – 390	141	

Abbreviations: L/W - length-width ratio; RVA - Rapid Visco-Analyser; RVU - Rapid Visco Unit; BU - Brabender Unit.

Table 8. Comparison of Waxy (Wx) alleles, apparent amylose content (AC) in ammonium buffer, starch synthase IIa (SSIIa) alleles and alkali spreading value: 27 farmers' specialty rice samples.

Sample name and source province	*Wx* alleles[a]	Apparent amylose content[b] (%)	*SSIIa* alleles[c]	Alkali spreading value
Waxy and low-AC				
Improved *Malagkit Sungsong* 2, Nueva Ecija	TAC	0.1 ± 0.0 Wx	AAGC	6.0 L
Balatinaw, Mt. Province	TAC	0.2 ± 0.1 Wx	GGGC	3.3 H
Jasmin, Or Mindoro	TAC	12.9 ± 0.3 L	AGTT	6.9 L
Jasponica, Laguna	TAC	13.0 ± 0.6 L	AGTT	6.0 L
Miponica, Laguna	TAC	14.2 ± 0.5 L	AGTT	5.1 H
Dinorado, Or Mindoro	TAC	15.7 ± 0.1 L	AGTT	6.8 L
Sampaguita, Tarlac	TAC	16.6 ± 0.4 L	AGTT	5.8 L
IR841 selection, Laguna	TAC	17.0 ± 0.7 L	AGTT	4.7 H
Minerva, Or Mindoro	TAC	16.4 ± 0.4 L	AGTT	6.6 L
Mean		**11.8 ± 0.3**		
Intermediate-AC				
Kinadoy, Palawan	GCC	17.5 ± 0.7 I	GGGC	4.3 I
Balsamo, Ilocos Norte	GCC	18.5 ± 0.3 I	AGGC	4.8 I
Perurutong, Laguna	GCC	19.3 ± 0.2 I	AGGC	4.7 I
Improved BPI, Camarines Sur	GCC	19.6 ± 0.2 I	GGGC	5.4 I
La Castellana, Negros Occ	GCC	19.9 ± 0.3 I	GGGC	4.9 I
Mimis, Isabela	GCC	20.3 ± 0.5 I	AGGC	5.0 I
Blondie, Camarines Sur	GCC	20.5 ± 0.5 I	GGGC	4.0 I
Palawan, Iloilo	GCC	20.6 ± 0.2 I	AGGC	5.0 I
Wagwag Paran, Nueva Viscaya	GCC	21.1 ± 0.4 I	GGGC	5.7 L
Bulao, Sorsogon	GCC	21.2 ± 0.7 I	GGGC	5.0 I
Mean		**19.9 ± 0.4**		
High-AC				
Malido, Iloilo	G T	22.5 ± 0.0 H	AGGC	5.0 I
Kaltao, Isabela	GCC	22.7 ± 0.1 H	AGGC	5.0 I
PSB Rc10, Occ Mindoro	GAT	23.6 ± 0.7 H	GGGC	4.9 I
Azucena, Nueva Ecija	GAT	23.8 ± 0.6 H	AGGC	6.6 L
Raminad, Nueva Ecija	GAT	23.9 ± 0.4 H	GGGC	6.0 L
Wagwag Señorita, Cagayan	GAT	23.9 ± 0.4 H	GGGC	5.0 I
Gurong Gurong, Camarines Sur	GAC	24.2 ± 1.1 H	GGGC	5.0 I
Wagwag Magasal, Nueva Viscaya	GAT	25.6 ± 0.4 H	GGGC	6.0 L
Mean		**23.8 ± 0.5**		

[a] *Wx* alleles combination [*e.g.* TAC, where: intron 1 SNP is T, exon 6 SNP is A, and exon 10 SNP is C].

[b] Mean ± SD of three replications. 1 mL 0.9 M NH_4Cl and 2 mL 0.15% I_2 in 1.5% KI for ammonium buffer method based on Juliano et al. (2012).

[c] *SSIIa* alleles combination (*e.g.* GGGC, where: SNP2 is G, SNP3 is G and FNP4 is GC).

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

JM Niones, LM Perez, NB Lucob, MCN Julaton, and RR Suralta

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 have identified enhanced aerenchyma and promoted lateral root (LR) development as key traits for maintaining root system, dry matter production and yield under soil moisture fluctuations and progressive drought. 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 by means of fine-mapping. Specifically, to develop recombinant inbred line (RIL) mapping population of DHL96 and CSSL47 crossed with IR62266 and IR64 (F3 and BC1F2), respectively and to develop RIL mapping population of DHL96 and CSSL47 crossed with NSIC Rc160 as background.

Highlights:

- In 2015DS, 95 F1 seeds from NSIC Rc160/DHL96 were developed for mapping populations. Backcrossed from IR62266/DHL96 and IR64/CSSL47 were also generated producing 26 and 359 BC1F1 seeds, respectively. Meanwhile, F2 plants from IR64/CSSL47 (17 plants); Nipponbare/CSSL47 (11 plants) and IR62266/DHL96 (11 plants) were evaluated in the field. Furthermore, 262 plants (IR62266/DHL96), 226 plants (IR64/CSSL47) and 226 plants (NSIC Rc160/DHL96) F2 populations were subjected to rapid generation advance (RGA) screening.

- Parentals such as CSSL47 and Nipponbare were tested for anther culturability. Boots were collected and anthers were plated in N6 callus induction medium. Total of 2340 and 3060 anthers were plated for CSSL47 and Nipponbare, respectively (Figure 3). Nipponbare (17 callused transferred) have 3 regenerants (green) and 1 albino plant while CSSL47 (134 callused transferred) have 1 regenerant, 6 greening (developing shoots) and 1 albino plant.

- In 2015WS, field evaluation of 2000 F2-3 populations for each crosses of IR62266/DHL96 and IR64/CSSL47 while 433 from NSIC Rc160/DHL96. Entries from each cross were collected

with leaf samples for DNA analysis and will be subjected to marker aided selection (MAS) in the future. The same crosses and generation (F2-3) were simultaneously subjected to RGA nursery: IR62266/DHL96 (262p), IR64/CSSL47 (226p) and NSIC Rc160/DHL96 (226p).

- BC1F1 generated in 2015DS were also evaluated in the field: 105 plants (IR64*2/CSSL47); 15 plants (IR62266*2/DHL96) and 29 plants (Nipponbare*2/CSSL47). Leaf samples were collected for DNA analysis and genotyping.

- Polymorphism survey results in 2015 showed that 25 (8.28%) Nipponbare/CSSL47 polymorphic to 302 SSR markers; 65 (21.59%) IR62266/DHL96 out of 301 SSR markers; 70 (23.26%) IR64/CSSL47 out of 301 SSR markers and 94 (25.82%) NSIC Rc160/DHL96 out of 364 SSR markers.

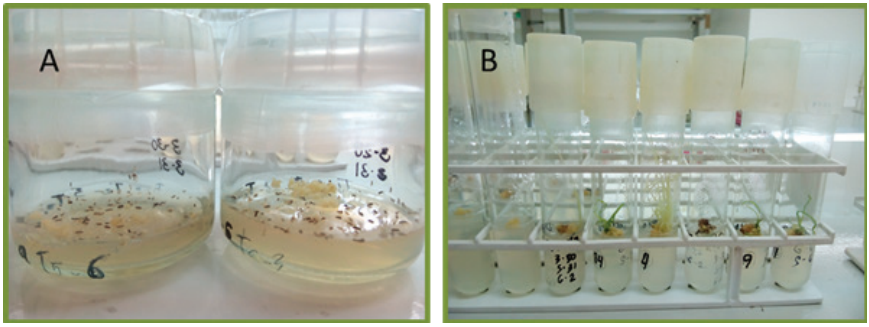

Figure 3. Plated anthers in N6 callus induction medium and MS regeneration medium showing (A) callus formation; (B) callus developing shoots and necrosis.

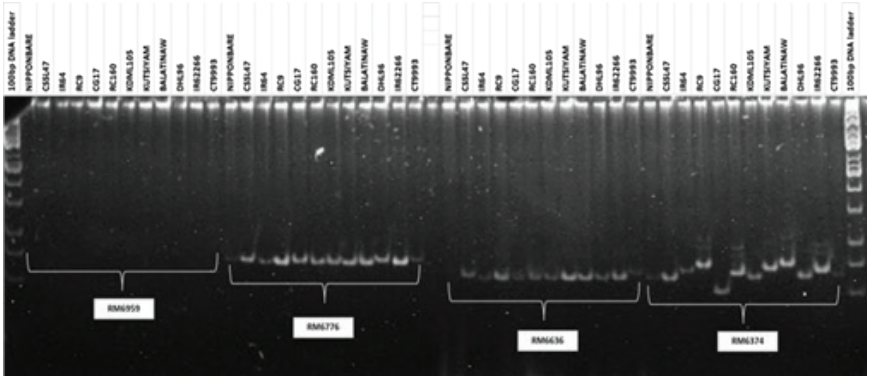

Figure 4. Survey on SSR markers showing polymorphism on different markers used.

Table 9. Distribution of SSR markers on 12 chromosomes in different target crosses.

Target Crosses	Chromosome											
	Chr1	Chr2	Chr3	Chr4	Chr5	Chr6	Chr7	Chr8	Chr9	Chr10	Chr11	Chr12
IR62266/DHL96	7	8	5	3	0	7	11	7	7	0	3	6
IR64/CSSL47	9	11	9	6	1	8	7	5	6	2	2	4
NSIC Rc160/DHL96	9	11	11	8	5	6	12	11	4	1	4	12
Nipponbare/CSSL47	4	3	4	3	0	3	0	3	0	0	2	3

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

JM Niones, MCN Julaton, RR Suralta, AB Aguelo, NB Lucob, LM Perez, and NV Desamero

Drought refers to shortage or less of water availability in the root zone affecting plants growth thus reduces 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 traits to enhance 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. This study aims to develop doubled-haploid mapping population from the cross CG17 x NSIC Rc9 and to identify QTL associated with functional stay-green trait.

Highlights:

- In 2015DS, NSIC Rc9 (relatively high functional stay green) and CG-17 (low stay green), were crossed however, no seeds generated due to high sterility. Reciprocal cross was then suggested (CG17/NSIC Rc9) and produced only 19 F1 seeds. But only 4 plants survived when planted in the field.

- Two new F1 crosses were also developed: NSIC Rc160/ Kutsiyam (13 F1 seeds) and Balatinaw/NSIC Rc160 (8 F1 seeds). Balatinaw and Kutsiyam were identified to have medium stay green capacity in a previous study (Suralta et al).

- Total of 3420 anthers of CG17 were plated in N6 callus induction medium. Twenty callused have been transferred to MS regeneration medium and 1 callus showed greening that eventually turned necrotic (Figure 5).

- In 2015WS, generated F1 from NSIC Rc160/Kutsiyam (13 seeds) and Balatinaw/NSIC Rc160 (8 seeds) and F2 of CG17/

NSIC Rc9 (1p) were planted in the field. Four plants survived from NSIC Rc160/Kutsiyam, however, none for Balatinaw/ NSIC Rc160. Each plant from crosses were collected with leaf samples for DNA analysis and genotyping.

