Philippine Rice R&D Highlights 2013

CROP BIOTECHNOLOGY CENTER

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CROP BIOTECHNOLOGY CENTER

Center Director: AAAlfonso

Executive Summary

The world has a rapid increase in population coupled with changing climate not optimum to rice growing setting. The consequences of these developments may have made the next century witness serious global rice shortage problems. Consequently, there is a need for an increase in rice grain yield yet the prevailing climate change is doing the opposite. There is a growing need to study and breed rice varieties that can tolerate climate change-driven biotic and abiotic stresses.

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

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. 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 the rice's important traits, rice production and food security are 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 these stresses. Nevertheless, genetic systems responsible for tolerance remain largely unknown yet.

Molecular markers have been proven effective in increasing selection efficiency most especially for traits that are simply inherited. Over the years of gene discovery in the model crop species Oryza sativa, several genes controlling economically important traits have been fine-mapped and tagged with closely linked or gene-specific markers. The best example is bacterial leaf blight resistance, which now has a series of genes with marker tags that have been proven to be useful for MAS: Xa4, xa5, Xa7, xa13, and Xa21. In the case of tungro, the most important viral disease in the country, flanking markers are available for rtv, the RTSV resistance QTL from ARC11554. In addition, the RTSV resistance gene from Utri merah, tsv1, has been very recently fine-mapped (Lee et al 2010) and markers very close to the gene just need to be evaluated for usefulness in line development.

Recognizing the economic importance of bacterial blight and tungro, the inbred irrigated lowland and hybrid rice breeding projects of PhilRice share the general aim to incorporate routine marker-assisted selection for resistance genes to these two diseases during line development leading up to variety release. In addition, it is equally important to introgress bacterial blight and tungro resistance genes into popular inbred and hybrid varieties, which are widely planted across the country but are not disease resistant, because they contribute to the development of disease epidemics eventually leading to crop failure. Yield losses that run in the thousands of metric tons do not bode well for a country that is struggling to be rice self-sufficient.

Genome-wide selection utilizes dense genome-wide marker data to predict the breeding values of individuals without the need for QTL information (i.e., no need for QTL analysis), making it suitable for complex traits controlled by many genes. The trait is improved by increasing the frequency of favorable alleles achieved by recombining the best individuals from generation to generation, which is basically a population improvement approach. Selection for the best individuals is made more precise by using genotypic data made possible by genome-wide markers, in addition to phenotypic data. Marker effects are estimated and breeding values are predicted through mixed models, which require complex computations and have only become more achievable by applied science researchers through high-speed computing and more user-friendly statistical programs.

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

Project Leader: LMPerez

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 study is to identify a set of STR markers for rice identification and develop allele ladders that will be used in standard profiling of cultivars.

The utilization of germplasm, including wild rice species, is necessary to enhance genetic diversity and broaden the genetic base of future cultivars that will be developed. This is important to provide a wide genepool for selection by breeders particularly in developing rice varieties tolerant to drought and other abiotic stresses. Wide-cross derived lines from

O. glaberrima are being evaluated for drought tolerance and identification of possible breeding lines with tolerance to vegetative and drought stress as 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 for databasing and identification of PhilRice-bred rice varieties and rice germplasm accessions in PhilRice genebank

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 have been done using different types of DNA markers. However, none of these studies focused on establishing a method of cultivar identification based on the 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 reference population for comparative analysis. STR, also known as 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:

- Alleles of different sizes in each locus were identified, pooled and used in the construction of allele ladder per marker. Monoplex and multiplex PCR strategies were employed to confirm and verify the selected alleles and to optimize the procedures in the construction of standard allele ladder. Allele ladder for 14 STR markers were constructed (total of 20 STR markers to date). PCR product of each allele was sent to 1st Base Sequencing Co. in Malaysia to verify and confirm sequence structure and actual size of the alleles in each STR primers. A total of 98 alleles included in the allele ladder were sequenced.

- Results confirmed the sequence variation in terms of the number of repeats in each allele. Table 1 shows the results of sequencing done on STR-M26 allele ladder. Figure 1 shows the banding pattern of STR-M26 allele ladder in monoplex and multiplex PCR.

- Allele alignment of 186 rice varieties using the constructed allele ladder of 14 STR markers was also done. Allele alignment was done by direct comparison of the allele ladder with amplified DNA samples. This procedure allowed us to correct the assignment of allele scores in the population. Allele scores are being finalized in preparation for the statistical analysis of match probabilities to further validate the candidate marker for rice identification.

- New alleles identified in the allele alignment will be subjected to verification by sequencing and for inclusion in the allele ladder. Figure 2 shows the DNA profile of rice varieties identified that contain possible new alleles.

Table 1. Information on the verified number of repeats present and PCR product size of alleles included in the constructed allele ladder of marker STR-M26.

Marker Code/ Allele	Repeat Motiff	PCR product size (in bp)
STR-M26_A	(CAT)9	137
STR-M26_B	(CAT)15	151
STR-M26_C	(CAT)21	161

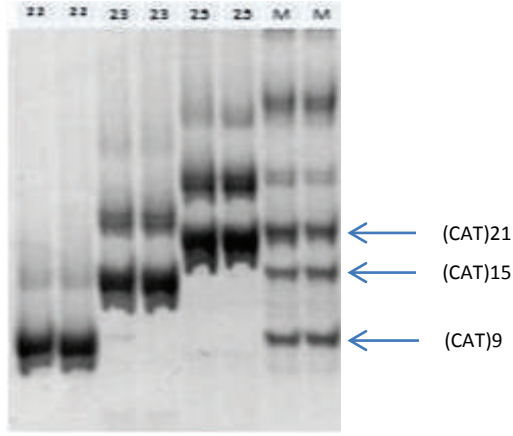


Figure 1. STR-M26 allele ladder obtained from monoplex and multiplex PCR analysis. Lanes 1-6, PCR products of alleles obtained from monoplex PCR (only 1 STR locus used); lanes 7-8, alleles obtained from multiplex PCR (3 STR loci used). Labels on the right indicate the core repeat motif and corresponding number of repeats in each allele verified by sequencing.

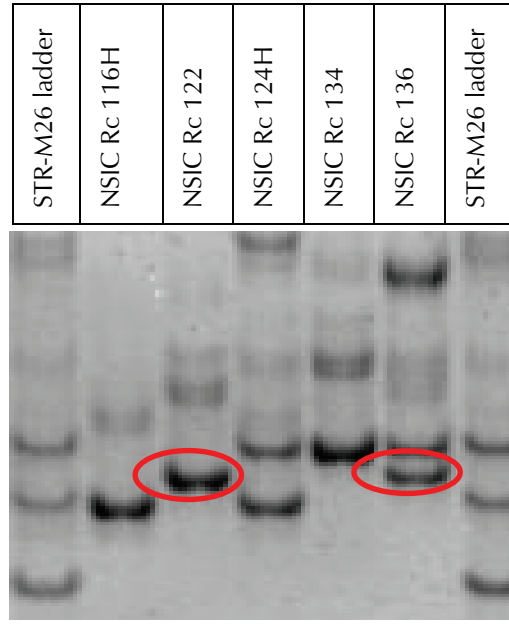


Figure 2. New alleles (encircled) identified in the alignment of allele scores of rice varieties using the allele ladder.

Utilization of wild rice species as gene sources for drought tolerance and other traits

AA Alfonso, AML Agustin, ES Avellanoza, and RT Miranda

Drought is the single most detrimental abiotic factor that hinders productivity in rainfed areas. With modest increases in productivity and decreasing physical area for favorable rice production, increasing production in the vast rainfed areas in the country is crucial in achieving rice self-sufficiency. Increased production can be achieved by construction of new irrigation facilities to address the problem in water scarcity, however, breeding of crops with built-in resistance is a cost-effective strategy to stabilize high yield under stress conditions.

The wild relatives of rice are rich sources of resistance and tolerance to biotic and abiotic stresses one of which is drought tolerance. Several wild rice accessions have been identified possessing drought tolerance and these can be used to develop drought tolerant rice varieties through wide hybridization. However, there are known barriers that limit the introgression of desirable traits from wild into cultivated species such as hybrid sterility, hybrid break down, and limited genome recombination. Nevertheless, there are available biotechnology tools that can address these problems such as embryo rescue and protoplast and somatic cell culture. Furthermore, several

techniques, such as backcross method and marker assisted breeding, can increase fertility recovery and other traits in early generation of inter-specific progenies. Superior progenies in stable generation should undergo replicated yield trials to measure the differences in their performance with precision and accuracy under target condition. Identification of even small difference in performance is very important to select superior lines to be advanced for higher breeding phase.

Highlights:

- Thirty-six wide-cross derived lines from IRGC 9118 (wild rice derivative), IRGC 11462, AUS 338, Nam Sa Gui 19, Chinois 6 and IR55419-04 composed of 18 early- and 18 intermediate-maturing lines were evaluated under drought stress and well-watered conditions with five rainfed varieties as drought tolerant checks (PSB Rc14, NSIC Rc192, Rc276, Rc282, and Rc288) and one susceptible check IR64. These were screened separately for vegetative and reproductive drought tolerance and were both laid-out using Randomize Complete Block Design replicated into three for reproductive and two for vegetative under drought stress and two under well-irrigated conditions. Agro-morphological data, drought responses of the cultivars as well as water table and soil moisture content were recorded.

- For vegetative drought tolerance screening, among different parameters evaluated, only plant height had significant difference among means. Three breeding lines with 62 - 66 cm plant height were significantly taller over IR64 but not to other checks.

- For reproductive drought tolerance screening (Figure 3), days to flowering of all breeding lines were not significantly different to all checks except NSIC Rc192 indicating the minimal effect of drought escape among entries. Under drought stress condition, drought parameters and hundred grain weight had good correlation to yield. Hundred grain weight and panicle harvest index had positive strong linear relationship with the same r value of 0.63 followed by leaf rolling (r=-0.52) and drying (r=-0.48) with negative moderate linear relationship. The same result was found under well-irrigated condition, all parameters had weak to very weak linear relationship to yield (Table 3). Four promising lines (RF-5, 17, 1, and 9) were selected based on yield under drought stress condition as well as yield stability and yield under fully-irrigated condition. Other lines such as RF-39, 25, 26, and 31 were among the top yielding under well-irrigated condition and, therefore, will be

further evaluated under the said condition (Table 2).

- Among the new tolerant checks evaluated, NSIC Rc276 had superior performance in terms of drought response and yield and, therefore, will be included as new drought tolerant check.

- Another 313 breeding lines composed of 58 PN, 119 ON, and 136 AON were selected under drought stress condition.

- Nine F1 populations from crosses between drought-tolerant and high-yielding progenies were advanced to F2.

- Eight O. glaberrima accessions were evaluated for vegetative drought tolerance. These were evaluated under drought-stress and well-watered conditions with four rainfed varieties as drought-tolerant checks (PSB Rc14, NSIC Rc192, Rc276, and Rc282) and susceptible check IR64. These were laid-out using Randomize Complete Block Design replicated into three under drought-stress and two under well-irrigated conditions. Based on the results, no accessions were identified as drought tolerant.

- In 2013 WS, 37 PYT (including four checks; PSB Rc14, IR64, NSIC Rc192 and NSIC Rc276), 58 PN, 119 ON and 133 AON drought selected lines from 2013 DS screening were forwarded for generation advance and for evaluation of yield potential and other agronomic traits.

- Of the 33 PYT test entries, PYT 15 have yield advantage of 7 to 33 percent over the four checks, (IR64=18%; PSBRc14=10%; NSIC Rc192=33% and NSIC Rc276=7%) with an average yield of 6.5 tons/ha.

- A total of 445 breeding lines composed of 58 PN, 119 ON, and 268 AON were forwarded for 2014 DS for drought tolerance evaluation.

Table 2. Drought response and agro-morphology of breeding lines under drought stress condition and their yield advantage over checks under drought stress and fully irrigated conditions.

Rank (D)	Code	DF	FD	LR	LD	PH (cm)	PT	PPT	NS	PL (cm)	PHI	RPHI	DY (tha^{-1})	YA over PSB Rc14	YA over NSIC Rc276	IY (tha^{-1})	YR	YA over IR64
1	RF-5	63	1	0	3	80	17	96.73	116	22	0.68	0.44	4.64	129.58	136.85	10.80	42.96	96.41
2	RF-17	64	2	3	3	78	20	97.40	113	24	0.69	0.32	4.36	121.90	128.73	13.86	31.49	123.74
3	RF-1	64	2	1	5	83	19	97.54	123	23	0.64	0.39	3.75	104.77	110.64	9.72	38.60	86.75
4	RF-9	64	-2	1	1	80	20	99.46	106	24	0.66	0.32	3.67	102.44	108.18	11.52	31.85	102.82
5	PSB Rc14	67	2	3	1	80	24	97.32	116	22	0.63	0.38	3.58	100.10	105.71	9.49	37.76	84.73
6	RF-8	61	1	0	1	82	22	94.49	94	21	0.74	0.34	3.54	98.82	104.36	10.55	33.54	94.17
7	RF-18	64	0	1	1	83	22	97.11	104	24	0.63	0.34	3.51	98.18	103.68	10.38	33.88	92.64
8	RF-27	63	1	3	1	75	22	99.03	141	22	0.63	0.34	3.49	97.61	103.08	10.35	33.76	92.42
9	RF-28	60	3	3	3	83	26	97.72	121	22	0.54	0.35	3.43	95.92	101.30	9.79	35.08	87.40
10	NSIC Rc276	68	3	1	1	84	24	96.25	126	22	0.66	0.34	3.39	94.74	100.05	10.07	33.68	89.91
11	RF-4	66	2	0	1	80	22	98.39	115	22	0.58	0.34	3.34	93.39	98.63	9.83	34.01	87.78
12	RF-6	64	2	0	3	79	19	97.23	110	22	0.67	0.30	3.26	91.06	96.17	11.00	29.64	98.21
13	RF-39	64	8	7	5	75	18	98.16	134	23	0.50	0.25	3.25	90.84	95.93	13.22	24.60	118.06
14	RF-26	66	7	5	3	69	18	97.82	122	22	0.65	0.27	3.23	90.34	95.40	12.03	26.87	107.45
15	RF-40	68	9	7	3	70	25	98.08	135	25	0.59	0.29	3.22	89.95	94.99	11.28	28.54	100.74
16	RF-11	66	0	1	1	80	23	98.83	121	24	0.65	0.38	3.15	87.92	92.85	8.24	38.19	73.58
17	RF-7	62	4	0	3	76	17	98.69	97	22	0.71	0.32	3.08	86.06	90.88	9.68	31.82	86.45
18	RF-16	65	0	3	1	80	22	97.09	139	25	0.59	0.26	2.94	82.17	86.77	11.40	25.80	101.78
19	RF-37	63	3	7	5	71	20	91.11	134	22	0.49	0.30	2.94	82.03	86.62	10.01	29.33	89.40
20	RF-10	65	-1	1	1	81	21	98.18	125	25	0.63	0.28	2.93	81.84	86.43	10.45	28.03	93.35
21	RF-29	60	3	3	3	80	28	97.03	123	21	0.50	0.30	2.87	80.22	84.71	9.52	30.17	85.00
22	RF-32	65	5	5	3	71	24	92.72	114	21	0.49	0.27	2.87	80.07	84.56	10.61	27.01	94.77
23	RF-38	65	6	7	5	72	20	90.02	118	22	0.52	0.31	2.86	80.00	84.48	9.19	31.18	82.01
24	RF-24	67	7	5	3	64	19	98.84	135	23	0.48	0.26	2.83	79.13	83.57	11.11	25.50	99.19

* Significant at 5% level; DY – yield in drought; IY – yield in irrigated; YR – yield reduction; YA – yield advantage

Continuation (Table 2)

Rank (D)	Code	DF	FD	LR	LD	PH (cm)	PT	PPT	NS	PL (cm)	PHI	RPHI	DY (tha^{-1})	YA over PSB Rc14	YA over NSIC Rc276	IY (tha^{-1})	RY	YA over IR64
25	RF-3	64	1	3	3	81	19	98.70	105	21	0.57	0.28	2.81	78.45	82.85	10.18	27.59	90.89
26	RF-31	65	4	3	1	75	26	99.02	112	21	0.54	0.23	2.76	77.18	81.51	11.97	23.09	106.86
27	RF-2	65	3	1	1	81	19	96.44	122	22	0.63	0.25	2.68	74.84	79.04	10.75	24.92	96.00
28	RF-30	66	6	3	1	73	24	95.90	137	22	0.52	0.23	2.67	74.59	78.77	11.39	23.44	101.71
29	RF-12	63	-2	0	1	82	21	96.84	116	23	0.65	0.26	2.50	69.81	73.72	9.86	25.35	88.00
30	NSIC Rc288	64	4	5	5	105	11	81.85	192	23	0.59	0.23	2.42	67.65	71.44	10.33	23.44	92.25
31	RF-33	67	8	5	3	72	24	96.92	127	23	0.41	0.24	2.41	67.27	71.04	10.17	23.69	90.76
32	RF-41	67	8	9	3	81	22	94.12	131	24	0.49	0.24	2.35	65.70	69.38	9.73	24.18	86.85
33	RF-34	65	6	7	5	71	25	90.67	125	21	0.43	0.21	2.32	64.83	68.46	11.14	20.84	99.45
34	NSIC Rc282	66	2	7	5	94	19	92.62	138	23	0.55	0.22	2.17	60.61	64.00	10.40	20.86	92.86
35	RF-36	65	5	7	3	72	24	94.67	123	23	0.42	0.20	2.09	58.32*	61.59	10.56	19.78	94.26
36	RF-35	66	6	7	3	70	25	89.94	135	22	0.44	0.21	2.05	57.16*	60.37	10.18	20.10	90.93
37	RF-25	66	6	5	3	66	17	99.17	119	22	0.51	0.17	2.04	57.09*	60.29	12.36	16.53	110.39
38	NSIC Rc192	57	2	9	7	99	12	86.30	169	24	0.48	0.16	1.73	48.25*	50.95*	10.23	16.88	91.37
39	IR64	66	2	5	5	75	21	97.31	112	22	0.54	0.12	1.37	38.19*	40.33*	11.20	12.20	100.02

* Significant at 5% level; DY – yield in drought; IY – yield in irrigated; YR – yield reduction; YA – yield advantage

Table 3. Correlation coefficients between grain yield and all other traits under both drought stress and fully-irrigated conditions.

	LD	FLW	FD	PH	RPH	PT	RPT	PPT	RPPT	NS	RNS	PL	RPL	PHI	RPHI	HGW	RHGW	Y	RY
LR	0.60**	0.16	0.50**	-0.16	-0.36**	-0.06	0.12	-0.42**	-0.42**	0.39**	0.23*	0.05	0.26**	-0.67**	-0.64**	-0.52**	0.54**	-0.52**	-0.54**
LD		-0.08	0.23*	0.08	-0.04	-0.31**	-0.22*	-0.47**	-0.46**	0.34**	0.22*	-0.05	0.14	-0.53**	-0.52**	-0.48**	0.45**	-0.48**	-0.45**
FLW			0.63**	-0.37**	-0.45**	0.10	0.25**	0.01	0.01	0.03	0.01	0.11	0.00	-0.20*	-0.18*	-0.09	-0.10	-0.09	-0.10
FLW (I)			-	0.08	-	0.02	-	-0.02	-	-0.04	-	0.36**	-	-0.30**	-	0.19*	-	-0.07	-
FD				-0.42**	-0.46**	0.05	0.17	-0.16	-0.15	0.24*	0.20*	-0.10	0.11	-0.52**	-0.54**	-0.24**	-0.27**	-0.24**	-0.28**
PH					0.68**	-0.26**	-0.25**	-0.13	-0.13	0.33**	0.06	0.25**	0.17	0.28**	0.27**	0.04	0.1	0.04	0.10
PH (I)						-0.55**	-	-0.06	-	0.52**	-	0.28**	-	-0.06	-	0.21*	-	-0.16	-
RPH							-0.20	0.14	0.14	-0.11	0.00	0.01	0.17	0.28**	0.27**	0.25**	0.26**	0.25**	0.26**
PT								0.42**	0.41**	-0.25**	0.00	0.00	0.15	-0.06	0.01	-0.36**	-0.03	0.12	0.14
PT (I)								0.05	-	-0.42**	-	-0.33**	-	0.01	-	-0.38**	-	0.03	-
RPT									0.31**	0.01	0.04	0.22*	0.15	-0.09	-0.02	-0.25**	-0.20*	0.00	0.02
PPT									1.00**	-0.39**	-0.22*	0.10	-0.09	0.35**	0.31**	0.12	0.14	0.31**	0.21*
PPT (I)										0.09	-	-0.05	-	-0.06	-	-0.10	-	0.06	-
RPPT										-0.40**	-0.22*	0.10	-0.09	0.35**	0.31**	0.00	0.03	0.30**	0.21*
NS											0.59**	0.35**	0.28**	-0.41**	-0.40**	0.31**	0.21*	-0.31**	-0.28**
NS (I)												0.46**	-	0.05	-	0.04	-	0.15	-
RNS												0.09	0.45**	-0.40**	-0.38**	0.30**	0.21*	-0.24**	-0.16
PL													0.45**	0.04	0.11	-0.31**	-0.28**	0.06	0.03
PL (I)														-0.22*	-	0.47**	-	0.14	-
RPL														-0.21*	-0.20*	-0.24**	-0.16	-0.01	0.01
PHI															0.90**	0.06	0.03	0.63**	0.57**
PHI (I)																-0.05	-	0.25**	-
RPHI																0.01	0.01	0.52**	0.51**
HGW																	0.88**	0.63**	0.52**
HGW (I)																		0.25**	-
RHGW																			0.51**
Y																			0.88**

* Significant at 5% level; ** significant at 1% level; I – fully-irrigated condition

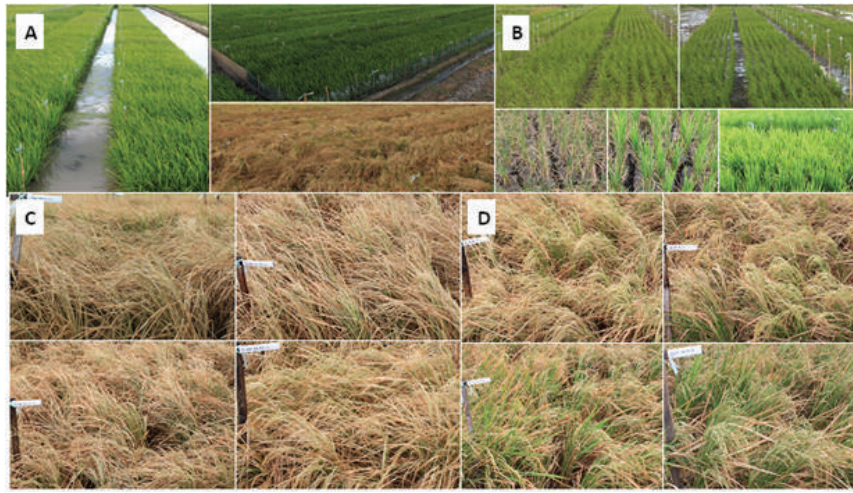


Figure 3. Reproductive and vegetative drought tolerance screening set-up (A-B); checks and selected breeding lines at 35 DADI (C-D).

II. Gene Discovery and Marker Development for Agronomically Important Traits

Project Leader: OEManangkil

In response to drought stress, changes in shoot dry matter production under simulated rainfed condition were strongly influenced by the ability to maintain root system developments. Total root length, total lateral root length and deepest nodal root below the gravel significantly contributed to dry matter production while total nodal root length and mean nodal root length did not, an evidence of root plasticity in drought stress.

Thirty putative QTLs associated to seedling vigor with LOD value of each from 3.1- 9.4 and phenotypic variance of 3.4- 66.7% were detected while 6 putative QTLs were identified associated to heat tolerance (pollen sterility) with LOD value ranging from 3.2-10.1 and phenotypic variance ranging from 1.0-51.1%. Both these traits require additional markers to fill the unfilled bins for a better QTL detection.

Phenotype and genotype data of different plant materials (varieties and F2s) to crack resistance will be used in the linkage and QTL analysis of crack resistance. Finally, marker system for molecular-aided backcrossing will be designed according to QTL data of the study.

Optimization of MHd_CAPS marker and EcoRII restriction enzyme for OS-lpa-MH86 gene was successfully carried out in the evaluation of markers for low phytic acid in brown rice. Distinct amplification of PCR

products of wild type and mutant plant was observed using different digestion time. The link marker for OS-lpa XQZ gene can now be used in MAS for developing lpa brown rice.

Twenty-one new cross combinations were generated in pyramiding of blast resistance genes into elite and high-yielding irrigated lowland rice cultivars. Blast resistance gene donors were selected in the population. The F1 plants with the same recipient background will be used in double cross combinations to pyramid two or more blast resistance genes. The BC1F1 plants generated will be established and tagged individually for foreground genotyping using pBA14 for Pi9 and z565962 for Piz gene.

Four QTL were identified, two QTL for RTSV resistance and two QTL for GLH resistance. ICIM revealed that RTSV resistance is tightly linked with the RM335-RM16428 region with LOD = 39.0 and R2 = 17.43%, with a secondary peak in the RM16434-RM3471 region with LOD = 13.3 and R2 = 3.60% (Table 3). GLH resistance was tightly associated with the RM335-RM16428 region with LOD = 46.3 and R2 = 19.19%, and with a secondary peak in the RM16434-RM16497 region with LOD = 14.8 and R2 = 3.56%. The primary signal (LOD peak) of the QTL for RTSV and GLH resistance was located between RM16425 and RM16427 as depicted in the ICIM line graph. The second peak was located in the RM16466-RM3471 region with the size of 655 kb. Correlation coefficient showed significant relationship ($r = 0.322$, $P < 0.0001$) which suggests that the two QTL detected were controlled with two different genes sitting in the same chromosome region. Thus, the QTL for both traits were tightly linked in above mentioned region.

QTL analysis on the plasticity of root traits in response to drought stress in rice

RR Suralta, N Lucob, and LM Perez

Plastic root system development in rice is a key trait for crop adaptation and productivity under drought-prone environments. In our previous study using CT9993/IR62266 double-haploid lines (DHLs), we precisely identified that L-type lateral root length and number at 20-40 cm depth strongly influenced plasticity in root system development based on total root length.

A field experiment was conducted to determine the functional roles of plastic root system development on biomass production and grain yield of ten selected DHLs and parents under continuously waterlogged (CWL) and simulated rainfed (DR) field conditions. Plasticity was estimated as the difference between values of the trait in DR and CWL for each DHL.

Highlights:

- Tables 4 and 5 show the above-ground traits under CWL and DR conditions. Significant effects of water treatment and genotype were observed for all the traits, except for the effect of treatment on the number of tillers. Interaction of water treatment and genotypes was significant in total biomass only. Total biomass production under CWL and DR ranged from 0.23-0.35 t ha-1 and 0.15-0.25 tha-1, respectively. Under DR, IR62266 showed significant high biomass production among the genotypes. DHL96, however, had higher biomass than CT9993. The same trend was observed in grain yield.

- In our previous experiment, we showed that changes in shoot dry matter production under DR condition were strongly influenced by the ability to maintain root system developments. Figure 4 shows the change in shoot dry weight of the different genotypes. DHL96 had more production of straw under DR than CWL; hence, it had the highest value of change in shoot dry weight. Scanning and analysis of root samples using WinRhizo software is on-going. Thereafter, relationship of yield and total biomass production to different root traits will be evaluated.

- Another set of selected DHLs with potential deep rooting ability was evaluated under simulated upland rainfed using plastic tubes with 5-cm-thick gravel layer placed at 30 cm below soil surface to validate the relationship of TLRL below gravel layer and dry matter production during drought stress. Figure 5 shows that at 0-30 cm depth (above gravel), soil moisture was lower than 30-90 cm depth (below gravel), an evidence that gravel layer prevented the capillary rise of water underneath gravel.

- Result showed that total root length, total lateral root length and deepest nodal root below the gravel significantly contributed to dry matter production while total nodal root length and mean nodal root length did not (Table 6).

- The same set of DHLs from plastic tubes is currently evaluated under PVC tube set-up. This experiment allows the simultaneous evaluation of root branching and rooting ability. The SMC treatments are well-watered (WW) and drought rewatered (DR). In WW, SMC is being maintained at 22 % until maturity of the plants. In DR, on the other hand, the soil in the tubes was first maintained at 22% SMC. When the plants reached 10th leaf stage, watering was withheld

and then the soil was allowed to dry up to 10 % SMC and then SMC was again reset back to 22%. Drought treatment imposed through drought-rewatered (soil moisture fluctuation) will be terminated at maturity of the plants. Thermal images of the tubes are periodically gathered for the analysis of canopy temperature and to relate this to photosynthesis and transpiration. After termination of the experiment, whole root system will be sampled for scanning and analysis.

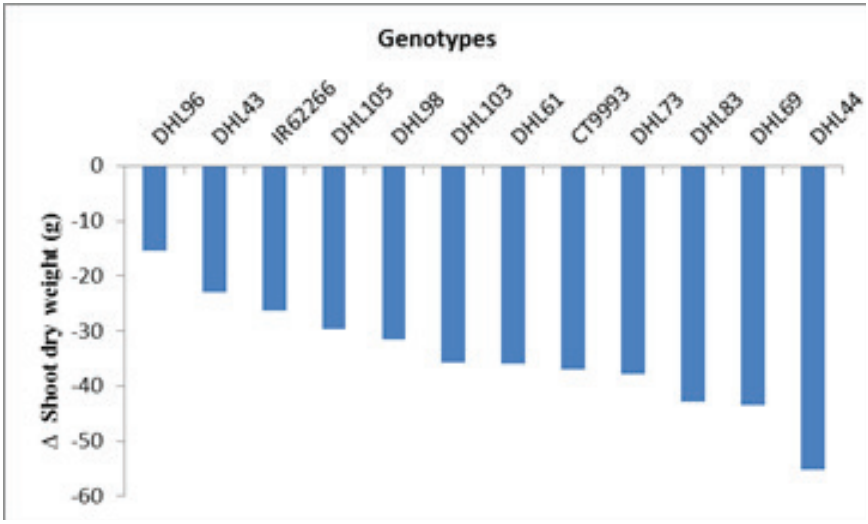


Figure 4. Change in shoot dry weight of CT9993, IR62266 and selected DHLs.