- Polymorphism survey results showed that 101 (27.74%) of 364 SSR markers were polymorphic to NSIC Rc9/CG17; 53 (14.56%) of 364 SSR markers to Balatinaw/NSIC Rc160; 61 (16.76%) of 364 SSR markers to NSIC Rc160/Kutsiyam and 35 (11.59%) of 302 SSR markers to IR64/NSIC Rc9.

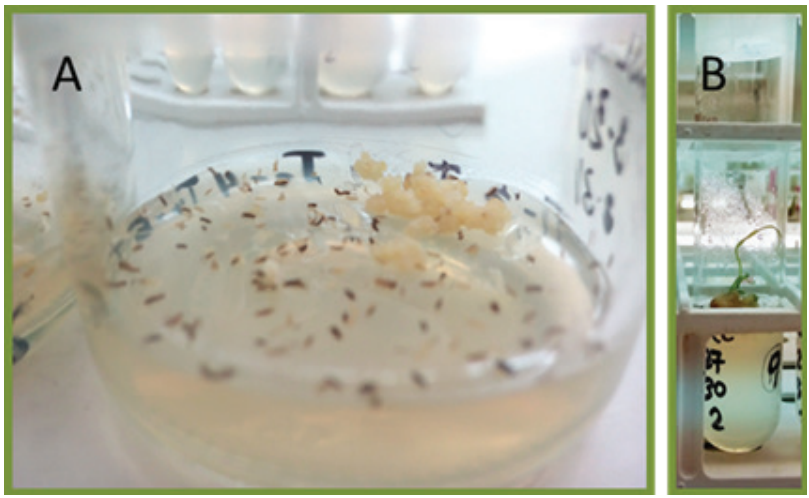

Figure 5. Anthers plated showing (a) callus formation and (b) callus showing shoot formation.

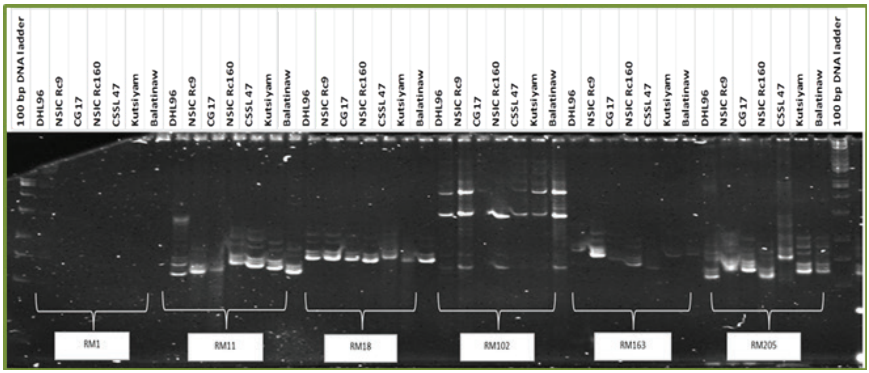

Figure 6. Survey on SSR markers showing polymorphism on different markers used.

Table 10. Distribution of SSR markers on 12 chromosomes in different target crosses.

Target	Chromosome											
Crosses	Chr1	Chr2	Chr3	Chr4	Chr5	Chr6	Chr7	Chr8	Chr9	Chr10	Chr11	Chr12
NSIC Rc9/ CG17	11	12	5	7	7	8	13	10	3	5	7	13
NSIC Rc160/ Kutsiyam	8	7	7	3	4	4	6	6	4	1	3	8
Balatinaw/ NSIC Rc160	5	9	6	2	5	2	6	5	2	2	2	7
IR64/ NSIC Rc9	5	5	3	2	0	1	4	5	1	1	2	6

Evaluation of drought tolerance QTL effects in adapted genetic background

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

In the onset of global warming causing irreversible effects of climate change, rice production in the world has been affected by several abiotic stresses. 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 which carry single quantitative trait loci (QTL) conferring resistance to drought are available for breeding. These could be utilized to develop drought tolerant lines through QTL pyramiding. Pyramiding is a process that could fast-track the development of drought tolerant lines by simultaneously combining multiple genes/QTLs together into a single genotype. This scheme could decrease the period of plant selection in comparison with conventional phenotypic selection wherein individual plants are screened for all traits of interest. Pyramiding is extensively employed in combining multiple genes in the development of lines with durable resistance to different plant diseases. Similarly, pyramiding could also be used to combine stress-response genes/ QTLs for abiotic stresses such as drought.

QTLs with major effects on grain yield under drought stress in rice have been reported in numerous studies (Babu et al. 2003; Lafitte et al. 2004b; Lanceras et al. 2004; Yue et al. 2006; Kamoshita et al. 2008) but limited work has been done to evaluate their direct effect in yield stability. Identification of these QTLs in improved genetic backgrounds is one of the essential requirements not often met in marker-assisted introgression which, when discovered, may greatly contribute in increasing rice productivity in drought-stressed environments.

The study aimed to: (1) pyramid three QTLs for grain yield under drought stress in NSIC Rc160 and NSIC Rc222 backgrounds; (2) identify and

evaluate the individual effects of the QTLs associated to grain yield under drought-stress conditions, and (3) evaluate the field performance of potential drought tolerant lines that will be developed.

Highlights:

- Polymorphism survey of four (RM 250, RM450, RM215, RM573) simple sequence repeat (SSR) markers flanking the putative QTL, qDTY2.3, was done in dry season. Out of four SSR markers, two (RM250 and RM573) markers flanking the QTL, qDTY2.3, were found polymorphic between Kali Aus and NSIC Rc160 and NSIC Rc222. The selected SSR markers were used for the selection of plants carrying the target QTLs.

- In 2015 DS, a total of 473 F1 plants were generated from the crosses between the near-isogenic lines IR92800, derived from the cross Kali Aus/IR64, and NSIC Rc160. Also, 454 F1 plants were generated from the crosses between IR92800 (qDTY2.3) NSIC Rc222. At 14 days after transplanting (DAT), leaf samples were collected individually from each plant for DNA extraction and marker genotyping using RM250 and RM573. The true F1 plants based on marker data were selected and used in backcrossing to their corresponding recurrent parent lines to increase the recovery of desirable characters.

- Figure 7 shows sample of DNA banding pattern of entries genotyped using RM573 and RM250. Samples of selection based on genotyping is shown in Figure 8. Table 11 shows the number of backcross lines generated and F1 seeds produced from various cross combinations. These backcrosses were established in 2015 WS for another cycle of backcrossing. Furthermore, the F1 plants that were not used for backcrossing were harvested into bulk and will be established in the field as F2 populations.

- The 46 F2 populations were established in 2015WS. Leaf samples from these populations were collected at 14 DAT for DNA extraction and marker genotyping using RM250 and RM573. Out of 1,200 plants that were genotyped, 180 plants were found to have alleles that were homozygous to the resistant donor (Table 12). Molecular genotyping of F2 lines is shown in Figure 9 and samples of line selection was shown in Figure 10.

- Five hundred twenty-four (524) drought-tolerant near-isogenic lines (NILs) and four checks (NSIC Rc160, Rc222, IR64-NIL and Vandana-NIL) were evaluated under field drought

condition at PhilRice Negros during the dry season of 2015 (Table 13). These lines were progenies from crosses of NSIC Rc222*3/Vandana and NSIC Rc160*3/IR64NIL and possessed quantitative trait loci (QTLs) for reproductive drought tolerance Lines. Lines were subjected to reproductive stage drought stress and phenotypic response in terms of agronomic traits such as plant height, productive tiller, days to heading, days to maturity, and grain yield were recorded. Drought recovery rate, leaf rolling, and leaf tip drying was also recorded every after drought imposition (Figure 11). Observation wells were installed strategically in the field to monitor the parched water table level, if water level fell below 100 cm for about three weeks, flash irrigation is done. Drought was successfully imposed during the reproductive stage of the lines as shown in Figure 12.

- Crop-stand of selected backcross populations in the field experimental setup is shown in Figure 13. Days to heading was relatively longer (85 DAS) and so the days to maturity (115 DAS) eventually, which is a common response of rice under drought stress – either earlier or later than the normal days to heading and maturity. NSIC Rc222*3/Vandanna NILs have shorter average maturity (108 DAS) than NSIC Rc160*3/IR64NIL NILs (117 DAS). Average plant height recorded was only 87cm and average productive tillers was 10/hill. Majority of the lines have good drought recovery rate and only few showed slight leaf rolling and leaf tip drying. Grain yield (<1000 kg/ha) of almost 50% of the lines was severely affected by drought stress. Grain yield ranges from 39 to 6006 kg/ha with an average grain yield of 1103 kg/ha. DT-462 got the lowest yield while DT 102 got the highest yield. NSIC Rc222*3/Vandana NILs out-yielded NSIC Rc160*3/IR64NIL NILs with 1490 kg/ha and 943 kg/ha yields, respectively. Moreover, the NILs out-yielded all the parent lines (Figure 14). Correlation analysis using Pearson's correlation revealed that grain yield is positively correlated to plant height and productive tiller but negatively correlated to days to flowering and maturity (Table 14). These information can be used in selecting for ideal phenotypes for drought tolerance.

Table 11. Number of backcrosses and F1 seeds generated in the introgression of qDTY2.3 derived from IR92800 (Kali Aus/2*IR64 NILs) in NSIC Rc160 and NSIC Rc222 background in dry season.

2015 DS F1 code	Cross combination	Recurrent Parent	No. F1 seeds
DT-13-11-1	NSIC Rc160/IR92800-285-B-B	NSIC Rc160	53
DT-14-4-1	NSIC Rc160/IR92800-304-B-B	NSIC Rc160	46
DT-15-1-13	NSIC Rc160/IR92800-314-B-B	NSIC Rc160	27
DT-15-4-1	NSIC Rc160/IR92800-314-B-B	NSIC Rc160	55
DT-5-10-2	NSIC Rc160/IR92800-89-B-B	NSIC Rc160	89
DT-5-3-3	NSIC Rc160/IR92800-89-B-B	NSIC Rc160	14
DT-5-6-3	NSIC Rc160/IR92800-89-B-B	NSIC Rc160	68
DT-5-7-3	NSIC Rc160/IR92800-89-B-B	NSIC Rc160	42
DT-5-8-22	NSIC Rc160/IR92800-89-B-B	NSIC Rc160	26
DT-5-8-6	NSIC Rc160/IR92800-89-B-B	NSIC Rc160	42
DT-5-9-3	NSIC Rc160/IR92800-89-B-B	NSIC Rc160	32
DT-7-7-1	NSIC Rc160/IR92800-121-B-B	NSIC Rc160	14
DT-4-8-2	NSIC Rc222/IR92800-85-B-B	NSIC Rc222	2
DT-4-9-3	NSIC Rc222/IR92800-85-B-Bs	NSIC Rc222	12

Table 12. Number of selected lines from F2 populations based on genotype data.

Code	Cross combination	No. of selected lines
DTF2-4	NSIC Rc160/IR92800-24-B-B-10	16
DTF2-5	NSIC Rc160/IR92800-24-B-B-22	23
DTF2-6	NSIC Rc160/IR92800-24-B-B-27	12
DTF2-7	NSIC Rc160/IR92800-24-B-B-32	21
DTF2-8	NSIC Rc160/IR92800-24-B-B-34	7
DTF2-9	NSIC Rc222/IR92800-24-B-B-11	11
DTF2-14	NSIC Rc160/IR92800-89-B-B-1	9
DTF2-15	NSIC Rc160/IR92800-89-B-B-2	16
DTF2-16	NSIC Rc160/IR92800-89-B-B-11	24
DTF2-17	NSIC Rc160/IR92800-89-B-B-21	19
DTF2-18	NSIC Rc160/IR92800-89-B-B-23	15
DTF2-19	NSIC Rc160/IR92800-89-B-B-24	7

Table 13. Number of BC4F3 lines from various cross combinations in NSIC Rc160 and NSIC Rc222 background evaluated for reproductive-stage drought-stress in PhilRice Negros.

Designation	Cross combination	QTL	Number of lines
PR47201-A102A-3	NSIC Rc222*3/Vandana	*DTY12.1*	47
PR47201-A102A-28			52
PR47201-A102A-29			57
PR47202-A103A-17	NSIC Rc160*3/IR64 NIL	*DTY2.2 & DTY4.1*	109
PR47202-A103A-18			78
PR47202-A103A-19			116
PR47202-A103A-25			65

Table 14. Pearson correlation analysis of agronomic traits of Near Isogenic Lines (NILs) under reproductive drought stress.

	GY	DTF	DTM	PLHT	PRDTIL
GY		-0.2921**	-0.3021**	0.3839**	0.3140**
DTF	0.2921**		0.9200**	-0.2204**	-0.2154**
DTM	-0.3021**	0.9200**		0.2404**	0.2245**
PLHT	0.3839**	-0.2204**	0.2404**		0.2560**
PRDTIL	0.3140**	-0.2154**	0.2245**	0.2560**	

** -highly significant at 1% probability; GY- grain yield; DTF- days to flowering; DTM- days to maturity; PLHT- plant height; PRDTIL- productive tiller

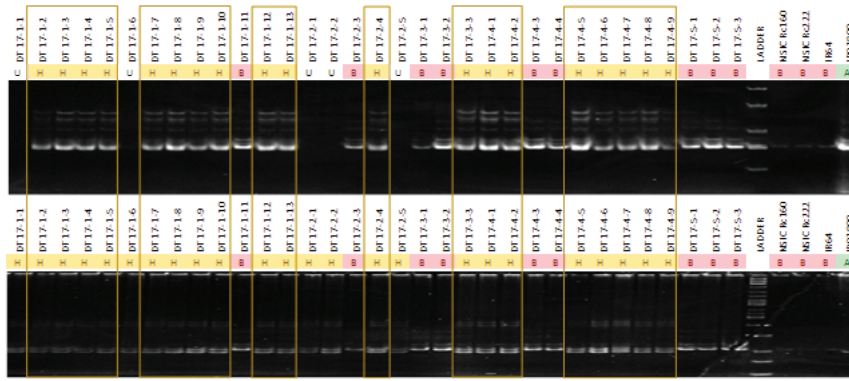

Figure 7. DNA banding pattern of F1 lines using RM250 and RM573 generated from the introgression of qDTY2.3 derived from IR92800 (Kali Aus/2*IR64 NILs) in NSIC Rc160 and NSIC Rc222 background. Selected true F1 lines are distinguished. A- donor type allele, B- non-donor type allele, and H- heterozygous.

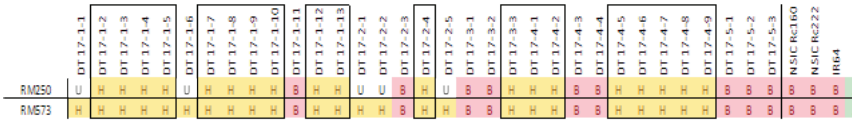

Figure 8. Selected entries based on the result of the molecular genotyping of F1 lines generated in the introgression of qDTY2.3 derived from IR92800 (Kali Aus/2*IR64 NILs) in NSIC Rc160 and NSIC Rc222 background. Selected true F1 lines are distinguished. A- donor type allele, B- non-donor type allele, and H- heterozygous.

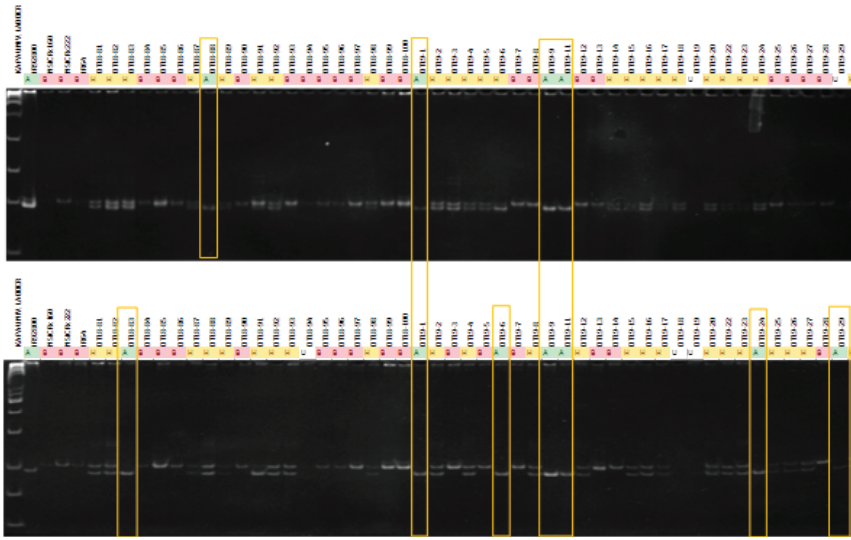

Figure 9. DNA banding pattern of F2 lines using RM250 and RM573 generated from the introgression of qDTY2.3 derived from IR92800 (Kali Aus/2*IR64 NILs) in NSIC Rc160 and NSIC Rc222 background. Selected true F1 lines are distinguished. A- donor type allele, B- non-donor type allele, and H- heterozygous.

Figure 10. Selected entries based on the result of the foreground genotyping of the F1 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 F1 lines are distinguished. A- donor type allele, B- non-donor type allele, and H- heterozygous.

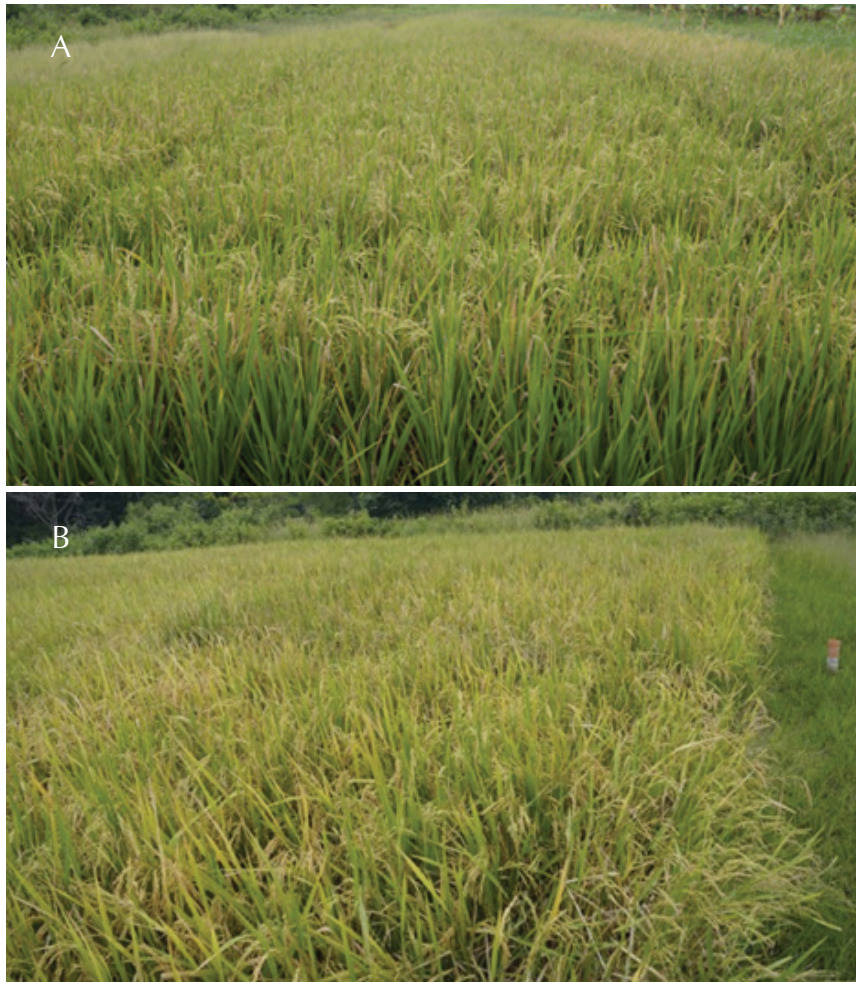

Figure 11. Experimental setup for drought stress screening at reproductive-stage in PhilRice Negros (A). Piezometers were strategically installed in the field for the daily monitoring of parching water table (B).

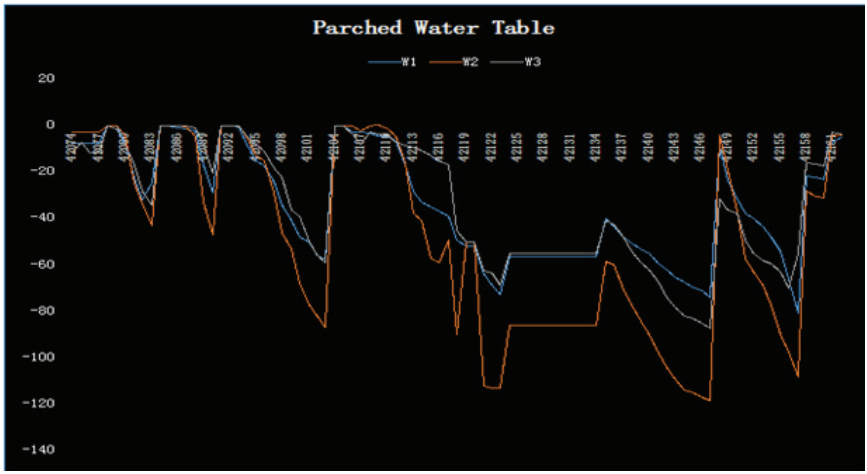

Figure 12. Parch water table (cm) indicating the drought stress was successfully imposed during the reproductive stress.

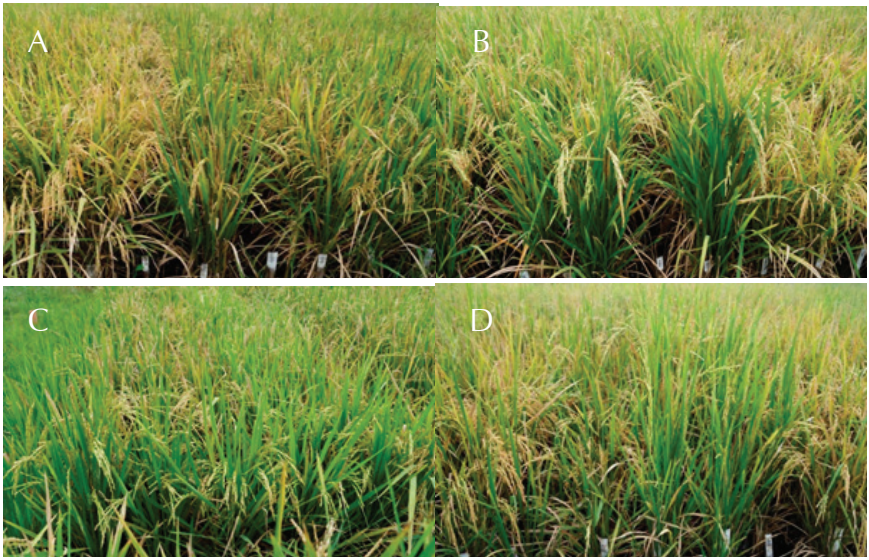

Figure 13. Crop-stand of selected backcross populations in the field (A) PR47201-A102A-29, (B) PR47201-A102A-3, (C) PR47202-A103A-17 and (D) PR47202-A103-25.