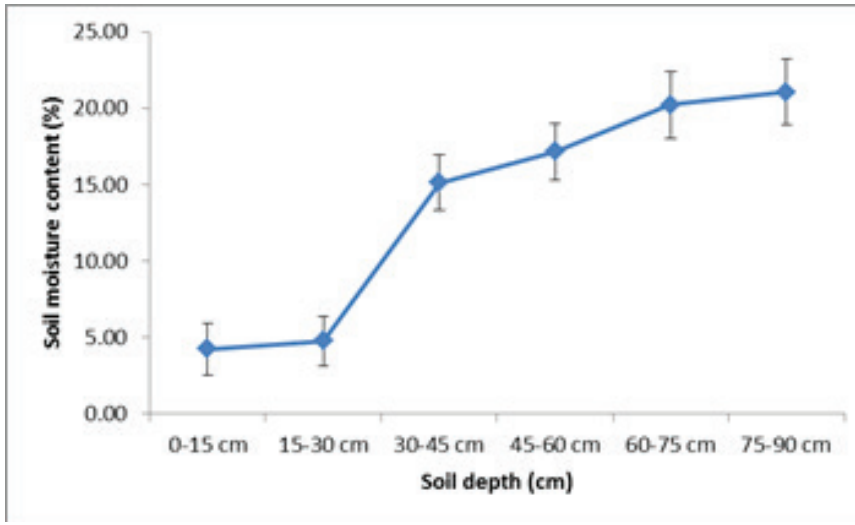


Figure 5. Soil moisture dynamics at different depths in plastic tube with 5-cm gravel layer embedded at 30 cm below soil surface.

Table 4. Shoot traits of selected CT9993/IR62266 DHLs and parents under CWL and DR conditions during 2013 dry season.

Genotype	Height (cm plant^{-1})		Number of tillers (plant^{-1})		Number of panicles (plant^{-1})		Straw weight (g plant^{-1})	
	CWL	DR	CWL	DR	CWL	DR	CWL	DR
CT9993	137.33 a	107.22 d	11.56 d	12.00 d	10.33 d	9.67 bcd	36.12 a	23.82 ab
DHL103	116.22 bcd	92.67 ab	18.44 abc	15.67 abc	18.00 abc	12.89 b	30.83 ab	18.93 ab
DHL105	80.56 g	62.00 c	23.44 a	23.44 a	22.56 a	18.33 a	29.52 ab	19.61 ab
DHL43	95.78 ef	82.56 bc	17.44 bc	17.33 bc	16.67 bc	15.00 ab	27.91 ab	20.26 ab
DHL44	123.22 abc	94.33 ab	16.78 bcd	13.89 bcd	15.67 bcd	10.78 bc	36.90 a	18.48 b
DHL61	114.56 cd	91.89 ab	17.33 bc	17.56 bc	14.89 bcd	13.11 ab	36.92 a	24.98 a
DHL69	116.22 bcd	102.33 ab	20.56 abc	16.78 abc	19.44 ab	15.11 ab	36.04 a	21.53 a
DHL73	122.00 bc	94.22 ab	16.89 bcd	15.67 bcd	15.78 bcd	12.89 b	35.22 ab	22.64 ab
DHL83	130.44 ab	97.33 ab	15.67 bcd	13.22 bcd	13.11 cd	11.11 bc	35.16 ab	20.86 ab
DHL96	121.67 bc	95.67 ab	11.56 d	14.78 d	10.67 d	13.00 ab	24.62 b	19.51 ab
DHL98	89.44 fg	69.44 c	15.33 cd	15.67 cd	13.44 cd	14.44 ab	28.57 ab	18.08 bc
IR62266	106.22 de	92.22 ab	21.11 ab	17.33 ab	20.00 ab	16.33 a	34.31 ab	25.56 a
Genotype	***		***		*		**	
Treatment	**		ns		***		**	
G x T	ns		ns		Ns		ns	

Values followed by the same letter in a column within each treatment are not significantly different ($p < 0.05$).
*, ** and ns indicates at the 5%, 1% level and no significance, respectively.

Table 5. Spikelet fertility, total biomass and grain yield of the different selected CT9993/IR62266 DHLs and parents under CWL and DR conditions during 2013 dry season.

	Spikelet fertility (%)		Total biomass (ton ha^{-1})		Grain yield (ton ha^{-1})	
	CWL	DR	CWL	DR	CWL	DR
CT9993	30.47 a	28.20 a	0.35 a	0.19 ab	5.45 ab	4.30 abc
DHL103	24.29 bc	22.36 ab	0.31 abc	0.18 ab	6.05 a	4.15 abc
DHL105	24.28 c	19.98 ab	0.27 bc	0.15 b	5.36 ab	3.87 c
DHL43	20.76 bc	19.99 ab	0.26 bc	0.19 ab	5.28 ab	3.71 ab
DHL44	23.28 bc	24.36 ab	0.31 abc	0.18 ab	6.25 ab	3.33 abc
DHL61	19.87 cd	18.42 ab	0.30 abc	0.23 ab	4.84 ab	3.16 abc
DHL69	21.23 bc	22.41 ab	0.32 abc	0.21 ab	4.84 ab	2.89 ab
DHL73	17.53 ab	13.43 b	0.28 abc	0.17 ab	5.57 b	2.76 bc
DHL83	25.90 bc	22.95 ab	0.28 abc	0.17 ab	4.63 ab	2.47 abc
DHL96	22.04 bc	20.72 ab	0.23 c	0.21 ab	4.76 ab	2.41 abc
DHL98	24.24 bc	19.42 ab	0.33 ab	0.19 ab	4.03 ab	1.89 a
IR62266	20.69 de	21.00 ab	0.31 abc	0.25 a	5.32 a	1.53 a
Genotype	***		***		***	
Treatment	ns		***		*	
G x T	ns		*		ns	

Values followed by the same letter in a column within each treatment are not significantly different ($p < 0.05$).
*, ** and ns indicates at the 5%, 1% level and no significance, respectively.

Table 6. Correlation matrices among root parameters below the gravel layer and shoot dry weight of selected doubled-haploid lines under drought stress only.

Root traits below gravel layer	Shoot dry weight
Deepest nodal root	0.43 **
Nodal root length	0.28 ns
Total root length	0.32 *
Total lateral root length	0.32 *
Mean nodal root length	0.22 ns

QTL mapping and expression profiling of genes associated to seedling vigor in direct wet-seeded rice system

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

Seed germination is an important and dynamic process in plant development. Rice, generally, has a high ability to germinate under water-submerged conditions. Despite this, however, high mortality of germinating rice in submerged condition during continuous monsoon rain is always observed in rice growing areas. Germination tolerance under partial or complete anoxia is thus a critical trait in direct seeding rice cultivation.

Submergence is a major stress causing yield losses particularly in the direct-seeded rice cultivation system and necessitates the development of rice cultivars with tolerance against submergence stress. Bioassay methods

that were based primarily on the seedling vigor evaluated by the ability of fast shoot elongation under submerged conditions were recently developed. Screening for anaerobic germination tolerance was also developed by the project and have been used for bioassay of lines that are being developed.

This study aimed to: (a) carry out gene expression profiling of candidates genes, and (b) determine genes/loci responsible to rice seedling vigor.

Highlights:

- A total of 97 polymorphic simple sequence repeats (SSR) markers were used to genotype the 186 F2 populations from a cross between Italica livorno and IR64 using 3Mb bin system (Figure 6). The QTLs associated with seedling vigor were analyzed by the Composite Interval Mapping (CIM) method using QTL IciMapping software version 3.2. In this method, the log-likelihood (LOD) score indicates the strength of the data supporting the hypothesis about the existence of the QTLs. A LOD threshold of 3.0 was used to detect putative QTLs and Kosambi were used to estimate map distances. Thirty (30) putative QTLs were associated to germination, root and shoot length and biomass (Table 7). LOD value of each QTL ranges from 3.1- 9.4 while the phenotypic variance ranges from 3.4- 66.7%.

- Additional markers to cover the gap in each chromosome were used. Polymorphism survey was done using 139 markers and 41 markers were found to be polymorphic (Figure 7). Out of 41 polymorphic markers, only 19 will be used to fill up bins that have no markers. Additional polymorphism survey of markers is on-going to fill up bins that have not yet representative markers.

- Selection was carried out based on phenotypic acceptability and uniformity among populations every season. A total of 17 advance lines from the cross Italica livorno and IR64 were selected and will be established for further evaluation in 2014 DS. Phenotypic data such as days to heading, pests and diseases were observed and recorded. Advance lines will be further evaluated under observational nursery (ON) to generate possible donor parent of seedling vigor trait.

- Additional markers will be procured, surveyed for polymorphism and polymorphic markers located in the unfilled bins will be used in the population.

Figure 6. Distribution of 97 polymorphic SSR markers used in 186 F2 population of seedling vigor.

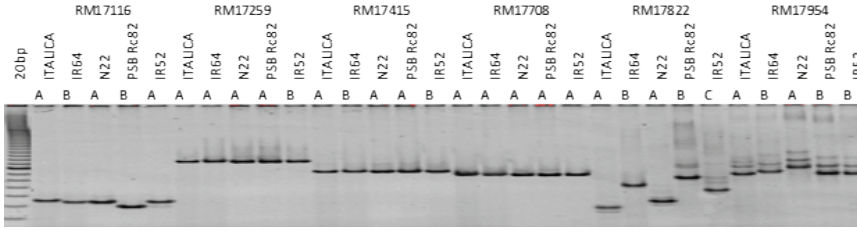


Figure 7. Polymorphism survey in the cross of Italica Livorno and IR64 using 139 SSR markers.

Table 7. Putative QTLs identified for seedling vigor trait.

QTL	Chr	Marker Peak	Flanking Markers	[a]LOD	[b]PVE(%)
%Germination	2	RM3178	RM3178-RM13500	3.2	6.0
	3	RM520	RM3601-RM520	4.9	66.7
	4	RM124	RM349-RM124	3.2	43.4
	5	RM178	RM188-RM178	3.5	59.7
	6	RM162	RM454-RM461	4.3	60.4
	7	RM214	RM214-RM18	3.1	21.3
	8	RM502	RM408-RM477	3.8	60.4
	9	RM434	RM3644-RM215	4.5	61.6
	11	RM552	RM286-RM144	4.7	63.7
Root	2	RM530	RM6-RM530	5.0	24.9
	3	RM282	RM282-16250	3.6	48.2
	6	RM454	RM3183-RM162	3.2	51.0
	8	RM477	RM544-RM477	5.3	34.9
	11	RM286	RM181-RM286	3.8	53.9
Shoot	1	RM489	RM495-RM489	3.3	10.8
	2	RM530	RM6-RM530	4.0	4.8
	3	RM16250	RM468-RM16250	8.6	23.8
	5	RM305	RM305-RM188	3.1	3.4
	7	RM214	RM214-RM18	3.6	11.6
	8	RM477	RM544-RM477	3.6	15.8
	9	RM242	RM288-RM215	3.2	3.8
Biomass	1	RM259	RM259-RM104	5.8	42.6
	2	RM6374	RM6374-RM3178	3.0	39.1
	3	RM523	RM523-16250	3.1	36.8
	6	RM3183	RM3183-RM461	3.0	44.3
	7	RM214	RM6776-RM214	4.6	46.4
	8	RM544	RM544-477	3.7	43.8
	9	RM5777	RM5777-RM205	9.4	45.1
	11	RM558	RM286-RM552	5.3	43.5
	12	RM247	RM247-RM277	3.8	40.0

[a] Threshold LOD score established at p=0.05 significance level.
[b] Percentage explained in the phenotypic variance.

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

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

Rice grows optimally between 20 to 35oC 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:

- Sixty six (66) simple sequence repeats (SSR) markers were used to genotype the 200 F2 populations of a cross between N22 and PSB Rc82 using 3 Mb bin system (Figure 8). QTLs associated with heat tolerance were analyzed by the composite interval mapping (CIM) method using QTL IciMapping software version 3.2. In this method the log-likelihood (LOD) score indicates the strength of the data supporting the hypothesis about the existence of the QTLs. An LOD threshold of 3.0 was used to detect putative QTLs and Kosambi was used to estimate map distances. A total of 6 putative QTLs were identified associated to pollen sterility (Table 8). LOD value for each QTL ranges from 3.2-10.1 and phenotypic variance ranges from 1.0-51.1%.

- Polymorphism survey was done using 139 markers and 39 markers were found to be polymorphic. Out of 39 polymorphic markers, only 26 will be used for bins that have not yet markers. Additional polymorphism survey of markers is on-going especially to place markers on bins that have no representative markers.

- Leaf samples were collected and DNAs were extracted on 425 F5 lines in 2013 DS. Extracted DNA of 425 F5 lines were PCR assayed using RM3586. Two hundred eight (208) F5 lines homozygous to N22 were selected and established during 2013 WS (Figure 9). Phenotypic data, such as days to heading, pests and diseases observed, were gathered and recorded.

- Additional markers will be surveyed for polymorphism and polymorphic markers located in the unfilled bins will be used in the population.

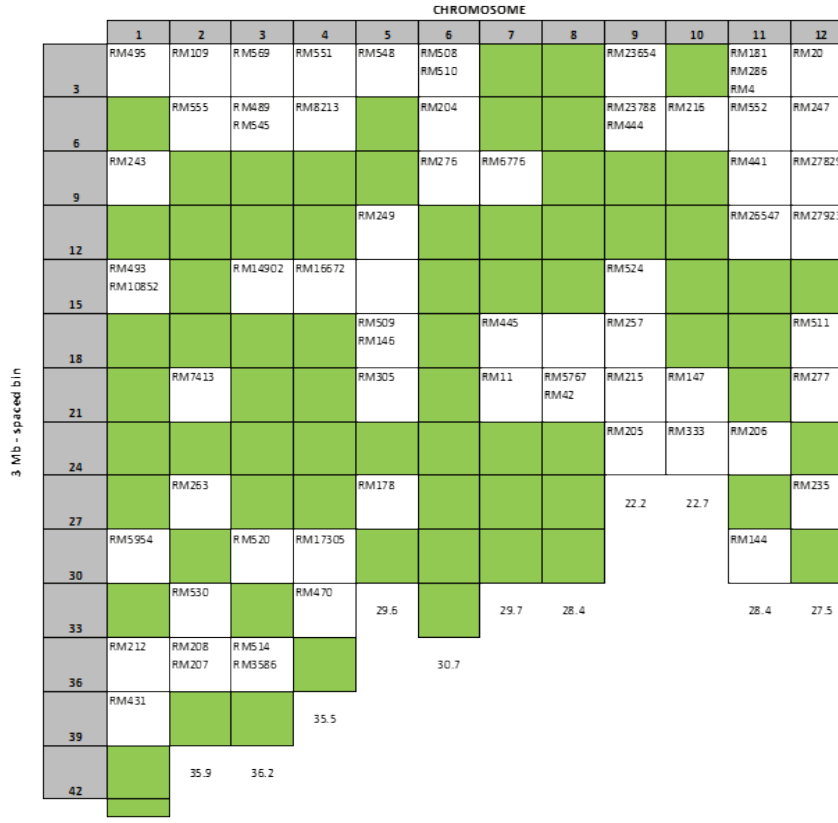


Figure 8. Distribution of 66 polymorphic SSR markers used in 200 F2 population of heat tolerance.