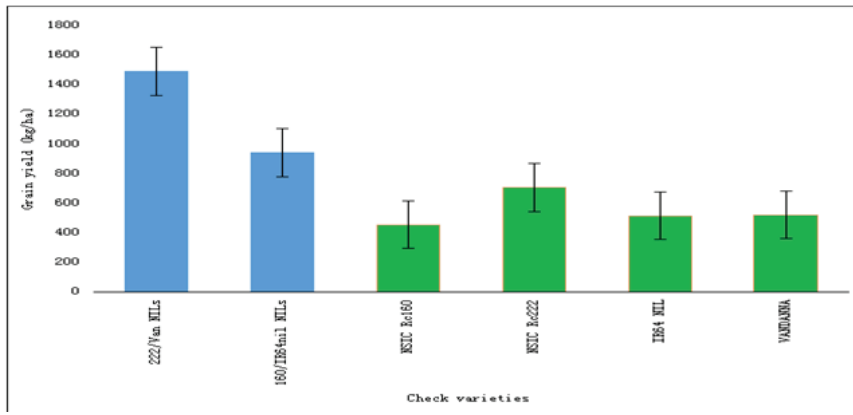

Figure 14. Grain yield performance of NILs along with check varieties.

Novel gene identification for rice crack resistance

VG Dalusong, RP Mallari, TE Mananghaya, AP Tuaño, LM Perez 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. Rice varieties with translucent, long, slender and less broken grains (less chalkiness) are some of the characteristics that consumers look for a quality rice. Rice grains are usually broken during milling thereby reducing the head rice yield and the milling quality. Environment, mechanical processing and genetic variation are the main factors that affects cracking or fissuring of rice grain. Chalkiness, imbalance of pressure during water penetration, hull tightness and bran diffusivity are some of the effects of these factors that contributes to cracking of grain before or after milling. Quantitative trait loci (QTL) for rice crack resistance were identified recently where 4 to 80% of the observed phenotypic variance was explained by the identified QTLs. This study specifically aims to fine-mapped the region of candidate QTLs and eventually find the genes responsible for the crack/fissure resistance. This study also aims to develop allele-specific markers that can be used for marker-assisted breeding and identify the proteins that confers to the mechanism of crack resistance.

Highlights:

- Seven QTLs were verified by reanalysis of genotype and phenotype data from of PSB Rc52xNSIC Rc160 F2 mapping population. Figure 15 shows the chromosome location and position of putative QTLs. These QTLs are all candidates for fine-mapping of crack resistance gene. Results of QTL analysis

from PSB Rc38xNSIC Rc160 are still on verification process.

- 715 SSR markers identified within the 3 Mb upstream and downstream region of the candidate QTLs were screened for polymorphism between the donor parent PSB Rc52 and recurrent parent NSIC Rc160. There are 82 polymorphic SSR markers identified on chromosome 2, 28 on chromosome 3 and 37 on chromosome 7. No SSR markers were screened in the QTL region on chromosome 9. These polymorphic markers will be used in fine-mapping of QTL regions for candidate gene analysis. Table 15 shows the chromosome location, size of QTL region and number of polymorphic and monomorphic SSR markers screened for fine-mapping. Graphical genotype representation of SSR markers screened was shown on Figure 16.

- For verification of QTL on multiple mapping populations, F1 populations were generated from a cross between crack resistant varieties NSIC Rc152 and NSIC Rc152, and susceptible rice variety BPI Ri10. A total of 23 F1 populations were generated; 4 from NSIC Rc154xBPI Ri10, 9 from BPI Ri10xNSIC Rc154, 1 form NSIC Rc152xBPI Ri10 and 9 from BPI Ri10xNSIC Rc152.

- Forty-five F2 populations were generated, where 7 populations were selected to advance on the next generation to produce F2:3 seeds for fine-mapping activity.

- Other characteristics of rice grain that manifest crack resistance and its phenotyping methods are currently evaluated to include in our study. These include but not limited to grain size, grain weight, grain shape, grain filling, kernel dimension, kernel hardness and bran thickness.

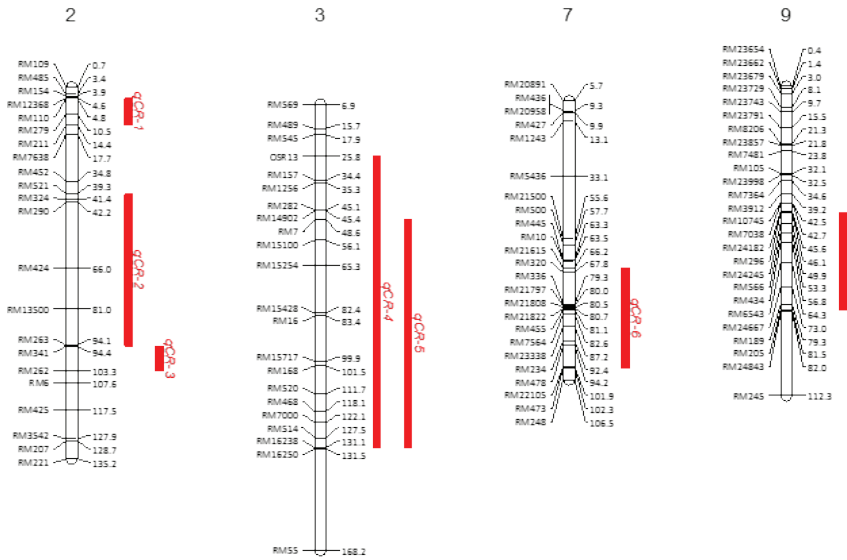

Figure 15. Location of putative QTLs for mapping of crack resistance gene.

Table 15. Chromosome location, size of QTL region and SSR markers screened for fine-mapping

Chromosome	Size of QTL region (in Mb)	Polymorphic	Monomorphic	Total SSRs screened
2	2.6, 15.0 & 2.4	82	260	342
3	28	28	188	216
7	10	37	120	157
Total		147	568	**715**

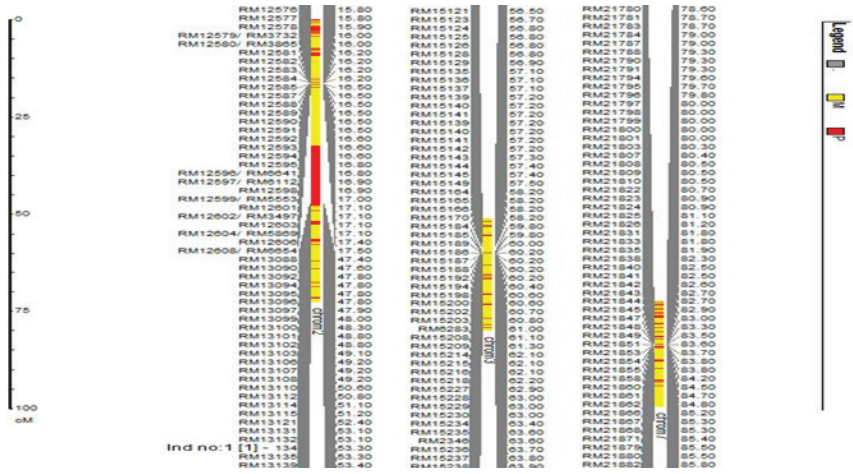

Figure 16. Graphical Genotype of the polymorphism survey between PSB Rc52 and NSIC Rc160 for fine mapping of the identified QTLs along chromosome 2, 3, and 7 from the cross PSB Rc52xNSIC Rc160 using Graphical Genotyping version 2.5.

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

JM Niones, MCN Julaton, RR Suralta, LM Perez, DAA Tabanao, AB Aguelo and NB Lucob

Fluctuation of soil moisture and progressive drought at varying degrees are regularly recurring stresses 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).

Highlights:

- In 2015 DS, 9 F1 seeds produced from CSSL47/NSIC Rc160 and 110 F1 CSSL47/KDML105. These F1 crossed will be backcrossed on the recurrent parents in 2015WS.

- In 2015WS, F2's of CSSL47/NSIC Rc160 (6 individuals) and CSSL47/KDML105 (18 individuals) was evaluated and seed

increase in the field. Individual plants were collected with leaf samples for DNA analysis and genotyping. For 2016DS, generation of NSIC Rc160/KDML105 will be done.

- Polymorphism survey results in 2015 identified 38 (12.71%) out of 299 SSR markers were polymorphic for NSIC Rc160/ KDML105 crosses while 83 (27.48%) out of 302 SSR markers of CSSL47/KDML105 crosses and 101 (27.75%) out of 364 CSSL47/NSIC Rc160 crosses.

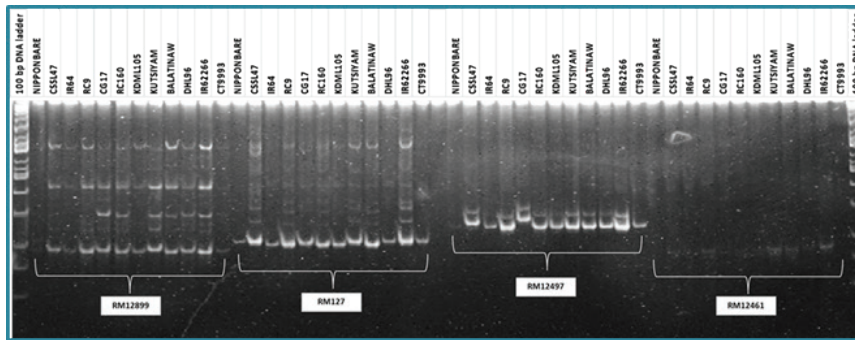

Figure 17. Representative SSR markers showing polymorphic bands.

Table 16. SSR markers distribution on 12 chromosome in different target crosses.

Target Crosses	Chromosome										
	Chr1	Chr2	Chr3	Chr4	Chr5	Chr6	Chr7	Chr8	Chr9	Chr10	Chr11
CSSL47/ KDML105	10	12	10	5	1	9	7	8	6	2	3
CSSL47/ NSIC Rc160	13	15	11	4	4	7	11	11	4	4	4
NSIC Rc160/ KDML105	3	3	7	2	0	4	2	6	4	0	4

Cloning of salinity tolerant gene/s from Nypa (Nypa fruticans)

SE Abdula, HJ Lee, DAA Tabanao, and ET Rasco, Jr.

Nypa fruticans, commonly known as nipa palm, is a species of palm native to the coastlines and estuarine habitats of the Indian and Pacific Oceans. Because of its adaptation to salinity and other adverse conditions, gene mining of agronomically important traits in nipa is of interest to researchers. This study is being conducted to clone salinity tolerant gene/s through reverse genetics approach.

Highlights:

- Based on the previously obtained 1.6Kb-long partial sequence of the Nipa Salt Overly Sensitive (SOS) gene, a set of primers were created for conducting rapid amplification of cDNA ends (RACE) PCR, using the SMARTTM RACE procedure by Clontech. The primer sequences were selected based on the specification found in the SMARTTM RACE cDNA Amplification Kit user's manual. Aside from this, two sets of backup primers were also made, though not employing SMARTTM RACE PCR. The formulated primers, for both sets, were also tested for any possible formation of unwanted secondary structures, such as hairpins, homo- and heterodimers. Two sets of backup primers were also formulated, though not employing SMARTTM RACE PCR.

- Rapid amplification of cDNA ends, in order to obtain the full length sequence, will be conducted.

- From the previously obtained and sequenced Nipa Indole-3-acetic acid-amido synthetase (Figure 20), primers were formulated for the isolation of the gene, in order to be used for the upcoming production of transgenic rice.

Primer Information for SOS RACE PCR

Primer Name	Sequence	Purpose
SOS-3'RACE1	5'-GCTGAGGGAGAGGAGGCTAGAAA-3'	3' RACE PCR
SOS-3'RACE2	5'-GACTGGGTTGCTGGAAGAAAAGG-3'	
SOS-5'RACE1	5'-GCATTGTCCAGTACATCTTCGGC-3'	5' RACE PCR
SOS-5'RACE2	5'-GAGCAGTGAAGTACGCAAGGTAGC-3'	

>NipaSOS|1606bpfragment

AGACCAAAAGATCATTTGGACTCTTGCTGTCAGCTACCTTGCGTACTTCACTGCTCAAGATGGGGCAGACATTTCAGGTGTC
TTGACAGTAATGACTCTAGGAATGTTCTATGCAGCTGTTGCAAGAACAGCTTTTAAGGGTGATGGGCAGCAAAGTTTGCATC
ATTTCTGGGAAATGGTAGCCTACATTGCCAATACTCTAATTTTTATCTTGAGTGGGGTTGTTATTGCCGAAGATGTACTGGACA
ATGCTAACCACTTTGAAAGACATGGTGCTTCATGGGGCTACCTCATTTTGCTTTATGTTTTTGTTCAATGCTCTCGAATCATAG
TTGTTGGGCTGCTGTTTCCATTTTTGCGGTACTTTGGCTATGGTTTGGAATGGAAAGAAGCAATTATTCTTGTGTGGTCTGGA
CTGCGAGGGGCTGTGGCTCTTTCGCTATCACTATCTGTTAAACGTGCTAGTGATAACGCAGACCAGACTCATCTCAGACCAG
AAGTTGGAACATTGTTTGTATTCTTCACTGGTGGGATTGTTTTCCTGACGCTGATTATCAATGGATCAACTACTCAGTTCTTCT
TGCATCTTCTAGAAATGGATAAACTCTCAGCGACAAAGATTCGTATATTGAACTATACAAGGTATGAAATGTTAAACAAGGCA
TTAGAGGCATTTGGTGATCTTGGGGATGATGAGGAACTAGGGCCTGCTGATTGGCCCACTGTGCAGAGATATATCACTTGCT
TAAGTAACTTGGATGAAGGACAGGTCCATCCTCACAGTGTTACTGAAAGTGAGTATCATCTGCAGTCTATGAATTTGAGAGA
CATTCGAGTACGCCTTCTAAATGGTGTCCAAGCTGCTTACTGGGGAATGCTTGAAGAAGGACGAATAACTCAAGCCACTGCA
AATATTTTAATGAGATCGGTTGATGAAGCTATGGATCTTGTTCCTACCCAAGAATTATGTGACTGGAAGGGTTTGCGGTCCAA
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AGTGAGGTTGCAAGAATTGTTATTGATGAAAGTAATGCTGAGGGAGAGGAGGCTAGAAAATTCTTGGAAGATGTTCGTGTT
ACATTCCCTCAGGTGCTTCGTGTGCTGAAGACTCGACAAGTAACATATTCGGTATTGACCCACTTGAGTGAGTATATTCAAAA
TCTCCAGAAGACTGGGTTGCTGGAAGAAAAGGAAATGGCCCATCTAGATGATGCTTTGCAGACAGACTTGAAGAAGTTCAA
GAGGAATCCACCATTGGTAAAAATGCCAAGAGTCAGTGATCTTTTGAACACTCATCCGTTAGTTGGTGCACTGCCTGCTGCG
ATGCGTGATCCTTTATTAAATAGCACAAAGGAAACAGTAAAAGGACATGGCACAATCCTTTATAGAGAGGGCTCAAGGCCAA
CTGGTATATGGCTTGTTTCGATTGGAGTAGTAAAGTGG

Figure 18. Formulated primers for Nipa SOS RACE PCR using the specifications for the SMARTTM RACE procedure.

Primer Name	Sequence	Purpose
SOS-P	5'-/Phos/GCTTCTTTCCATTCC-3'	5' RACE PCR
SOS-A1	5'-GCAGTGAAGTACGCAAGGTA-3'	
SOS-A2	5'-CTGTCAAGACACCTGAAATG-3'	
SOS-S1	5'-GCCGAAGATGTACTGGACAA-3'	
SOS-S2	5'-CTGGGAAATGGTAGCCTACA-3'	
SOS-S3	5'-GGTGATGGGCAGCAAAGTTT-3'	
SOS-3R	5'-CTGGGTTGCTGGAAGAAAAG-3'	3' RACE PCR

Primer Name	Sequence	Purpose
SOS-P	5'-/Phos/GCT TCT TTC CAT TCC-3'	5' RACE PCR
SOS-1A	5'- CATTGTCCAGTACATCTTCGGC -3'	
SOS-2A	5'- GCATGGGGCTACCTCATTTTG -3'	
SOS-1S	5'- GCGGTACTTTGGCTATGGTTTG -3'	
SOS-2S	5'- GTTGTTGGGCTGCTGTTTCC -3'	
SOS-3R	5'-CGGTATTGACCCACTTGAGT-3'	3' RACE PCR

Figure 19. Backup primer sets for the RACE PCR procedure.

NipaIAA|1971bpsequence

ATGGAGGAGGCCGGGTCCAGAGGGAGACCCTTCGAAGGATTCTCGAACAGAACGGTGGTGCAGAATACTTGCAGAATTTGGGCCTCGGAGGAAGAA
CCGACCCCGAGAGTTTTAAGGCCCATGTCCCCTTGGTCACTCATGAAGTTCTGGCGCCCTACATTCAGAGGATCATCGACGGTGATGCTTCGCCCA
TCCTCACCGGGAGGCCTATATCATCGATCTCGTTGAGTTCTGGCACCACGCAAGGAAAGCCCAAGTTTTTGCCATTCAACGAGGAACTAGTTCAAT
CCACCATGCAGATCTATAGGACTTCATTTGCTTTCAGAAACCGAGAATATCCAATTGGCAATGGAAAAGCTCTGCAATTCATCTACAGCAGCAAGC
AGTCCAAAACAGTTGGGGGCCTCACTGCTACAACGGCCACGACAAATGTGTACCGAAGTGAACAATTCAAATGCACGATGGAGGATATCCAGTCTC
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TTCTCTCCAGCAGGATTACTGTCCCATCGATCCGTGCGGCTGTTAGCAAACTTTTAAGTCCAAATCCCACACTGGCTGATTCTATATACAAAAAAT
GTATGGGTTTGAGTAATTGGTATGGTGTGATTCCAGAACTCTGGCCCAATGCCAAATATGTCTATGGCATTATGACAGGATCCATGGAACCATATT
TGAAAAAAATGAGACATTATGCAGGAAGCTTACCACTGTTGAGTGCCGATTATGGTTCTTCGGAGGGATGGATCGGTGCAAATGTAAACCCTGGGC
TGCCCCCGGAACTGGCGACCTTTGCGGTGCTTCCCAACATTGGTTACTTTGAATTTATTCCTCTGGAGAAACCTGAGGGGCAGGAGCTGGAGAATT
GTGCCTCCATTCACTATATAGAATCGGAGCCGGTTGGTCTTGCAGAAGTCGAGGTTGGCAAAGAGTATGAAATTGTTGTCACCAGTTTTGCAGGTC
TATATCGGTACCGGCTGGGGGATGTCGTCAAGGTAGCGAGATTCCATAACTCGACGCCGGAGCTGCAATTCATATGCCGAAGGAGCCTGCTCCTAT
CCATCAACATTGACAAGAACACGGAGAAAGACCTGCAGCTGGCCGTGGAAGAGGCGACCAAGCTCCTGGCGGCCGAGAAGCTCGAGGTCGTGGACT
TCACCAGCTACGTCGATCTCTCGACGGACCCTGGCCACTATGTGATCTTCTGGGAGCTGAGCTCCGACGCCGGCGAGGAGGTCCTGAGCGGCTGCT
GCAATTGCTTGGACCTGGCGTTCGTGGATGCAGGCTATGTTAGCTCGAGGAAGGCGCGCGCCATTGGACCGCTGGAGCTCCGCGTACTCCGGAAGG
GAACGTTCCAGATGATCCTGGATCATTATCTCAGCCTCGGGGCCGCGGTGGGCCAGTTCAAGACGCCGCGCTTCGTCGGCGCTTCAAACGGCAAGG
TCTTGCAGATCCTGCGCAGGAACGTCGTCGAGAGCTATTTCAGTACCGCCTATGGCTGCTAAGGGCGCCGTATCTATGCCCTCCTGTAATAATATT
GTTCTCTGTGCCGCCCTTCTAGCTCTCTCTAGAAGACCTTTTCATGAGCTTGTTGTCAAGGGGAAATAACTCCTCTCTTAAGTTTTCATGTGTGGT
TTTTCTACAGCAGCAAGCAATGTTAGAACCGGAGGGACAATTACTATGTGATTAGGATCTCGGTGCATGTCAAGCCTATCAACAGTTCAACTAATC
TTTGTAAAAAAAAAAAAAAAAGGATCCGGTACCTCTAGATCAGAATCACTAG

Figure 20. Formulated Primers for Nipa IAA.

Association of GLH and RTSV resistance to GLH14, TSV1, and ELF4G SNP type in selected popular Philippine traditional rice varieties and mapping of novel GLH and RTSV resistance genes

AA Dela Cruz, MM Rosario, and MJC Duque

RTD is considered the most serious rice viral disease in the Philippines and in the South and Southeast Asia in terms of major yield losses. 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 and RTBV eventually cause RTD. Imbe et al. (1993) reported that even if the RTSV resistant plants become infected with RTBV at a high rate, they still exhibit low RTD incidence since the transmission of RTBV is highly dependent on the presence of RTSV.