Table 8. Putative QTLs identified for heat tolerance trait.

QTL	CHR	Peak marker	Flanking markers	[a]LOD	[b]PVE(%)
%Pollen	1	RM493	RM493-RM10852	3.7	4.3
sterility	2	RM263	RM6374-RM263	3.2	1.0
	3	RM569	RM569-RM338	10.1	51.1
	6	RM435	RM435-RM204	4.6	16.1
	9	RM257	RM257-RM215	3.4	2.9
	10	RM216	RM216-RM147	6.8	44.7

[a] Threshold LOD score established at p=0.05 significance level.
[b] Percentage explained in the phenotypic variance.

Figure 9. Selected F6 breeding lines of heat tolerance established in the field.

Molecular genetics of crack resistance in rice and developing marker system for MAB

LM Perez, TE Mananghaya, AP Tuaño, VG Dalusong, IG Pacada, TF Padolina, and BO Juliano

One of the most important qualities of milled rice is the percentage of whole or unbroken rice. Whole rice kernels have two to three times more market value than broken rice grains, which means that any reduction in milling yield results in financial losses for both rice producers and millers (Pinson et al 2010). Introgression of fissure resistance to high yielding varieties will improve head rice recovery of the improved line/cultivar. This study aimed to characterize fissure resistance in PSB Rc38 using molecular genetics approach and identify linked or associated DNA markers that can be used in marker-assisted breeding (MAB) to improve fissure resistance in Philippine rice varieties.

Highlights:

Phenotyping

- 280 F2 plants of the cross PSB Rc38 and NSIC Rc160 and 300 F2 plants of the cross PSB Rc52 and NSIC Rc160 were screened for crack resistance using the Rough Rice Soaking Method at Rice Chemistry and Quality Laboratory, PhilRice-Los Baños).

- For PSB Rc38 x NSIC Rc160, 1 F2 plant has % stressed head rice yield value equal to that of PSB Rc38 (crack resistance

donor) and 27 with higher % stressed head rice yield were determined. Figure 10 shows the frequency distribution of the % stressed head rice of 280 F2 plants of the cross PSB Rc38 and NSIC Rc160.

- For PSB Rc52 x NSIC Rc160, 5 F2 plants has % stressed head rice yield equal to PSB Rc52 (crack resistance donor) and 58 F2 plants with higher % stressed head rice yield value. Figure 11 shows the frequency distribution of the % stressed head rice of 300 F2 plants of the cross PSB Rc52 and NSIC Rc160.

Genotyping

- As of 2014 February, genotyping of 280 F2 plants were done on 30 of 61 polymorphic SSR markers identified between PSB Rc38 and NSIC Rc160, and 18 of 65 polymorphic SSR markers identified between PSB Rc52 and NSIC Rc160.

- Target date of completion of genotyping activity is March 2014.

- Linkage and QTL analysis will be conducted once genotyping activity is completed.

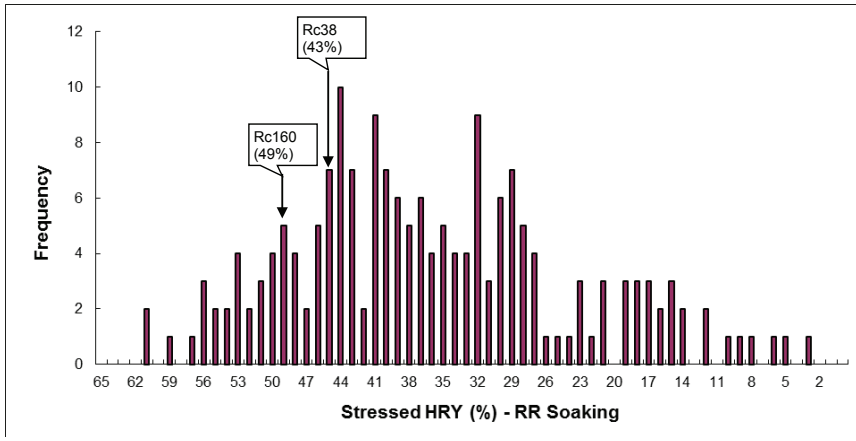


Figure 10. Frequency distribution of stressed head rice (HRY in %) using the Rough Rice (RR) Soaking method of 280 PSB Rc38xNSIC Rc160 F2 plants.

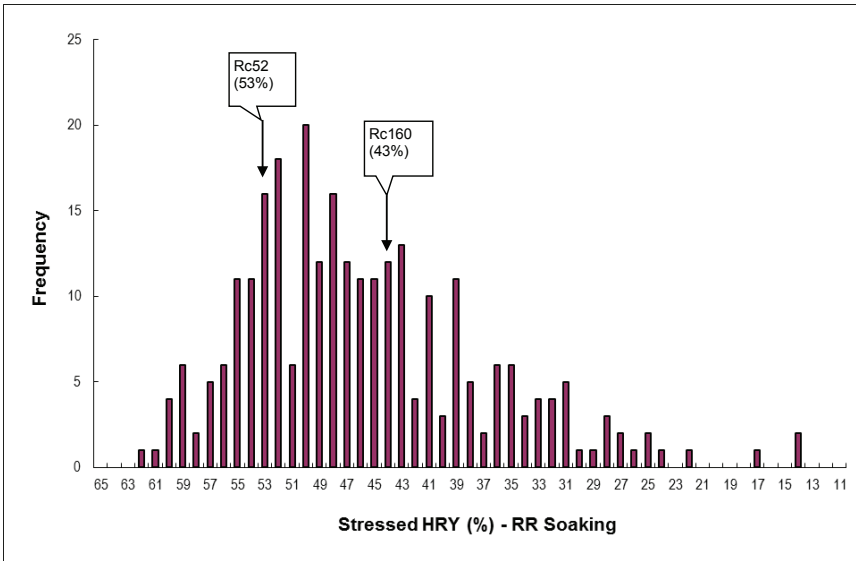


Figure 11. Frequency distribution of stressed head rice (HRY in %) using the Rough Rice (RR) Soaking method of 300 PSB Rc52xNSIC Rc160 F2 plants.

Establishment of marker system for low phytic acid (LPA) in brown rice for improved mineral bioavailability

LM Perez, TE Mananghaya, VG Dalausong, AP Tuaño, IG Pacada, TF Padolina, and BO Juliano

Phytic acid (myo-inositol 1, 2, 3, 4, 5, 6-hexakisphosphate (IP6) or phytate/phytin when in salt form), is the main storage form of phosphorous in various plant tissues, particularly in the bran and seeds. Phytic acid is strongly negatively charged over a wide pH range, thus, there is high possibility of binding with positively charged molecules including the important minerals like Ca, Zn, Fe, Mg, and many others. Phytic acid is also known as 'anti-nutrient' because it blocks the absorption of certain minerals into our body. In brown rice, phytic acid is mostly found in the aleurone layer and germ (embryo) in the form of phytic acid bodies or globoids. Since phytic acid is associated in normal seed germination process, lowering its content may become lethal to the rice seed because it has direct effect on the seedling growth.

Two lpa mutants, Os-lpa-Minghui 86 or Os-lpa-MH86 and Os-lpa-Xiequingzhao or Os-lpa-XQZ, its wild type, and associated molecular markers were obtained from Zhejiang University, China through Prof. Qing-Yao Shu. In this study, our goal is to conduct initial investigation of lpa mutants and evaluate its link molecular markers to detect lpa genes and determine the possibility of developing lpa rice cultivars. It also aims to

conduct molecular genetic studies of lpa, and establish marker system that can be used in marker-assisted breeding approach for developing lpa brown rice in the Philippine.

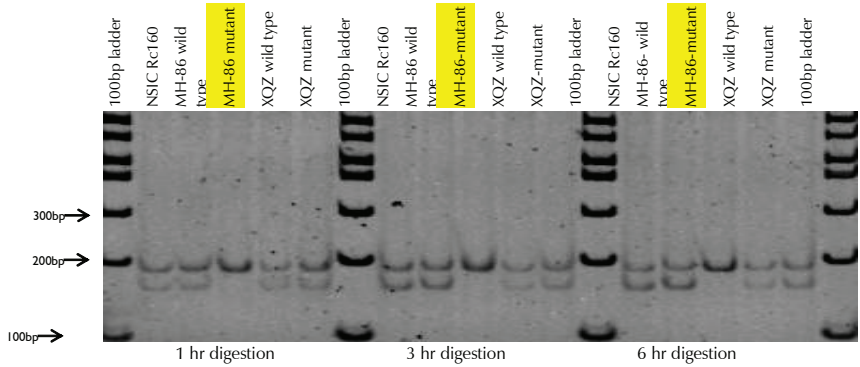
Highlights:

- Optimization of MHd_CAPS marker and EcoRII restriction enzyme for OS-lpa-MH86 gene was successfully carried out. Distinct amplification of PCR products of wild type and mutant plant was observed using different digestion time. However, the information of DNA fragments provided by Prof. Shu (Unpublished) was different to the expected DNA fragments size for wild type and mutant (Figure 12). Allele detection for OS-lpa-MH86 can be verified when the DNA of BC progeny and F2 plants from the OS-lpa-MH86 and NSIC Rc160 crosses will be analyzed.

- In general, the link marker for OS-lpa XQZ gene can now be used in MAS for developing lpa brown rice.

- From 106 BC2F1 seeds generated from the OS-lpa-XQZ and NSIC Rc160 crosses, only five germinated using culture media. The BC1F1 source of these germinated seeds has ~0.30μg inorganic phosphate content. However, when the DNA of BC2F1 plants were validated using link marker, no OS-lpa-XQZ alleles were detected. This can be possible because lpa is a recessive gene and the genotyping for the lpa gene should be done in BC2F1 generation not in BC1F1.

- Another breeding population of OS-lpa XQZ and NSIC Rc160 were developed. Out of 203 F2 seeds, only 79 exhibited low phytic acid with inorganic phosphate content that ranged from ~0.30 to 2.31μg. These 79 seeds were pre-germinated and only 15 successfully germinated and grown. These 15 plants were validated using the optimized InDel marker for OS-lpa XQZ gene. Results showed that four plants exhibited heterozygous alleles that correspond to OS-lpa-XQZ gene and its parent, NSIC Rc160. Further evaluation will be carried out for F3 population to obtain OS-lpa-XQZ homozygous alleles.



*Note: Mhd_CAPS primer sequence from Prof. Shu (unpublished)

Figure 12. DNA fragment size of OS-lpa-MH86, recurrent parent NSIC Rc160, MH-86 wild-type and mutant in Mhd_CAPS marker digested using EcoRII restriction enzyme.

Establishment of marker system and marker-assisted selection (MAS) for blast resistance in irrigated lowlands

LM Perez, MER Fabreag, TE Mananghaya, JT Niones, and AA Alfonso

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 much attention. Through the years, many blast resistant 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. Pyramiding or stacking of identified blast resistance genes (Piz, Piz-5, Pish, Pi9, Pi40 and SHZ-2 QTLs) into high-yielding rice varieties using DNA based markers will accelerate the development of rice cultivars with broad spectrum resistance to blast.

Highlights:

- One of the most useful breeding strategies for controlling diseases is to focus on the pyramiding of different resistance genes into a single elite cultivar through marker-assisted selection (MAS). Evaluation and validation of tightly linked, functional and DNA based markers were conducted initially. The three markers identified useful for marker-assisted selection (MAS), z565962 for Piz, pBA14 for Pi9, and 9871. T7E2b/MluC1 for Pi40 will be used in detecting blast resistance genes in the progenies of the developed cross combinations. In BC2F1 of NSIC Rc152/IRBL9W, plants showed heterozygous

bands using pbA14, SCAR marker, were preceded to BC3 (Figure 13). BC3F2 segregating populations of NSIC Rc152/ IRBL9W, NSIC Rc226/SHZ-2, and PSB Rc82/IRBL9W were established in blast nursery constructed in BSU, La Trinidad, Benguet for blast resistance evaluation (Table 9).

- The new cross combinations generated using five high-yielding irrigated lowland rice cultivars (NSIC Rc160, NSIC Rc224, NSIC Rc226, NSIC Rc238, and NSIC Rc240) and selected blast resistance gene donors (Table 10). The F1 plants with the same recipient background will be used in double cross combinations to pyramid two or more blast resistance genes. The BC1F1 plants generated will be established and tagged individually for foreground genotyping using pBA14 for Pi9 and z565962 for Piz gene. For utilization of Pi40 gene, MluC1, a restriction enzyme will be used in replaced to Hinf1 for DNA analysis of progenies developed with Pi40 gene donor.

Table 9. Advanced lines generated for introgression of blast resistance genes into irrigated lowland rice cultivars.

Parentage	Generation	Gene present
NSIC Rc154/RBLz5-CA	BC_3F_2	Piz5
NSIC Rc152/IRBL9W	BC_3F_2	Pi9
NSIC Rc226/SHZ-2	BC_3F_2	QTL
PSB Rc82/IRBL9W	BC_3F_2	Pi9
NSIC Rc218/IR65482-4-136-2-2	BC_3F_1	*Pi40*
NSIC Rc152/IR65482-4-136-2-2	BC_2F_1	*Pi40*
NSIC Rc160/IR65482-4-136-2-2	BC_2F_1	*Pi40*
NSIC Rc128/IR65482-4-136-2-2	BC_2F_1	*Pi40*
NSIC Rc158/IR65482-4-136-2-2	BC_2F_1	*Pi40*
NSIC Rc148/IRBLzFu	BC_2F_1	Piz
NSIC Rc218/IRBLzFu	BC_2F_1	Piz
NSIC Rc226/SHZ-2	BLF_4	QTL

Table 10. List of new cross combinations generated in pyramiding of blast resistance genes into elite and high yielding irrigated lowland rice cultivars.