The breeding program of PhilRice for RTD resistance has been so limited on use of either ARC11554 or Utri Merah as donors of GLH and RTSV resistance. Even though various Philippine traditional rice varieties (TRV's) were been pre-observed with RTD resistance, breeders hesitate to use them in breeding because genes conferring the traits were yet determined. This study is being conducted to help diversify the sources of RTD resistance genes, through identifying alternative donors of Glh14 and tsv1 and discovering novel resistance genes among Philippine TRVs. As an initial step, the selected TRVs are being screened for reactions to GLH to determine the resistance mechanisms involved, and the presence or absence of Glh14 and tsv1 in the varieties will be established. The involvement of another gene, coding for a translation initiation factor (eIF4G), in the resistant reactions of plants to RTSV will also be explored. The second phase of this study will be mapping of novel GLH and RTSV resistance genes.

Highlights:

- A total of 72 Philippine traditional rice varieties (PTRV) were screened for reactions to GLH using preference and antibiosis tests (Figure. 21). The molecular characterization of the PTRVs for presence or absence of the Glh14 resistance locus and tsv1 was based on SSR markers RM8213 and RM5494, respectively. Included in the tests as check varieties were ARC11554, Matatag 6, Utri Merah and TN1.

- Table 17 shows the association of reactions to GLH and molecular marker data of PTRVs. Generally, the reactions observed in the antibiosis test during the 3rd and 5th days were the same, while the comparison of means of GLH alighted in the PTRVs and check varieties (n=10, using the Duncan's Multiple Range Test (DMRT) at 5% level of significance) consistently supported the results of antibiosis.

- There were 13 PTRVs identified as potential sources of GLH resistance gene(s) other than Glh14. These exhibited consistent resistant reactions to GLH even without the resistant ARC11554-type RM8213 allele. Meanwhile, 3 PTRVs with Glh14 were identified. These can be used as alternative sources of the Glh14, 1 of which also has tsv1.

- A total of 16 more PTRVs observed with consistent GLH resistance showed unique allele variants with RM8213 (different from ARC11554-type and TN1-type). Interestingly, allele variants present in these GLH-resistant PTRVs were likewise observed in other GLH-susceptible PTRVs. Thorough examination of the DNA sequences of these allele variants may be helpful in identifying the particular GLH-responsive gene/genes present in the Glh14 resistance locus. Moreover, 7 and 9 out of the 16 PTRVs were detected with- and without tsv1, respectively.

- Meanwhile, all GLH-susceptible PTRVs identified in this study are being considered for further screening for reactions to RTSV alone or RTSV+RTBV. Unlike the GLH-resistant PTRVs, the GLH-susceptible PTRVs are ideal materials for studying resistance to tungro viruses since no vector resistance will likely interfere in the virus inoculation.

- For a very long time, only ARC11554 and Utri Merah had been used as sources of Glh14 and tsv1 in PhilRice breeding programs for RTD resistance. Initial results of this study enabled selection of several alternative sources of Glh14 and/or tsv1 among our very own PTRVs, while promising materials for mapping novel RTD resistance genes were likewise pinpointed with certainty.

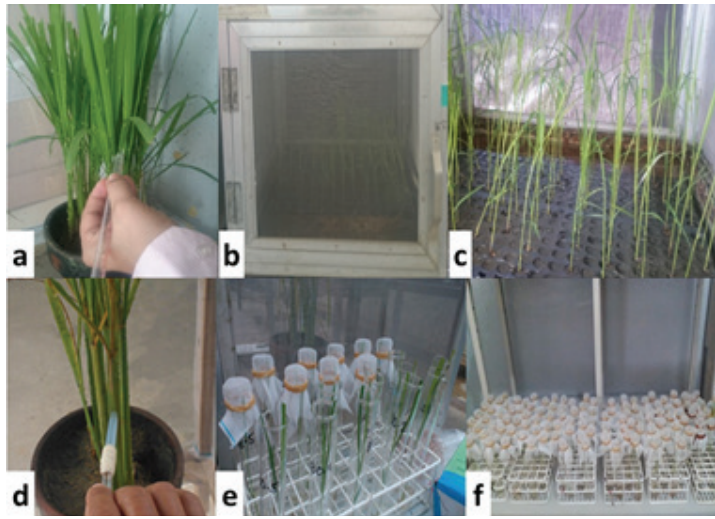

Figure 21. Screening of selected Philippine traditional varieties (PTRV) for reactions to GLH.For the preferences test, virus-free GLH (a) were added into caged 2-week old seedlings (b) at 5 GLH: 1 seedling ratio. The number of GLH alighted from the individual seedlings (c) were recorded on the 2nd day (in the early morning, noontime and late afternoon). For the antibiosis test, 5 4th-instar GLH nymphs (d) were added into single 10-day old seedlings in 75ml-test tubes containing 2 ml water. Test tubes were covered with nylon mesh and tied with rubber bands to prevent insect escape (e). Percent Nymph Mortality (%NM) was determined (0-30 %NM = Susceptible; 31-100 %NM = Resistant) on the 3rd and 5th day after addition of GLH (f).

Table 17. Association of reactions to GLH and presence/absence of Glh14 and tsv1 in selected PTRVs.

	PTRV	Accession Number	*tsv1*	*Glh14*	Antibiosis test*		Preference test**	Remarks
			RM5495	RM8213	Day3	Day 5		
A. GLH-resistant (w/o Glh14); w/o tsv1								
1	AMPIPIT	PRRI000250	B	B	R	R	R	
2	BALASANG	PRRI000255	B	B	R	R	R	
3	BARAO	PRRI000474	B	B	R	R	R	
4	BAYABAS	PRRI000485	B	B	R	R	R	
5	BINANGKURO (BALAYANG)	PRRI000442	B	B	R	R	R	
6	BOLASTOG	PRRI000264	B	B	R	R	R	potential sources of GLH resistance gene(s) other than Glh14
7	BUCAYAB	PRRI000265	B	B	R	R	R	
8	CIBUGAN	PRRI000420	B	B	S	R	R	
9	FISCAL	PRRI000500	B	B	R	R	R	
10	GINARACIA	PRRI000226	B	B	R	R	R	
11	LIMANGCA	PRRI000301	B	B	R	R	R	
12	PANGASINAN	PRRI000440	B	B	R	R	R	
13	STA. CATALINA	PRRI000463	B	B	R	R	R	
	Utri Merah		A	B	R	R	R	
B. GLH-resistant (with Glh14); w/o tsv1								
1	LUPING	PRRI000302	B	A	R	R	R	alternative sources of Glh14
2	PANESPES	PRRI000451	B	A	R	R	R	
	Matatag 6 (GLH-resistant check)		B	A	R	R	R	
C. GLH-resistant (with Glh14); with tsv1								
1	SIMPOLOT 1	PRRI000441	A	A	R	R	R	alternative sources of Glh14 and tsv1
	ARC11554 (RTD-resistant check)		A	A	R	R	R	
D. GLH-resistant allele variant (RM8213); w/o tsv1								
1	GORIT	PRRI000476	B	E	R	R	R	DNA sequences of allele variants for further examination; GLH-susceptible varieties are for evaluation of reactions to RTSV/RTD
2	INUWAK	PRRI000292	B	E	R	R	R	
3	KAMATI	PRRI000436	B	E	R	R	R	
4	KATIBONG	PRRI000548	B	E	R	R	R	
5	KAUTAK	PRRI000465	B	D	R	R	R	
6	PINUTYUKAN	PRRI000427	B	D	R	R	R	
7	SINAN MIGUEL	PRRI000437	B	C	R	R	R	
8	SURIGAO	PRRI000434	B	E	R	R	R	
9	TUKON-TUKOD	PRRI000547	B	D	R	R	R	
E. GLH-susceptible allele variant (RM8213); w/o tsv1								
1	BUWOO-WA	PRRI000460	B	E	S	S	S	
2	DAGSOL	PRRI000276	B	E	S	S	S	
F. GLH-resistant allele variant (RM8213); with tsv1								
1	AGRICULTURA	PRRI000248	A	D	R	R	R	DNA sequences of allele variants for further examination; alternative sources of tsv1
2	BONTOTAN	PRRI000418	A	D	R	R	R	
3	CAPULUNGAN	PRRI000560	A	D	R	R	R	
4	GUINAYANGANG	PRRI000433	A	D	R	R	R	
5	INIRAAN	PRRI000423	A	D	R	R	R	
6	LI-TOT (RED DEKOT)	PRRI000530	A	D	R	R	R	
7	ROMERO	PRRI000473	A	E	R	R	R	
G. GLH-susceptible allele variant (RM8213); with tsv1								
1	ALLILIK	PRRI000459	A	D	S	S	S	
2	INANIBONG	PRRI000422	A	D	S	S	S	
3	IPIS	PRRI000490	A	D	S	S	S	
4	KANTING	PRRI000491	A	D	S	S	S	
5	MALAPAY	PRRI000512	A	D	S	S	S	
6	MALOIT	PRRI000514	A	D	S	S	S	
7	PAC-ANG	PRRI000484	A	D	S	S	S	
H. GLH-susceptible (w/o Glh14); w/o tsv1								
1	ARINGMONGMONG	PRRI000232	B	B	S	S	S	for evaluation of reactions to RTSV/RTD
2	BAAY	PRRI000540	B	B	S	S	S	
3	BINURNAY	PRRI000445	B	B	S	S	S	
4	BULASTOG	PRRI000447	B	B	S	S	S	
5	CAIDOL	PRRI000469	B	B	S	S	S	
6	CANDENAVIA	PRRI000478	B	B	S	S	S	
7	DI-TINTA	PRRI000521	B	B	S	S	S	
8	EPIS (KINTOMAN)	PRRI000559	B	B	S	S	S	
9	GANG TOBAN	PRRI000487	B	B	S	S	S	
10	IMANG	PRRI000288	B	B	S	S	S	
11	KASINKAN	PRRI000432	B	B	S	S	S	
12	KAYANGA	PRRI000507	B	B	S	S	S	
13	KINOROS	PRRI000508	B	B	S	S	S	
14	MACABATO	PRRI000510	B	B	S	S	S	

Table 17. Association of reactions to GLH and presence/absence of Glh14 and tsv1 in selected PTRVs. Con't.

14	MACABATO	PRRI000510	B	B	S	S	S	
15	MALYOK	PRRI000561	B	B	S	S	S	
16	PINAS	PRRI000516	B	B	S	S	S	
17	RAGOL	PRRI000558	B	B	S	S	S	
18	WARAY-WARAY	PRRI000458	B	B	S	S	S	
I. GLH-susceptible (w/o Glh14); with tsv1								
1	BOCAO	PRRI000522	A	B	S	S	S	alternative source of tsv1
J. GLH-susceptible; w/o tsv1								
1	AMPIPIT	PRRI000569	B	B	S	S	S	
2	BALASANG	PRRI000570	B	B	S	S	S	
3	BALLUKOK	PRRI000443	B	B	S	S	S	
4	BOKAL	PRRI000574	B	B	S	S	S	
5	GINATUDAY	PRRI000571	B	B	S	S	S	for evaluation of reactions to RTSV/RTD
6	LA UNION	PRRI000450	B	B	S	S	S	
7	MALINIS	PRRI000572	B	B	S	S	S	
8	SALANAY (CARANIAG)	PRRI000566	B	B	S	S	S	
9	SALAYUSAY	PRRI000565	B	B	S	S	S	
10	SAMPAGA	PRRI000573	B	B	S	S	S	
	TN1 (RTD-susceptible check)		B	B	S	S	S	
K. With ambiguous or incomplete data								
1	SENILBERIO	PRRI000533	B	A	S	S	S	for verification/completion
2	KATUDAY	PRRI000534	B	-	S	S	S	

Legend: "A" resistant allele; "B" - susceptible allele; C-F = RM8213 allele variants ; "R" resistant; "S"=susceptible, "-" no available data
** n=10; **based on comparison of means of GLH alighted on varieties tested*

Field and screenhouse evaluation of individual and combined influences of GLH14 and TSV1 in ARC11554-derived elite lines infected with RTD

AA Dela Cruz, MJC Duque, MM Rosario, DK Donayre, SE Abdula, CG Flores, and CU Seville

Rice tungro disease (RTD) causes severe stunting and yellowing in a rice plant, resulting to heavy crop losses. RTD is a complex disease caused primarily by rice tungro spherical virus (RTSV) and aggravated by rice tungro bacilliform virus (RTBV), both transmitted by green leafhopper (GLH). In PhilRice, ARC11554 was long been used as source of RTD resistance but previous marker-assisted breeding only focused on introgression of a fragment from chromosome 4. Recently, the positions of Glh14 resistance locus and tsv1 gene in ARC11554 were found separately located in chromosomes 4 and 7, respectively. Moreover, the RTSV resistance in ARC11554 was also associated to eIF4G, a gene coding for a translation initiation factor. The eIF4G sequence variant in ARC11554 was found similar with the SNP type in RTSV-resistant Utri Merah 16882, TW16 and in other RTSV-resistant rice genotypes. In the previous study, rice breeding lines introgressed with Glh14 and/or tsv1, singly or combined, were examined for reactions to RTSV and significant resistance were observed in plants carrying at least a tsv1.

In this current study, the effectiveness of Glh14 and tsv1 against dual infection of RTSV and RTBV is being evaluated among selected rice breeding lines with various genetic backgrounds. Test lines are being evaluated for reactions to RTD in fields, where the disease is naturally occurring, and in the screen house under controlled conditions. Results of this study will validate the usefulness of Glh14 and tsv1, along with their corresponding

PCR-based molecular markers, in marker-assisted breeding. Promising lines introgressed with Glh14 and tsv1 will be recommended as common sources of traits for rice varietal development/improvement.

Highlights:

- A total of 14 selected rice lines (3 F7 lines derived from ARC11554 x TN1 and 11 PhilRice breeding lines) were evaluated for reactions to RTD under screen house and field conditions. Included in the evaluations as resistant and susceptible checks were ARC11554, TN1, Matatag 6, Utrimerah and Matatag 12. SSR markers RM8213 and RM5495 were used to screen the materials for presence/absence of the target locus/gene.

- In the screen house experiment, 14 days after sowing (das) seedlings were individually infested with 3 viruliferous GLH (vGLH, in mylar cages) for 24 hours. Ten seedlings per line were used. At 14 and 21 days post inoculation (dpi), the intensity of symptom development in the individual plants were assessed based on the scoring system of Narayanasamy and Muthulakshmi (1996).

- The field evaluations were conducted in PhilRice Experiment Stations in Negros (PhilRice NES) and Midsayap (PhilRice MES) during the wet season, however, materials established in MES were severely damaged by black bugs. In NES, the percent RTD infection was calculated using the formula: No. of plants with distinct yellow-orange discoloration / total number of plants x 100.

- Table 18 shows the individual and combined influences of Glh14 and tsv1 in ARC11554-derived rice lines infected with RTD under controlled and field conditions.

- Under controlled condition, almost no interveinal chlorosis and leaf discolorations were observed on the 2 rice lines F7-22-103-1-20 and 17-19-6-4-7. In terms of percent reduction in plant height, 14% and 15% were observed in F7-22-103-1-20 at 14 dpi and 21 dpi, respectively, while 17-19-6-4-7 plants efficiently recovered from 67% height reduction at 14 dpi to 19% at 21 dpi. Meanwhile in the field, the percent RTD infection in 17-19-6-4-7 was lower (5%) compared in F7-22-103-1-20 (35%). Likewise, the agro-morphological traits of 17-19-6-4-7 was far better than F7-22-103-1-20, which is acceptable since the latter materials was just developed for use in genetic analysis.

- Generally, rice lines introgressed with both Glh14 and tsv1 exhibited least expression of RTD symptoms whereas rice lines with single Glh14/tsv1 has lesser intensity of disease expression relative to rice lines without any of the 2 RTD resistance locus/gene.

- The use of resistance to GLH or RTSV alone does not assure the prevention of tungro although the combination of Glh14 and tsv1 might prolong the durability of rice plants against tungro in fields. The hypothesis that tsv1 suppresses RTSV multiplication in rice plants, inhibiting RTBV transmission and eventual expression of distinct tungro symptoms, has to be carefully investigated.

Table 18. Individual and combined influences of Glh14 and tsv1 in ARC11554-derived rice breeding lines infected with RTD under controlled and field conditions

	Elite lines/Varieties	Locus/Genes		*CONTROLLED CONDITION (Screenhouse)*					*FIELD CONDITION (PhilRice Negros, 2015WS)*					
				% height reduction		% leaves with discoloration		interveinal chlorosis (+/-)	Agro-morphological traits					
		Glh14	*tsv1*	14 dpi	21 dpi	14 dpi	21 dpi		%RTD infection *	Plant height (cm)	Average # of tillers	% productive tillers	% Filled grains	Ave. # of grains per panicle
1	F_7- 22-28-36-1-3	-	+	27	23	10	12	+/-	46	94	16	88	42	156
2	F_7-26-301-3-10	+	-	21	16	6	11	+/-	55	120	19	84	57	167
3	F_7-22-103-1-20	+	+	14	15	5	1	-	35	124	21	82	28	108
4	1-3-21	+	-	33	33	24	29	+/-	2	135	19	90	58	186
5	17-19-6-4-7	+	+	67	19	0	0	-	5	110	20	93	46	174
6	116-18-11	-	-	31	24	22	24	+	41	116	15	85	51	176
7	F_5-294-3-10	-	-	37	39	36	22	+	40	101	18	89	51	150
8	F_5-295-2-10	-	-	35	31	34	24	+	26	107	19	93	45	138
9	F_5-298-3-2	-	-	44	42	24	20	+	32	104	22	94	56	144
10	F_5-299-2-5	-	-	47	39	8	10	+	41	104	20	96	56	162
11	F_5-304-3-9	-	-	35	33	19	20	+	44	105	19	98	44	149
12	F_5-289-3	-	+	25	35	48	41	+	61	97	16	85	52	172
13	425-1-9	+	-	42	28	41	43	+	77	114	12	84	63	211
14	425-3-9	+	-	40	36	35	23	+	73	114	13	88	54	190
15	Matatag 6 (GLH-resistant check)	+	-	14	15	0	2	-	23	101	18	76	72	111
16	Matatag 12	+	-	13	13	1	2	-	10	110	16	79	65	167
17	Utri Merah (RTD-resistant check)	-	+	9	13	0	1	-	10	164	19	94	53	233
18	ARC11554 (Resistant check)	+	+	4	2	0	0	-	5	161	24	97	38	150
19	TN1 (Susceptible check)	-	-	41	41	24	29	+	82	92	16	84	60	140

*Legend: "A" ARC11554 allele type; "B" TN1 allele type; * mean of 3 reps with 100 individual plants per rep;*

SALT gene sequence and expression levels in selected Philippine traditional and modern varieties

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

The production of rice, being one of the most important crops, is under threat by different abiotic and biotic constraints such as salt stress and insect attack (Ismail et al., 2007; Brar et al, 2009; Fujita et al., 2009; Singh et al., 2010). Salt stress is a major problem in farming areas and one of the major obstacles in the production of rice worldwide (Thomson et al., 2010; Linh et al., 2012). According to Negrao et al. (2011). Although rice is one of the crops that can flourish in areas affected by high salinity, modern rice varieties cultivated these days are more sensitive to salt stress, particularly in the early vegetative and late reproductive stages (Ismail et al., 2007; Singh et al., 2010). Another constraint to the production of rice is the devastation caused by insect pests. The insects green leafhopper (GLH), Nephotettix virescens, and brown planthopper (BPH), Nilaparvata lugens Stål, are among the most devastating pests of rice. These insects feed by sucking the plant sap using their stylets, damaging the epidermis and parenchyma of the rice plant (Brar et al., 2009; Fujita et al., 2009). In addition, these insects are also vectors of devastating pathogens of rice. GLH act as vector for the causal agents of the rice tungro disease – rice tungro bacilliform virus (RTBV) and rice tungro spherical virus (RTSV) (Hull, 1996; Saito et al., 1976). On the other hand, BPH act as vectors for the grassy stunt virus and the ragged stunt virus (Fujita et al., 2009).

The Orysata or the Oryza sativa agglutinin is a mannose-binding jacalin-related lectin found in salt-stressed rice. This lectin was found to have insecticidal activities against biting-chewing and piercing-sucking insects (Al Atalah et al., 2014). The rice insect pests, 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). According to Zhang et al. (2000), Orysata corresponds to the protein product of the salt-stress-induced salT gene (LOC_Os01g24710) of the rice plant which is located at the short arm of the 1st chromosome at the around the 13.9 Mb region. A study by Thomson et al. (2010) was able to localize the QTL for saline tolerance or SalTol at 10.8 Mb to 16.4 Mb at the short arm of the 1st chromosome. Due to the location of the salT gene within SalTol, it is suggested to evaluate the strong possible association of the salT gene with the salt tolerance and insect resistance of the plant.

This study aims to determine variation in DNA sequence and gene expression levels of the salT gene in a panel of Philippine traditional and modern varieties. The following objectives are: (1) to determine tolerance or susceptibility to salinity and insect stress of different traditional and modern varieties (2) to compare gene expression levels of salT gene in different varieties under salinity and insect stress (3) to compare DNA sequences of

the salT gene (4)to analyze association between functional variation and genotype data.

Highlights:

- A rice panel consisting of 60 varieties were assembled in 2015 DS. Seed stocks were increased in 2015 WS for insect resistance and salt tolerance screening. Table 19 contains the assembled rice panel. Leaf samples were collected in 2015 WS and high quality genomic DNA was extracted from each entry for DNA sequencing.

- Gene-specific primers were designed in order to cover the entire salT gene DNA sequence of around 1.5 kb along with the upstream region which is hypothesized to be the promoter of the gene. The salT gene sequence of Nipponbare was used as reference for primer design. Primers were designed to have at least 150 to 200 overlapping bases in order to ensure the quality of the produced DNA sequence. The primer saltJE01 (861 bp) targets the hypthosized promoter region and the primers saltJE1 (948 bp), saltJE2 (1417 bp), and saltJE2.1 (969 bp) were created to cover the entire gene. Specificity of the primers were ensured through the alignment of the primer sequences with the Nipponbare reference sequence and also through Basic Local Alignment Search Tool (BLAST) analysis. Figure 22 shows the location of the primers and their targeted regions.

- The polymerase chain reaction (PCR) conditions for the designed primers were optimized in 2015 WS. Banding patterns of the PCR products using the designed primers are shown in Figure 23. In order to finally ensure the effectivity of the primers, the PCR products were purified and processed for DNA sequencing.