Parentage	Generation	Gene present
NSIC Rc160/SHZ-2	BC_1F_1	QTL, Pi9
NSIC Rc160/IRBL9W	BC_1F_1	Pi9, Pi40
NSIC Rc160/IRBLsh-S (CO)	BC_1F_1	Pish, Pi40
NSIC Rc160/IRBLz5-CA(CO)	BC_1F_1	Piz5,QTL
NSIC Rc160/IR65482-4-136-2-2	BC_1F_1	Pi40,QTL
NSIC Rc224/IRBL9W	BC_1F_1	Pi9,QTL
NSIC Rc224/IRBLsh-S (CO)	BC_1F_1	Pi9,Piz5
NSIC Rc224/IR65482-4-136-2-2	BC_1F_1	Pi40,QTL
NSIC Rc224/IRBLz5-CA(CO)	BC_1F_1	Piz5,QTL
NSIC Rc224/SHZ-2	BC_1F_1	QTL, Pish
NSIC Rc226/IRBLz5-CA(CO)	BC_1F_1	Piz5,QTL
NSIC Rc226/IRBL9W	BC_1F_1	Pi9, QTL
NSIC Rc226/IRBLsh-S (CO)	BC_1F_1	Pish, Pi40
NSIC Rc226/IR65482-4-136-2-2	BC_1F_1	Pi40,QTL
NSIC Rc226/SHZ-2	BC_1F_1	QTL,Pi9
NSIC Rc238/IR65482-4-136-2-2	BC_1F_1	Pi40,QTL
NSIC Rc240/IR65482-4-136-2-2	BC_1F_1	Pi40,QTL
NSIC Rc240/IRBLz5-CA(CO)	BC_1F_1	Piz5,QTL
NSIC Rc240/SHZ-2	BC_1F_1	QTL, Piz5
NSIC Rc240/IRBL9W	BC_1F_1	Pi9, Pish
NSIC Rc240/IRBLsh-S (CO)	BC_1F_1	Pish, Pi40

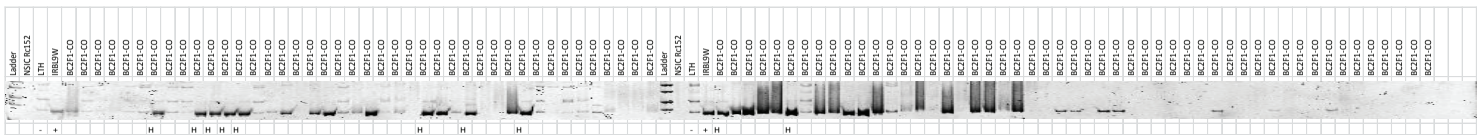


Figure 13. Foreground genotyping of BC2F1 plants of NSIC Rc152/IRBL9W using pBA14 marker for detection of Piz allele.

Molecular genetics and transfer of tungro resistance from ARC11554 to modern varieties

DA Tabanao, FP Waing, TC Fernando, and GO Romero

One of the major constraints in rice production is rice tungro disease (RTD). Its severe occurrence may lead to very high yield losses. This viral disease is difficult to forecast and control, and the use of host plant resistance remains the best control measure. Deployment of resistant varieties in the field is the most efficient and reliable method to combat the disease. The use of marker-assisted selection (MAS) in breeding can enhance the efficiency of selecting breeding lines with RTD resistance.

The rice tungro spherical virus (RTSV) resistance present in the Indian landrace ARC11554 had been mapped in the short arm of chromosome 4 (Sebastian et. al, 1996; Romero et. al. 2008). The resistance QTL linked to the RM8213 locus (10.7 cM) is corroborated in three resistant backcross lines, derived with phenotypic selection, from the ARC11554 x IR64 cross. Furthermore, initial analysis in diverse genetic backgrounds also showed decreasing tungro susceptibility as the ARC11554 allele dosage increased. In 2010, the identification of recombinant lines for fine-mapping was completed and the transfer of the resistance QTL into modern varieties was continued.

This study aimed to introgress tungro resistance into high- yielding but disease- susceptible commercial cultivars through marker-assisted breeding, to characterize the tungro resistance derived from ARC11554 through phenotyping and genotyping, and to develop a large population to fine-map the tungro resistance QTL.

Highlights:

- Fifty-six SSR markers in chromosome 4 were used to survey for nucleotide polymorphism among introgression lines together with their parents. Out of 56 SSRs, 12 SSRs were found polymorphic between the donor (ARC11554) and the recurrent parent (NSIC Rc138). These polymorphic markers were used to evaluate the haplotypes of 55 advanced introgression lines. Out of 55 advanced introgression lines, 22 lines (11 BC2F6 and 11BC4F4) were selected based on their individual haplotypes that most closely resembled ARC11554 in the resistance introgression region (flanked by RM8213 and RM3471). The extent of the introgressed region in chromosome 4 in advanced introgression lines are graphically displayed the consistent introgression of resistance QTL region is shown in Figure 14. Introgression lines were also evaluated to determine the similarity of the resistance of ARC11554 to the reported gene (tsv1) governing RTSV resistance mapped

from Utri merah (Indonesian cultivar) around 22.1 Mb of rice chromosome 7. However, no polymorphism was found between NSIC Rc138 and ARC11554 from the 10 SSR markers surveyed near and around the tsv1 region. This result suggests that there was no genome introgression from the ARC11554 in the introgression lines.

- The 22 lines selected based on haplotype analysis were further studied for whole-genome genotyping in order to determine the percentage recovery of NSIC Rc138 genome. A total of 271 SSR markers distributed across the rice genome were surveyed for polymorphism between NSIC Rc138 and ARC11554 (Table 11). Of the 271 SSR markers used, 108 were identified polymorphic. These markers were used for whole-genome fingerprinting of 22 selected advanced introgression lines (11 BC2F6 and 11 BC4F4). Genetic similarity was calculated using the simple matching coefficient in NTSYSpc 2.1 (Exeter Software, Setauket, NY) to determine the percentage similarity to the recurrent parent, NSIC Rc138. Marker similarity of introgression lines ranged from 81 to 96% in BC2F6 and 89 to 99% in BC4F4 (Fig. 15). Among introgression lines, ARC138-4-5-5-2-30 was selected as donor line in the generation of mapping population. The donor line has 96% percent genetic similarity to the recurrent parent. All the F1 plants derived from the cross between NSIC Rc138 and ARC138-4-5-5-2-30 were tested for heterozygosity. The true F1 plants were were self-pollinated and used as seed source of the F2 mapping population.

- Responses of the 1,014 F3 lines along with the parents NSIC Rc138 and ARC138-4-5-5-2-30 were assayed directly based on the antibiosis score, damage scale and disease index. The donor parent, ARC138-4-5-5-2-30, was found resistant to GLH and moderately resistant to tungro while the NSIC Rc138 was GLH and tungro susceptible. All the F3 lines were classified into three categories based on resistance scores as resistant, segregating and susceptible. The corresponding F2 plants were genotyped as “AA” (homozygous susceptible), “Aa” (segregating heterozygous) and “aa” (homozygous resistant). In Table 12, the segregation in the F2 population showed a good fit to the expected ratio of 1 AA:2 Aa:1 aa. Scores of F3 lines evaluated for test- tube antibiosis showed a continuous distribution, the arcsine transformed data ranged from a low value of 0.52 to a high value of 1.54 (Figure 16A). Among F3 lines screened for resistance, the percentage of resistant progeny was 71% of total number of progeny. Furthermore, damage rating ranged

from 0 to 9 and showed continuous distribution (Figure 16B). Recorded mean DI based on SES on rice was 3.88±2.51 with a variance of 6.35. X^2 and values were 0.987 in antibiosis test and 0.73 in damage rating. Both were not significant at 5% significance level. Chi-square goodness of fit showed that the pattern of resistance in the segregating F3 lines was observed in the ratio 1R:2MR:1S. Sebastian et al. (1996) reported that this kind of observation suggested that a single dominant gene confers GLH resistance in ARC11554. Visual scoring was used in the evaluation of disease severity in F3 lines. Based on the disease index (DI) values, ARC138-4-5-5-2-30 was classified as moderately tolerant and NSIC Rc138 as susceptible. Among F3 families evaluated, percentage of resistant progeny was 70% of total number of progeny (Figure 16C). Recorded mean DI based on SES on rice was 6.04±1.52 with a variance of 2.31.

- Two methods were employed in the detection of presence of tungro viruses. These techniques were serological (i.e., ELISA) and molecular (i.e., RT-PCR). ELISA was a tedious and laborious technique compared to RT-PCR wherein results were obtained in a day. However, in ELISA, inoculated plants can be classified as tolerant or susceptible based on the level of coat protein as measured through absorbance value at 405 nm (A405). This technique gave more definite assessment of virus infection in plants and has been very helpful in screening cultivars for their reaction to tungro virus (Hibino et al., 1990). Observed absorbance value in inoculated F3 families showed that 74.5% were classified as tolerant and 24.5% were infected with RTSV. On the other hand, in terms of RTBV coat protein level observed, F3 families were 42.7% tolerant and 57.3% infected. Results in this study coincide with the report of Sebastian et al. (1996), that ARC11554 was resistant to RTSV and with a low level of resistance to RTBV. Tungro virus specific bands were amplified in conventional PCR procedure and visualized in agarose gel electrophoresis from inoculated F3 families. In RTBV, 94.09% of total inoculated lines showed presence of specific bands (positive) and only 5.91% were found no amplicon showing absence of virus in inoculated plants (negative). On the other hand, presence of RTSV was 91.99% and 8.01% having no amplicon. Due to high sensitivity of RT-PCR technique, it can amplify even small fragments of tungro viruses present in inoculated plants. Thus, it cannot discriminate the susceptible from tolerant plants based on the presence of virus alone.

- A large population size was established and genotyped using 12 SSR markers. Phenotype and molecular data were analyzed using the newer version of mapping software. The advantage of using a larger population size in this study resulted to a higher resolution of QTL map. The putative QTL region was localized to only 0.5 kb compared to 2 Mb in previous literature (Romero et al., 2010). A genetic linkage map was constructed using genotype data in F2 population. In the F2 population, threshold LOD score was set at 5.0. QTL analysis was carried out using inclusive composite interval mapping (ICIM) with LOD scores ≥5.0. Graphical representation of QTLs located on the linkage map is shown in Figure 17 while list of putative QTL flanked by markers along with their LOD values, additive effects and phenotypic variance are presented in Table 13. Four QTLs were identified, two QTLs for RTSV resistance and two QTLs for GLH resistance. ICIM revealed that RTSV resistance is tightly linked with the RM335-RM16428 region with LOD = 39.0 and R2 = 17.43%, with a secondary peak in the RM16434-RM3471 region with LOD = 13.3 and R2 = 3.60% (Table 13). GLH resistance was tightly associated with the RM335-RM16428 region with LOD = 46.3 and R2 = 19.19%, and with a secondary peak in the RM16434-RM16497 region with LOD = 14.8 and R2 = 3.56%. The primary signal (LOD peak) of the QTL for RTSV and GLH resistance was located between RM16425 and RM16427 as depicted in the ICIM line graph (Figure 18). The second peak was located in the RM16466-RM3471 region with the size of 655 kb. Correlation analysis (SAS Institute Inc. 2012) using Pearson's coefficient was done to determine the relationship that exists between the two traits (GLH and RTD resistance). The correlation coefficient computed showed significant relationship ($r = 0.322$, $P < 0.0001$) which suggests that the two QTLs detected were controlled with two different genes sitting in the same chromosome region. Thus, the QTLs for both traits were tightly linked in above mentioned region.

- A total of 10 BC2F6 lines in NSIC Rc138 background were selected and evaluated in a replicated yield trial in 2013 DS. The grain yield of the backcross lines in NSIC Rc138 background ranged from 5.5 to 7.2 t/ha with an average of 6.5 t/ha which was lower than that of NSIC Rc138 (7.0 t/ha). Among the entries evaluated for three consecutive seasons (2012 DS, 2012 WS and 2013 DS) the backcross line ARC138-1-3-9-5 consistently performed in the top 20% with a maximum yield of 7.4 t/ha (Table 14).

Table 11. Simple Sequence Repeats markers with polymorphism between recurrent parent and donor parent.

CHROMOSOME	MARKERS	POLYMORPHIC MARKERS	POLYMORPHISM (%)
1	25	12	48.00
2	36	12	33.33
3	27	9	33.33
4	56	16	28.57
5	19	10	52.63
6	22	12	54.55
7	16	7	43.75
8	18	7	38.89
9	15	8	53.33
10	8	3	37.50
11	14	8	57.14
12	15	4	26.67
Total	271	108	39.85

Table 12. Marker segregation in the F2 mapping population.

MARKERS	POSITION (cM)	AA	%	Aa	%	aa	%	$??^{??}$	p-value
RM551	0.71	277	32.10	2	0.20	585	67.70	1077.10	0.00
RM335	2.75	276	29.00	482	50.60	194	20.40	14.20	0.00
RM518	8.12	235	25.50	469	50.90	218	23.60	0.84	0.36
RM16425	17.42	250	26.70	458	48.80	230	24.50	1.46	0.23
RM16427	17.42	240	26.80	427	47.70	228	25.50	2.29	0.01
RM16428	17.66	225	24.10	435	46.50	275	29.40	10.13	0.00
RM8213	17.77	223	24.80	444	49.40	232	25.80	0.37	0.54
RM16434	18.68	223	25.80	382	44.20	260	30.10	15.39	0.00
RM16466	22.62	253	26.30	484	50.30	226	23.50	1.52	0.22
RM3471	25.24	158	22.50	341	48.60	203	28.90	6.17	0.01
RM16497	26.29	239	25.40	469	49.90	232	24.70	0.12	0.73
RM16575	40.08	0	0.00	0	0.00	971	100.00	2919.00	0.00

A- denotes allele of NSIC Rc138
a- denotes allele of ARC138-4-5-5-2-30

Table 13. Putative QTLs for GLH and RTSV in F2:3 population derived from NSIC Rc138 and ARC138-4-5-5-2-30.

QTL	FLANKING MARKERS	PEAK MARKERS	PEAK LOD	ADDITIVE EFFECT	PVE (%)
qRTV4.1	RM335-RM16428	RM16425-RM16427	39.04	0.79	17.43
qRTV4.2	RM16434-3471	RM16466-RM3471	13.26	0.39	3.60
qGLH4.1	RM335-RM16428	RM16425-RM16427	46.30	0.15	19.19
qGLH4.2	RM16434-RM16497	RM16466-RM3471	14.82	0.07	3.56

Table 14. Yield performance of selected backcross lines in NSIC Rc138 background in replicated yield trial.