- Partial DNA sequence of the salT gene is shown in Figure 24 using the primer saltJE1. The alignment annotation revealed some haplotype patterns among the 10 varieties. Various polymorphisms, such as deletions, insertions, transversions, and substitutions are seen in the DNA sequence. Unfortunately, problems were encountered in DNA sequencing using the primers saltJE2 and saltJE2.1 in spite of great efforts exerted on the re-optimization of PCR conditions. Thus, new primers covering the downstream region of the gene were designed and re-ordered. Optimization of the new primers will be performed upon their arrival.

- The ordered laboratory and screening supplies are processed and are still awaiting delivery. Remaining activities of the study will be immediately performed upon the arrival of the ordered supplies. In 2016 DS, the DNA sequence of the salT gene in the assembled panel will be obtained. Also, the evaluation of tolerance, resistance, and susceptibility to insect attack and salinity stress will be performed in the same season.

Table 19. List of assembled rice panel consisting of modern and traditional Philippine varieties and known trait checks.

	ENTRY		ENTRY
1	PRRI000016	31	NSIC Rc184
2	PRRI000017	32	NSIC Rc190
3	PRRI000032	33	PRRI006783
4	PRRI000424	34	PRRI006826
5	PRRI000103	35	NSIC Rc328
6	PRRI000137	36	NSIC Rc330
7	PRRI000295	37	NSIC Rc334
8	PRRI000172	38	PRRI000386
9	PRRI000212	39	PRRI000303
10	PRRI000285	40	PRRI006558
11	PRRI000289	41	PRRI001182
12	PRRI002991	42	PRRI000510
13	PRRI002995	43	PRRI000538
14	PRRI003193	44	PRRI000548
15	PRRI003336	45	PRRI000376
16	PRRI004825	46	PSB Rc2
17	PRRI005276	47	PSB Rc4
18	PRRI005285	48	PSB Rc6
19	PRRI005397	49	PSB Rc8
20	PRRI005614	50	PSB Rc10
21	PRRI006311	51	PSB Rc20
22	PRRI006313	52	PSB Rc32
23	NSIC Rc82	53	PRRI 006560
24	NSIC Rc186	54	TN1
25	NSIC Rc188	55	ARC11554
26	NSIC Rc290	56	PRRI 001775
27	NSIC Rc292	57	FL478
28	NSIC Rc294	58	POKKALI
29	NSIC Rc340	59	IR29
30	PRRI000422	60	NSIC Rc222

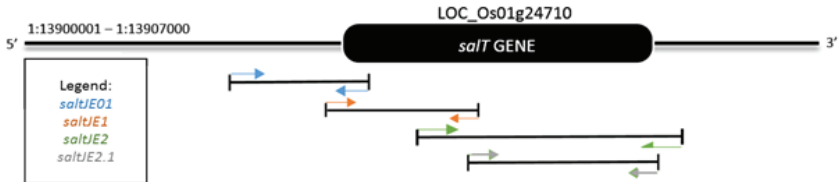

Figure 22. Visual representation of the salT Gene and the three designed primers, saltJE01 (861 bp), saltJE1 (948 bp), saltJE2 (1417 bp), and saltJE2.1 (969 bp) and their targeted regions.

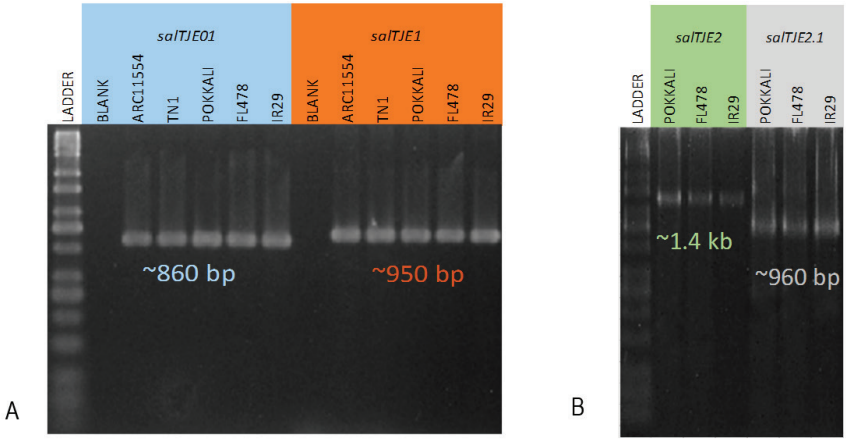

Figure 23. (A) Optimization of primers saltJE01 and salTJE1 using Pokkali, FL478, IR29 and 2 representative samples from the panel. (B) Optimization of primers salTJE2 and saltJE2.1 using Pokkali, FL478, and IR29.

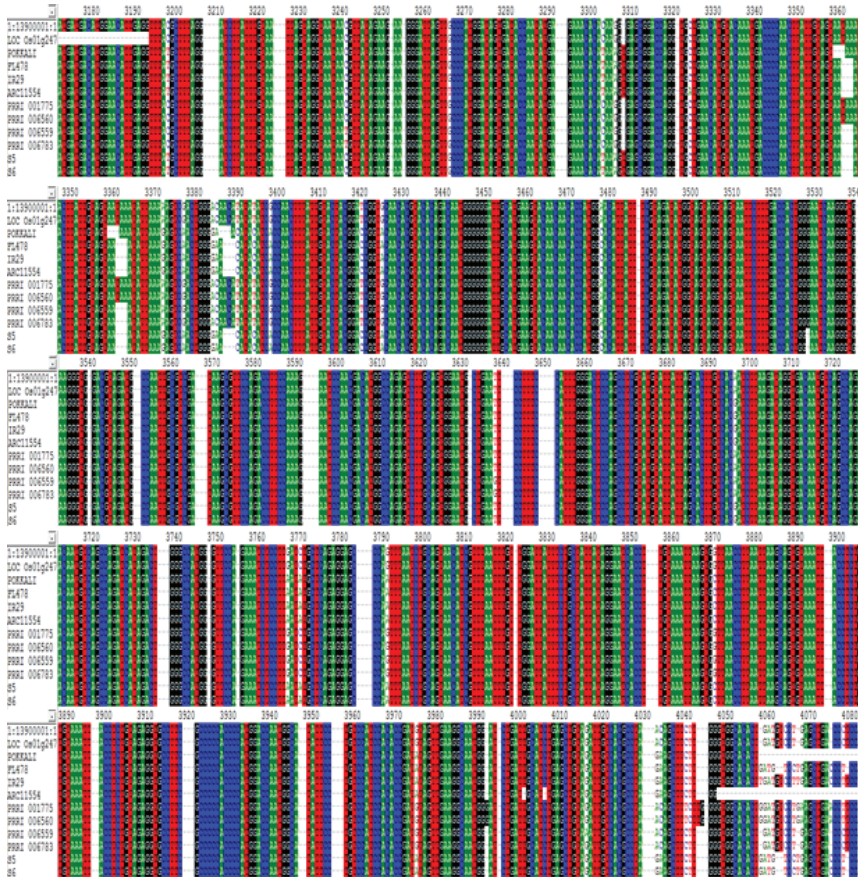

Figure 24. Multiple alignment of the partial DNA sequence of the salT gene using salTJE1 in ten rice varieties. Sequences were aligned with Oryza sativa cv. 'Nipponbare' sequence from 13,900,001 bp to 13,907,000 bp of the short arm of the 1st chromosome. Similar bases at each base position are highlighted with the same color. Nucleotide bases without colored backgrounds, on the other hand, represent polymorphisms in the DNA sequence such as deletion, transversions, insertions, and substitutions.

Establishment of marker system in developing Thermo-Sensitive Genic Male Sterile (TGMS) lines with BLB and RTD resistance

RT Miranda, NRL Sevilla, and CFS Te

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 Mestiso 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 Mestiso 19 and Rc204H known as Mestiso 20 for commercial cultivation.

This technology however, is faced with the two major rice diseases in the irrigated lowland environment which may cause signficant 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 Mestiso 1 and Mestiso 48 (IR58025B), Mestiso 29 (IR68897B) and other selected female parents through MAS; and (3) evaluate the sterility, resistance to BLB and tungro, and performance of the developed lines under male sterile growing condition.

Highlights:

- For second quarter of DS, molecular marker assay was conducted to verify the presence of genes/QTLs conferring resistance to tungro and green leaf hopper (GLH) in the elite donor line PR37171-1-1-1-2-2-1-1 or Line 27. Simple sequence repeat markers RM5495 linked to tsv1 loci for tungro resistance and RM8213 linked to glh14 loci for GLH resistance were utilized. In addition, genotypes used as checks were ARC11554 and TN1. Results show that only the resistance allele for GLH was present in Line 27 (Figure 25). Target gene genotyping to confirm presence of BLB resistance genes is on-going.
- PRUP-TG101 and PRUP-TG102, the S-lines of TGMS hybrids Mestiso 19 and Mestiso 20, Line 27 (2-in-1 line), with resistance to BLB and intermediate reaction to RTV and NSIC Rc120 (RTV resistant line) were identified as donor/sources of sterility gene (S-lines), resistance to Bacterial Leaf Blight (BLB) and Rice Tungro Virus (RTV) respectively.
- Produced three F1 crosses with cross combination, M20 x Line 27, M19 x Line 27 and M19 x NSIC Rc120 and seed increased for generation advance. F1 plants from these crosses were then crossed to two B lines (IR58025B and IR68897B) for the introgression of the S gene, BLB and RTV resistance producing six new F1 crosses, Table 20.

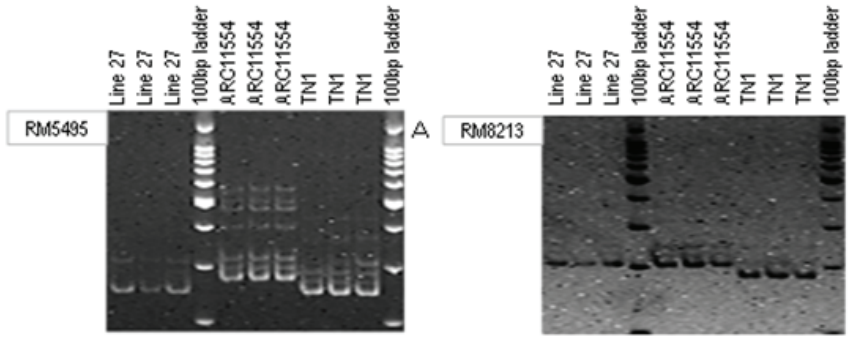

Figure 25. Target gene assay using linked markers for (A) tsv1 and (B) glh14.

Table 20. 2015 Generated F1 crosses using two S line donor, two B line and 2-in-1 line for BLB and RTV resistance donor.

Cross Combination	No. of F1 produced
Dry Season	
1. M19 x Line 27	14
2. M20 x Line 27	45
3. M19 x NSIC Rc120	57
Wet Season	
1. M19 x Line 27/IR68897B	2
2. M19 x Line 27/IR58025B	150
3. M20 x Line 27/IR68897B	81
4. M20 x Line 27/IR58025B	84
5. M19 x NSIC Rc120/IR68897B	40
6. M19 x NSIC Rc120/IR58025B	43
7. M19 x Line 27 x PYT-52	15
8. F1-59 x F1-53/IR68897B	15

Abbreviations and acronymns

ABA – Abscicic 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 – cystoplasmic 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
DWSR – 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
IRRI – 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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