Designation	Cross combination	Yield (t/ha)		
		2012 DS	2012 WS	2013 DS
ARC138-1-3-9-5	ARC11554/3*NSIC Rc138	7.4	6.0	7.2
ARC138-1-3-3-3	ARC11554/3*NSIC Rc138	7.2	5.0	5.5
ARC138-1-3-3-4	ARC11554/3*NSIC Rc138	6.5	4.7	6.8
ARC138-4-5-7-4	ARC11554/3*NSIC Rc138	5.9	5.4	6.4
ARC138-4-5-7-3	ARC11554/3*NSIC Rc138	5.8	3.4	-
ARC138-1-3-3-1	ARC11554/3*NSIC Rc138	5.1	4.2	-
ARC138-4-5-7-1	ARC11554/3*NSIC Rc138	5.1	4.2	6.7
ARC138-4-5-7-2	ARC11554/3*NSIC Rc138	4.8	4.0	-
ARC138-1-3-3-5	ARC11554/3*NSIC Rc138	4.0	-	-
ARC138-4-1-1-1	ARC11554/3*NSIC Rc138	2.5	-	-
NSIC Rc138		8.8	5.0	7.0

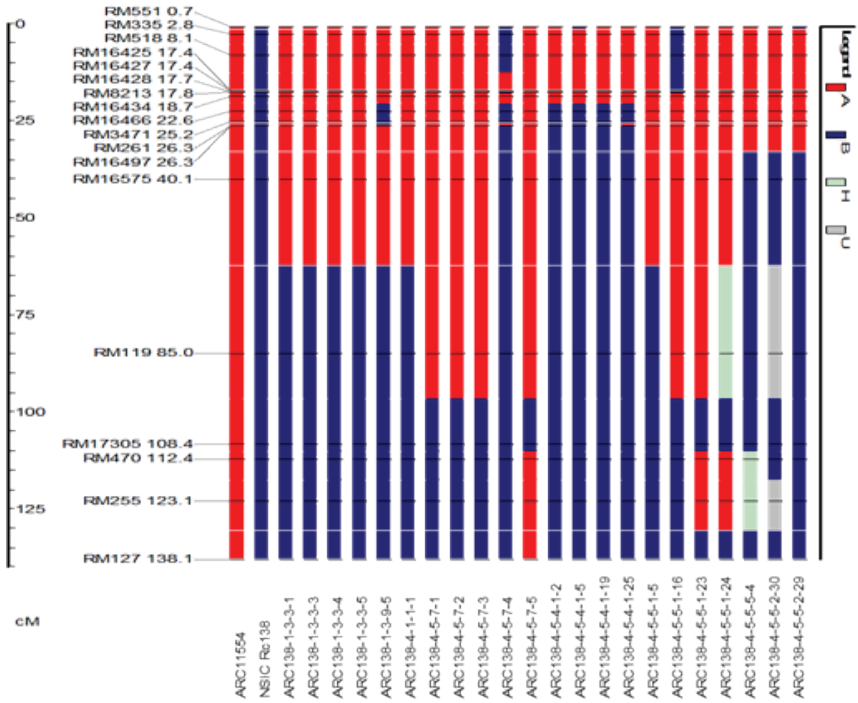


Figure 14. Graphical genotypes of 22 selected advanced introgression lines (11 BC2F6 and 11 BC4F4) in chromosome 4 together with the donor and recurrent parent lines.

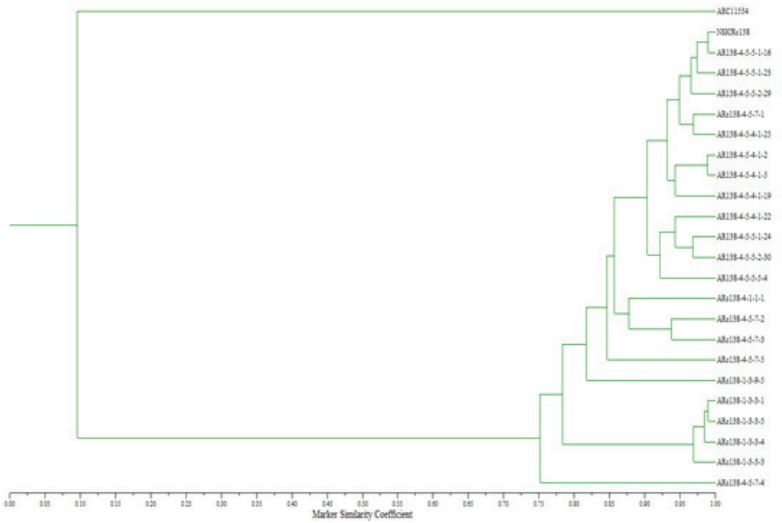
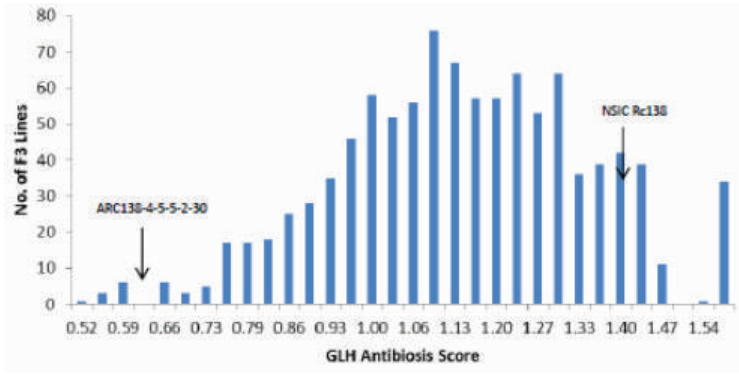
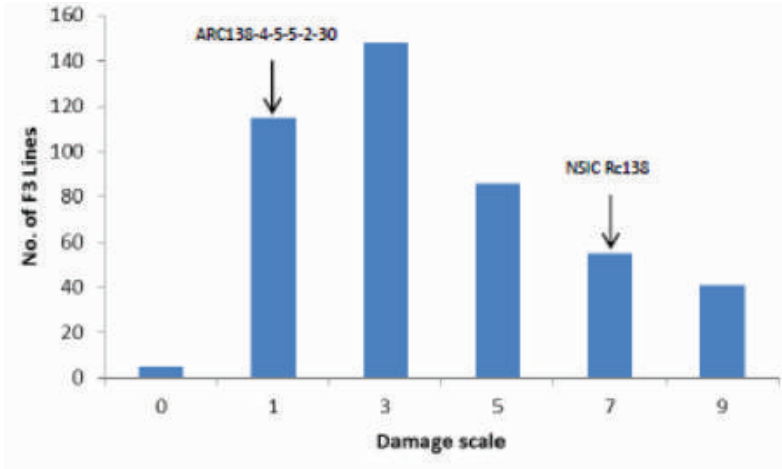


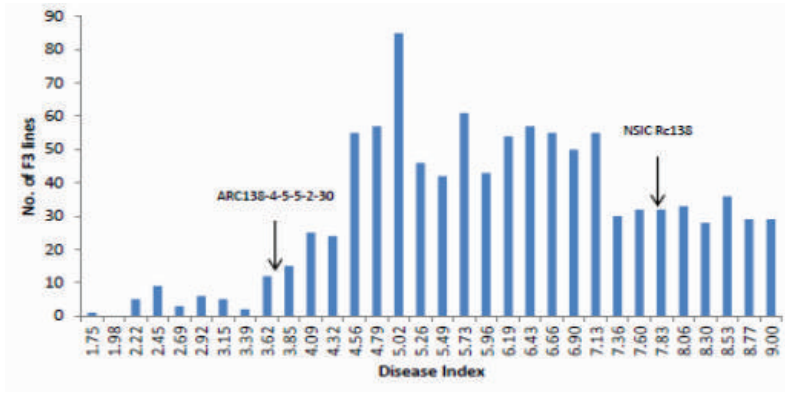
Figure 15. Unweighted pair group method with arithmetic mean (UPGMA) dendrogram of 24 rice genotypes genotyped with 108 polymorphic SSR markers based on simple matching similarity index.



A



B



C

Figure 16. Frequency distribution of F3 families (NSIC Rc138/ ARC138-4-5-5-2-30) in terms of: (A) GLH resistance based on test-tube antibiosis experiment (percent nymph survival is arcsine-transformed value), (B) compartment box method and (C) tungro resistance based on mass screening method.

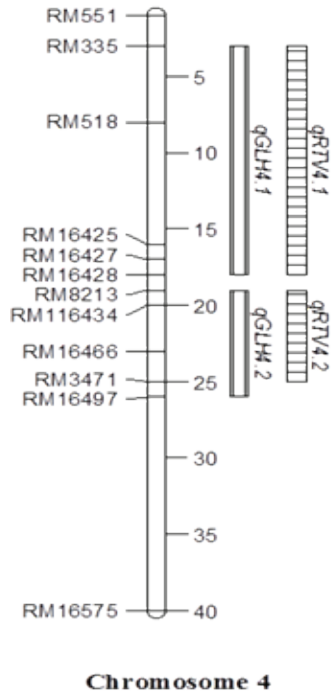


Figure 17. Linkage map showing the location of GLH and RTSV resistance on short arm of rice chromosome 4 using Mapchart 2.2 software (Voorrips, 2002). Marker names are listed on the left hand side of the chromosome with the QTLs indicated on the left.

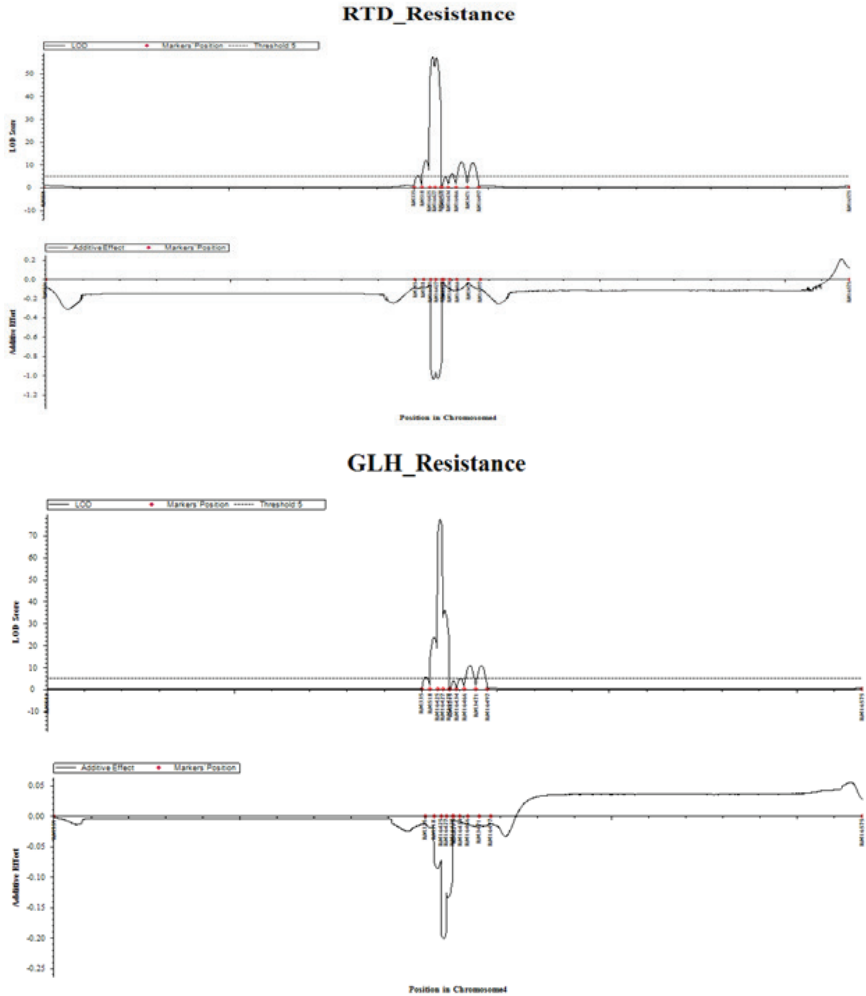


Figure 18. ICIM graph for QTLs detected for (A) RTSV resistance and (B) GLH resistance in the short arm of chromosome 4 in the F3 population derived from the cross between NSIC Rc138 and ARC138-4-5-5-2-30.

III. Marker-Assisted Line Development

Project Leader: DAATabanao

The main objective of this project was to develop inbred and hybrid parent lines with improved disease resistance through marker-assisted target gene selection and grain yield through genome-wide marker-assisted selection. Specifically, the objectives were: (1) to determine presence of target genes of tungro and bacterial blight resistance in inbred and hybrid parent lines using functional or linked molecular markers; 2) to assess disease resistance, morpho-agronomic traits, and recurrent parent genome recovery of breeding lines undergoing marker-assisted selection; (3) to establish a genome-wide selection approach in enhancing the yield level of rice through best linear unbiased prediction using phenotypic data and genome-wide marker effects; and (4) to further improve the tungro resistance of promising breeding lines for irrigated lowland breeding and direct seeding by introgression of both GLH resistant allele and tsv1 gene from ARC11554.

Marker-assisted selection for disease resistance genes in inbred variety development

DA Tabanao, FP Waing, EP Rico, Jr., MJC Duque, RC Braceros, and TF Padolina

This study aimed to: (1) incorporate resistance genes to bacterial blight and tungro in the breeding lines of inbred variety development by marker-assisted selection, (2) develop a common donor for disease resistance, (3) assess recurrent parent genome recovery, morpho-agronomic traits and disease resistance of backcross lines, and (4) assess applicability of published molecular markers for resistance genes Xa7, xa13, and tsv1 for marker-assisted selection in line development.

Highlights:

- A total of sixteen elite lines with at least two Xa genes were elevated to stage 1 of multi-environment trial (MET 1) in 2012 dry season and wet season. From these elite lines, five entries were elevated to stage 2 of multi-environment test (MET 2) in 2013 DS. In addition, nine elite lines with at least two Xa genes were elevated to Stage 1 of Multi-Environment Test (MET 1) in dry season. The summary data of genotype and morpho-agronomic traits in elite lines are presented in Table 15. The yield data is ranging from 3829 kg/ha to 7029 kg/ha. Based on the genotype and field data of the elite lines, 10 lines were nominated and elevated to preliminary multi-environment test (MET 0) in 2013 DS. In addition, pedigree nursery populations and elite lines were also established in the field and assayed with markers for target genes. They were comprised of 15 populations in F2 (total of 1755 plants), and 356 F6 and 157 elite lines. DNA extraction and target gene

assay for Xa4, xa5, Xa7 and Xa21 were done. The different gene combinations present in various generations are listed in Table 15.

- Field establishment of various pedigree nursery populations was done. These were comprised of: three populations in F5 generation denoted as B002, B007 and B009 and three populations in F3 generation denoted as B018, B019 and B020. The F3 populations were assayed for the presence of resistance genes (Pi40 in B018 and Xa23 in B019 and B020). In F5 populations, line selection was carried out based on phenotypic acceptability, while presence of target gene was the basis of selection in F3 populations for generation advance. The number of selected lines is shown in Table 16.

- One F4 population denoted as B013 and three F3 populations denoted as B015, B016 and B016 were screened for tungro disease resistance following the mass screening method. Plants rated as resistant were transplanted in the field. PCR assay was done to evaluate for the presence of resistance QTL using flanking markers. Plant selection was carried out based on genotype data. Segregating populations with introgressed resistance QTL (tsv1, rtv and GLH) for tungro were established in PhilRice branch stations (Isabela and Negros) in 2013WS. These were comprised of: one population in F5 generation denoted as B013, three populations in F4 populations denoted as B015, B016 and B017, and three populations in F3 populations denoted as H022, H024 and H025. The reaction of these populations under natural infection of tungro disease was observed and scored based on SES on rice. Resistant lines were selected for generation advance and for further disease screening. These selected lines will be evaluated through PCR assay to verify the presence of resistance QTL using flanking markers in 2014 DS.

- The F1 plants produced in 2012 WS through backcrossing to increase the recovery of the recurrent parent genome were planted in the field in 2013 DS. The backcross derived plants with introgressed Xa4, Xa7 and Xa21 were denoted as B028 (IRBB62/3*NSIC Rc160). A complex cross was done between B021 (IRBB52/NSIC Rc160//IRBB54/NSIC Rc160) with Xa4, xa5 and Xa21, and B022 (IRBB55/NSIC Rc160//IRBB54/ NSIC Rc160) with xa5 and xa13. This complex crossed was done to pyramid more than two Xa genes and denoted as B027. The F1 plants were subjected to target gene assay prior to backcrossing while the complex cross plants were self-

pollinated for generation advance in 2013 WS. A DNA marker, 9871.T7E2b, which was identified to be linked to the Pi40 gene, was used to evaluate the presence of blast resistance gene in F1 plants from the cross NSIC Rc160/IR65482-4-136-2-2 (denoted as H026). An endonuclease, HinfI, was used to detect polymorphism between parental lines. Two recently released varieties, NSIC Rc238 and NSIC Rc240, were crossed to IR65482-4-136-2-2 (Pi40). The F1 plants were established in the field and subjected to target gene assay in 2013 WS.

- Completion report of the DOST-BC Ref. No. 2008-0234 "Marker- assisted pyramiding of bacterial blight and tungro resistance genes from transgenic and landrace donors" was submitted to the DOST Biosafety Committee.

Table 15. Different gene combinations confirmed in various generations in 2013 DS. Lines with at least two genes were selected for generation advance.

	Gene combinations										
Generation	***Xa4, xa5***	***Xa4, Xa7***	***Xa4, Xa21***	***xa5, Xa7***	***xa5, Xa21***	***Xa7, Xa21***	***Xa4, xa5, Xa7***	***Xa4, xa5, Xa21***	***Xa4, Xa7, Xa21***	***xa5,Xa7, Xa21***	***Xa4,xa5,Xa7, Xa21***
HPSI 1	4	29	1	1	1	7	7	3	38	1	21
HPSI 2	4	16	9	7	6	0	13	2	20	8	20
HPSI 3	0	13	5	11	0	21	6	0	0	9	11
HPSI 4	11	4	10	4	3	4	5	16	2	2	4
HPSI 5	19	0	17	1	8	1	0	17	1	2	2
HPSI 6	7	25	6	6	5	7	21	4	11	3	13
HPSI 7	7	6	9	5	2	7	5	10	21	5	18
HPSI 8	0	22	0	12	1	8	23	0	14	7	11
HPSI 9	0	21	2	0	0	0	25	2	26	0	36
HPSI 10	16	14	9	0	0	0	30	10	7	0	7
HPSI 11	3	19	2	0	0	0	26	6	28	0	27
HPSI 12	23	0	28	0	8	0	0	35	0	0	0
HPSI 13	4	29	4	0	0	0	12	8	8	0	31
HPSI 14	4	3	14	0	0	0	4	41	7	0	10
HPSI 15	1	17	5	3	4	8	13	3	19	13	24
F_6	85	16	65	15	60	9	9	34	6	4	4
Elite	147	148	153	111	116	117	203	208	209	172	264

Table 16. Total number of selections in some of the IMAS populations in 2013 DS.

Generation	Population Designation	Cross combination	No. of Selections
TWF_5	PR40843-B002	IR BB54/Matatag 6/NSIC Rc160	15
F_5	PR40595-B007	PSB Rc14/TW16	21
F_5	PR40596-B009	NSIC Rc160/TW16	33
F_4	PR40598-B013	TW16/NSIC Rc160	52
F_3	PR40599-B015	NSIC Rc222/TW16	17
F_3	PR40600-B016	NSIC Rc216/MATATAG 6	51
F_3	PR40601-B017	NSIC Rc216/TW16	3
F_3	PR40602-B018	IR65482-4-136-2-2/NSIC Rc222	52
F_3	PR40603-B019	IRBB23/NSIC Rc160	51
F_3	PR40604-B020	IRBB23/NSIC Rc222	46
BC_1F_3	PR40597-B011	B002/NSIC Rc160	18
BC_1F_2	PR40618-B024	B006/NSIC Rc160	18
DCF_2	PR40619-B025	B009/B004	64
DCF_2	PR40616-B021	B003/B004	27
DCF_2	PR40617-B022	B005/B004	25

Marker-assisted selection for disease resistance in hybrid variety development

DA Tabanao, FP Waing, EP Rico, Jr., and NRL Sevilla

Hybrid rice is currently being grown in the Philippines and is still gaining significance given its yield advantage over inbred cultivars. The public hybrids Mestizo 1 and Mestiso 3 are among the most promising hybrid cultivars so the improvement of their parental lines is given high priority. However, prevalence of bacterial leaf blight and tungro viruses, RTBV (rice tungro bacilliform virus) and RTSV (rice tungro spherical virus), had continually threatened the su0ccess of hybrid rice technology. The bacterial blight disease is caused by the pathogen Xanthomonas oryzae pv. oryzae while tungro virus is transmitted by the green leafhopper (Nephotettix virescens). These diseases cause severe damage to the crop often leading to a significant yield loss, and breeding for varieties with durable disease resistance provides the best management option. With the advent of molecular marker technology, introgression of two or more resistance genes to susceptible cultivars has become possible. Likewise, it hastens the process of morpho-agronomic characterization and selection.

This study aimed to pyramid bacterial blight and tungro resistance genes in hybrid parent lines through marker-assisted selection, to determine which disease-resistant lines are most genetically similar to recurrent parents by SSR genome wide genotyping, and to perform yield of improved parent lines and hybrids. IRBB62, donor parent of the bacterial blight resistance genes Xa4, Xa7 and Xa21, was crossed to maintainer and restorer parent lines. IRBB54 was used as source of xa5. Improved hybrids were crossed to PJ25-ARC lines to combine tungro and bacterial blight resistance genes.

Functional markers MP (Xa4), M5 (Xa7), and U1I1 (Xa21) were used to select lines with 2-3 Xa genes; linked SSR marker RM8213 was used to detect the GLH resistance QTL from ARC11554; and, flanking markers (RM21800 and RM336) were used to detect the tsv1 tungro resistance gene from Utri Merah.

Highlights:

- Backcross-derived maintainer and restorer lines of Mestizo 1 and Mestiso 3 were established in the field, and morpho-agronomic data such as plant height, panicle number, and grain yield were gathered (Table 17). These lines were also screened for the presence of bacterial blight resistance genes. The numbers of lines were as follows: 110 maintainer and 6 restorer lines of Mestizo 1, and 57 maintainer and 4 restorer lines of Mestiso 3. These maintainer and restorer lines are now being used in hybrid rice breeding program. From these lines, selected improved lines were used for testcrossing to evaluate their maintaining and restoring ability. Selected maintainer lines were used in testcrossing to evaluate combining ability. A total of 23 cross combinations were generated. The F1 seeds were integrated in the hybrid breeding.

- Original and improved CMS lines of Mestizo 1 and Mestiso 3 were established in the field and crossed to their corresponding improved maintainer lines for A□B seed production and to their corresponding restorer lines for A□R for seed production of improved hybrids with bacterial blight resistance (Table 18 and Figure 19). Pollen sterility of the improved CMS lines was evaluated. Percent pollen sterility of the improved CMS lines ranged from 96.84 to 99.75% (Table 18). The improved Mestiso 3 hybrids seed produced in 2012 DS and WS were evaluated in replicated yield trials at PhilRice CES (2012 WS and 2013 DS) and in Magsaysay, Davao del Sur (2012 WS, Table 19). The grain yield of improved Mestiso 3 ranged from 4.37 to 5.34 t/ha and performed better over the original Mestiso 3 with 4.36 t/ha in 2012 WS. In addition, from these cross combinations two improved F1 hybrids were also evaluated in Magsaysay, Davao del Sur. The grain yield of the two improved hybrids were 10.7 t/ha in M3BB2-57-10A/19R76 and 12.7 t/ha in M3BB4-2-39-3A/19R52 while in original Mestiso 3 was 10.8t/ha. The improved hybrid M3BB4-2-39-3A/19R52 performed very well in Davao del Sur and had recorded a yield advantage of 17.6% over the original Mestiso 3. Moreover, improved Mestiso 3 hybrids seed produced in 2012 WS were evaluated in a replicated yield trial in 2013 DS. The average grain yield of the improved F1 hybrids was 7.90 t/

ha and performed better over the local checks PSB Rc82 with 7.42 t/ha and PSB Rc18 with 7.40 t/ha.

- PR40638H, an improved Mestiso 3, was entered in the multi-location yield trial (MYT) at PhilRice CES (Figure 20) and PhilRice Isabela in 2013 DS. It performed well in both locations and obtained the third highest grain yield, with 7.37 t/ha at PhilRice CES and 5.65 t/ha at PhilRice Isabela, out of 20 entries evaluated. The improved Mestiso 3 parent lines (CMS, maintainer and restorer lines) are currently included in the pipeline of hybrid breeding. The improved Mestiso 3 F1 hybrid can be promising to combat the bacterial blight disease especially in wet season cropping.

- Field establishment of various pedigree nursery populations for hybrid parent line development with introgressed tungro and bacterial blight resistance were done. These were comprised of: one population in F6 generation denoted as H012 and six populations in F7 generation denoted as H001, H002, H003, H004, H006, and H007. Selection was also carried out based on PAcp. From these F7 populations in IR68897B background, two F7 populations (H001 and H002) were assayed for the presence of two Xa genes. Plants with both xa5 and Xa21 were used for testcrossing in 2013 DS. Eight cross combinations were generated. These F1 plants were included in the materials used in hybrid breeding and genetics project.

- Introgression of bacterial blight resistance genes in the parent lines of two line hybrid system was done in 2013. Two populations of TGMS parent lines in F2 generation denoted as H028 (PRUPTG101/IRBB54) and H029 (PRUPTG102/IRBB54) were established in the field at NEUST in Gabaldon for backcrossing. This was done to further increase the recovery of the recurrent parent genome. Prior to backcrossing, target gene assay was done to select plants that contained both xa5 and Xa21 gene. A total of 11 plants in H028 and 8 plants in H029 with both genes were selected and backcrossed. The BC1F1 seeds generated from various crosses will be planted in a male fertile environment in 2014 DS for another cycle of backcrossing.

- F1 plants with introgressed bacterial blight, tungro and insect resistance genes and QTLS were planted in the field in 2013 WS. F2 populations, each with 200 plants, were also established in the field. Leaf samples were collected separately from each F1 and F2 plants for DNA extraction and were

subjected to target gene assay. Published and functional markers for Xa genes (Xa4, xa5, xa13, Xa21 and Xa23), and SSR markers for tsv1 (RM5495 and RM336) and, rtv and Glh (RM16425 and RM16427) were used for heterozygosity test. The true F1 plants presented in Figure 21 were backcrossed to their recurrent parents. On the other hand, the F2 plants that were homozygous to the donor parent allele were selected for generation advance.

Table 17. Morpho-agronomic characteristics of backcross-derived parent lines of Mestizo 1 and Mestiso 3.

Cultivar	Designation	Cross Combination	No of Lines	Number of panicles	Plant height (cm)	Grain yield (t/ha)	*Xa* genes
Mestiso 1							
	IR58025B ORIG			9	100	-	
	25B-P-52	IR 58025 B/IRBB62	1	14	99	7.80	*Xa4*
	25B-P-65	IR 58025 B/IRBB62	1	14	103	4.89	*Xa4, Xa21*
	25B-P-71	IR 58025 B/IRBB62	1	12	97	5.88	*Xa4, Xa21*
	25B-P-96	IR 58025 B/IRBB62	1	10	114	6.59	*Xa4, Xa21*
	BB1-23	IR 58025 B/PR 39897-25 B-71	9	10-21	84-93	4.23-9.11	*Xa4, Xa21*
	BB1-29	IR 58025 B/PR 39897-25 B-71	8	14-21	76-94	5.52-8.31	*Xa4, Xa21*
	BB1-36	IR 58025 B/PR 39897-25 B-71	8	12-24	75-91	4.53-7.12	*Xa4, Xa21*
	BB1-37	IR 58025 B/PR 39897-25 B-71	1	-	-	4.89	*Xa4, Xa21*
	BB4-3	IR 58025 B/PR 39897-25 B-65	10	9-16	92-100	4.65-7.83	*Xa4, Xa21*
	BB4-6	IR 58025 B/PR 39897-25 B-65	12	10-24	80-92	4.64-8.42	*Xa4, Xa21*
	BB4-9	IR 58025 B/PR 39897-25 B-65	7	9-18	87-94	2.64-6.17	*Xa4, Xa21*
	BB4-15	IR 58025 B/PR 39897-25 B-65	3	10-21	96-97	3.85-4.70	*Xa4, Xa21*
	BB4-18	IR 58025 B/PR 39897-25 B-65	10	10-23	86-110	3.4-7.59	*Xa4, Xa21*
	BB4-21	IR 58025 B/PR 39897-25 B-65	8	12-25	91-99	3.14-7.57	*Xa4, Xa21*
	BB8-8	IR 58025 B/PR 39897-25 B-52	8	13-21	76-106	4.50-10.28	*Xa4, Xa21*
	BB8-14	IR 58025 B/PR 39897-25 B-52	3	16-17	83-102	5.82-7.40	*Xa4, Xa21*
	BB8-16	IR 58025 B/PR 39897-25 B-52	3	13-15	94-98	5.46-9.34	*Xa4, Xa21*
	BB8-17	IR 58025 B/PR 39897-25 B-52	1	11	91	3.93	*Xa4*
	BB8-24	IR 58025 B/PR 39897-25 B-52	2	17-22	83-94	5.91-6.45	*Xa4, Xa21*
	BB8-28	IR 58025 B/PR 39897-25 B-52	2	13-14	88-97	3.43-5.20	*Xa4*
	BB10-22	IR 58025 B/PR 39897-25 B-52	1	15	93	3.04	*Xa4*
	BB44-15	IR 58025 B/PR 39897-25 B-65	10	15-26	96-106	4.46-8.41	*Xa4, Xa21*
	IR34686R ORIG			13	112	-	
	86R-P-85	IR 34686R /IRBB62	1	10	107	6.14	*Xa4, Xa21*
	86R-P-86	IR 34686R /IRBB62	1	13	104	7.30	*Xa4, Xa21*
	86R-P-89	IR 34686R /IRBB62	1	14	105	6.54	*Xa4, Xa21 (H)*
	86R-P-113	IR 34686R /IRBB62	1	11	112	7.74	*Xa4, Xa21*
	M1RR5	IR 34686R/PR 39898-86 R-86	1	15	98	5.15	*Xa4, Xa21*
	M1RR-6	IR 34686R/PR 39898-86 R-89	1	19	105	8.37	*Xa4*
Mestiso 3							
	IR68897B ORIG			10	100	-	
	97B-P-25	IR 68897 B/IRBB62	1	11	105	5.42	*Xa4, Xa21*
	97B-P-36	IR 68897 B/IRBB62	1	14	96	4.42	*Xa4, Xa21*
	97B-P-50	IR 68897 B/IRBB62	1	18	77	8.68	*Xa4, Xa7*
	M3BB2-2	IR 68897 B/PR 39901-97 B-36	9	9-21	71-97	4.76-8.47	*Xa4, Xa21*
	M3BB4-2	IR 68897 B/PR 39901-97 B-36	20	12-22	66-96	4.35-10.10	*Xa4, Xa21*
	M3BB4-8	IR 68897 B/PR 39901-97 B-36	1	12	76	7.44	*Xa4*
	M3BB6-7	IR 68897 B/PR 39901-97 B-50	10	8-17	79-95	5.32-9.21	*Xa4, Xa21*
	M3BB6-9	IR 68897 B/PR 39901-97 B-50	11	8-23	76-90	5.26-7.85	*Xa4, Xa21*
	M3B20	IR 68897 B/PR 39901-97 B-36	3	-	-	5.76	*Xa4, Xa7, Xa21*
	IR60819R ORIG			111	12	-	
	19R-P-52	IR 60819 R/IRBB62	1	-	-	6.92	*Xa4*
	19R-P-56	IR 60819 R/IRBB62	1	99	7	9.15	*Xa4*

Table 18. Pollen sterility evaluation in the CMS line development of improved Mestiso 3 (2013 DS).

Male	Female	Pollen sterility (%)
PR39901-97B36	97A36	99.13%
PR39901-97B36-21	M3A21	98.93%
PR39927-97B36-M3BB4-2-31-4	PR39927-97A36-M3BB4-2-31-4	98.72%
PR39927-97B36-M3BB4-2-39-3	PR39927-97A36-M3BB4-2-39-3	98.36%

Table 19. Yield performance of four improved Mestiso 3 hybrids in the Science City of Muñoz, Nueva Ecija (2012 WS and 2013 DS), and Magsaysay, Davao del (2012 WS).

Cross Combination	Grain yield (t/ha)		
	2012 WS		2013 DS
	Muñoz	Magsaysay	Muñoz
PR39901-97A36/19R56	5.34	-	8.06
PR39901-97A36-21/19R76	5.21	10.7	8.08
PR39927-97A36- M3BB4-2-31-4/19R52	4.95	12.7	7.33
PR39927-97A36-M3BB4-2-39-3/19R115	4.82	-	8.12
PSB Rc82	-	-	7.4
PSB Rc18	-	-	7.42
Mestiso 3	4.36	10.8	-

Figure 19. AxR seed production of improved Mestiso 3 in the Science City of Muñoz, Nueva Ecija (2013 DS).

Figure 20. Improved Mestiso 3 with two bacterial blight resistance genes in MYT in the Science City of Muñoz, Nueva Ecija (2013 DS).

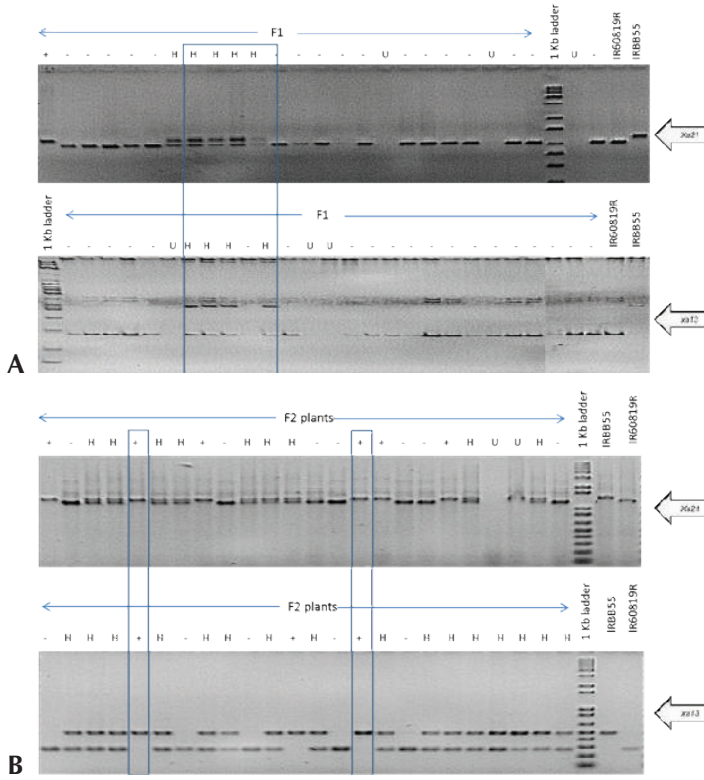


Figure 21. Target gene assay in (A) F1 and (B) F2 plants in IR60819R background using markers for Xa21 (upper gel) and xa13 (lower gel) showing the introgression of both genes from IRBB55. Heterozygote (H), donor allele (+), recurrent parent allele (-), no amplification (U).

Genomewide selection for grain yield

RA Millas, EP Rico Jr., and DA Tabanao

Genomewide selection (GWS) is a breeding method that has been initiated in plants only very recently as emerging technologies from different fields have become applicable to the area of genetic improvement (Bernardo and Yu, 2007). It is a combination of molecular genetics, advances in statistical applications in the life sciences, high-speed computing, and new software for plant breeding and genetics.

GWS utilizes dense genomewide marker data to predict the breeding values of individuals without the need for QTL information (i.e., no need for QTL analysis), making it suitable for complex traits controlled by many genes. The trait is improved by increasing the frequency of favorable alleles achieved by recombining the best individuals from generation to generation, which is basically a population improvement approach. Selection for the best individuals is made more precise by using genotype data made possible by genomewide markers, in addition to phenotype data. Marker effects are estimated and breeding values are predicted through mixed models, which require complex computations and have only become more achievable by applied science researchers through high-speed computing and more user-friendly statistical programs.

This study aimed to: (1) develop two mapping populations and detect QTLs for grain yield, (2) estimate marker effects of genomewide SSRs in RI population through best linear unbiased prediction (BLUP), (3) estimate marker-based breeding values (MBVs) of Cycle 1 and Cycle 2 plants in recurrent selection population, and (4) develop high-yielding inbred lines through marker-assisted recurrent selection.

Highlights:

- Two recombinant inbred populations were established in the field. PR40794-G006 DC F6 (Bulibod na puti/ PR36248-Hy-2-5-1//NSIC Rc158/PR36218-Hy-2-2-5) and PR40629-G021 DC F5 (NSIC Rc160/IR65600-27-1-2-2//NSIC Rc158/IR71700-247-1-1-2) with 200 inbreds each. These two populations were evaluated in replicated yield trials at PhilRice Central Experiment Station (CES). Phenotypic data, such as days to heading, grain yield (harvested from 5 hills) and plant height, were gathered. Grain yield data of PR40629-G021 ranged from 3.3 - 13.2 t/ha with an average yield of 8.3 t/ha. Shown in Table 20 is the predicted grain yield performance of the top performing G021 lines evaluated in replicated yield trials in 2013 DS with genotype considered as random effects factor, and replication and block considered as fixed effects factor. A new RIL's, G038 F3 (PR34641-2B-19-1-1/

Leuang) with 200 inbred lines was also established in the field.

- In 2013 DS, the selected PR40614-AC, PR40846 and PR40853 F6 lines were established and further evaluated in replicated yield trials at PhilRice CES (Figure 22), PhilRice San Mateo Isabela(SMI), PhilRice Negros, and in Magsaysay, Davao del Sur. Phenotypic data such as days to heading, grain yield and plant height were gathered. Grain yield data across the four locations ranged from 4.8 - 7.8 t/ha with an average yield of 6.0 t/ha. The predicted yield data of the top performing PR40614-AC, PR40846, and PR40853 F6 lines with genotype considered as random effects factor, and replication and block considered as fixed effects factor are shown in Table 21.Together with the phenotypic acceptability data (PAcp) gathered from PhilRice CES, the best performing lines were again selected for further yield trials in Magsaysay, Davao del Sur and at PhilRice CES in 2013 WS.

- The three genomewide selection (GWS) populations, PR40613-AB F8 (double cross, cycle 0), PR40614-AC F7 (intermated AB, cycle 1), PR40614-AD F5 (intermated AC, cycle 2), were established in the field. The 385 AD lines were laid out in a separate alpha lattice experiment at PhilRice CES. In addition, the 60 best performing AB, AC and AD lines selected from Magsaysay, Davao del Sur and at PhilRice CES in 2012 WS, and further selected based on PAcp data on 2013 DS were also established in replicated yield trials at PhilRice CES and in PhilRice SMI. Phenotypic data such as days to heading, panicle number per plant, grain yield (harvested from 5 hills) and plant height were gathered. Grain yield data analysis across two locations is underway.

- The MA and MB recombinant inbred populations, serving as C0, were recombined to produce GA and PA (C1 of MA) and GB (C1 of MB). Estimates of genomewide marker-based breeding values of the GA and GB F2 populations were calculated using the marker data generated. The top 20 genotypes were identified and intermated, generating cycle 2 populations denoted as PA for GA and MD for GB. Several evaluations in the pedigree nursery were done for both the C1 and C2 lines. In every season, selection was carried out based on PAcp among populations. The number of lines selected per population is shown in Table 22. Selected lines will be planted for further evaluation in the pedigree nursery in 2014 DS.

- In 2012 WS, the grain yield data of AB (cycle 0) lines obtained at PhilRice CES and PhilRice SMI during 2012 DS were analyzed using SAS. Together with the PAcp data,

the top 20 performing genotypes of CES and SMI were intermated separately producing a new cycle 1 F1 population denoted as AE. A new set of intermating was done in 2013 DS producing an additional cycle 1 AE population. The number of lines selected is also shown in Table 22 and this will be planted for further evaluation in the pedigree nursery.

- For initial yield evaluation, a replicated yield trial was done in 150 GF6 lines composed of nine populations created from a set of candidate founders in 2007 denoted as G007-G011 and G023-G026. PAcp data were gathered on all entries that will serve as basis for the selection of entries for further yield trials.

- Field establishments of various populations were done in 2013 DS and WS. Selections were done in 2013 DS for further evaluation in the pedigree nursery in WS. The selected lines in 2013 WS comprised six populations in F2 generation denoted as G044, G046, G047, G051, G052 and G053; eight populations in F3 generation denoted as G032, G035, G036, G037, G039, G040, G042 and A078; four populations in F4 generation denoted as G027, G028, G030 and G031; seven populations in F5 generation denoted as G014-G020. Selection was carried out based on phenotypic acceptability among populations. The number of lines selected in each population per generation is shown in Table 22.

Table 20. Predicted grain yield performance of the top performing PR40629-G021 (NSIC Rc160/IR65600-27-1-2-2//NSIC Rc158/ IR71700-247-1-1-2) lines evaluated in replicated yield trials at PhilRice Central Experiment Station during 2013 DS.

Designation	Grain yield (kg/ha)
IR65600-27-1-2-2	8,818.64
IR71700-247-1-1-2	13,171.82
NSIC Rc158	11,920.18
NSIC Rc160	11,491.75
PR40629-G021-108	13,253.74
PR40629-G021-96	12,920.65
PR40629-G021-99	12,539.10
PR40629-G021-38	12,092.15
PR40629-G021-158	11,886.48
PR40629-G021-50	11,860.71
PR40629-G021-39	11,845.40
PR40629-G021-36	11,639.73
PR40629-G021-54	11,505.89
PR40629-G021-135	11,326.54
PR40629-G021-124	11,322.19
PR40629-G021-166	11,203.64
PR40629-G021-34	11,013.60
PR40629-G021-55	10,973.14
PR40629-G021-70	10,878.75
PR40629-G021-42	10,835.79
PR40629-G021-160	10,753.71
PR40629-G021-86	10,561.30
PR40629-G021-12	10,554.74
PR40629-G021-23	10,511.39
PR40629-G021-29	10,502.99
PR40629-G021-53	10,421.27
PR40629-G021-52	10,397.62
PR40629-G021-79	10,310.41
PR40629-G021-31	10,277.69
PR40629-G021-178	10,275.75
PR40629-G021-82	10,256.32
PR40629-G021-106	10,254.32
PR40629-G021-186	10,177.16
PR40629-G021-13	10,128.76

Table 21. Predicted grain yield performance of the top performing F6 lines evaluated in replicated yield trials at PhilRice Central Experiment Station, PhilRice San Mateo Isabela, PhilRice Negros, and in Magsaysay, Davao del Sur during 2013 DS.

Designation	Cross Combination	Grain yield (kg/ha)
PSB Rc10		6373.94
PR32220-16-1-B-1-2		6252.23
Adron125		6215.68
IR69800-5-2-3		5139.29
PR40614-AC8-4-1	Adron 125/PR32220-16-1-B-1-2//PSB Rc10/IR69800-5-2-3	7776.46
PR40853-6-2-2	NSIC Rc158/PR36243-Hy-2-3-1	7182.47
PR40846-9-2-2	Dinalores/NSIC Rc160	6873.50
PR40614-AC5-8-2	Adron 125/PR32220-16-1-B-1-2//PSB Rc10/IR69800-5-2-3	6640.42
PR40853-4-1-1	NSIC Rc158/PR36243-Hy-2-3-1	6631.69
PR40846-22-1-3	Dinalores/NSIC Rc160	6618.39
PR40853-22-2-3	NSIC Rc158/PR36243-Hy-2-3-1	6614.08
GB2-7-1-3	PSB Rc10/IR69800-5-2-3	6612.91
PR40846-22-1-1	Dinalores/NSIC Rc160	6608.66
PR40846-22-1-2	Dinalores/NSIC Rc160	6556.95
PR40614-AC1-3-3	Adron 125/PR32220-16-1-B-1-2//PSB Rc10/IR69800-5-2-3	6473.14
PR40614-AC3-5-1	Adron 125/PR32220-16-1-B-1-2//PSB Rc10/IR69800-5-2-3	6467.12
PR40614-AC1-9-2	Adron 125/PR32220-16-1-B-1-2//PSB Rc10/IR69800-5-2-3	6442.09
PR40853-6-1-2	NSIC Rc158/PR36243-Hy-2-3-1	6410.49
PR40853-7-2-2	NSIC Rc158/PR36243-Hy-2-3-1	6382.83
PR40614-AC8-9-2	Adron 125/PR32220-16-1-B-1-2//PSB Rc10/IR69800-5-2-3	6327.65
PR40853-22-2-2	NSIC Rc158/PR36243-Hy-2-3-1	6324.96
PR40614-AC8-3-3	Adron 125/PR32220-16-1-B-1-2//PSB Rc10/IR69800-5-2-3	6319.51
PR40846-19-2-2	Dinalores/NSIC Rc160	6291.66
PR40614-AC8-10-2	Adron 125/PR32220-16-1-B-1-2//PSB Rc10/IR69800-5-2-3	6270.12
PR40614-AC1-5-1	Adron 125/PR32220-16-1-B-1-2//PSB Rc10/IR69800-5-2-3	6228.85
PR40614-AC8-10-3	Adron 125/PR32220-16-1-B-1-2//PSB Rc10/IR69800-5-2-3	6225.61
PR40853-7-1-2	NSIC Rc158/PR36243-Hy-2-3-1	6219.20
PR40614-AC8-6-3	Adron 125/PR32220-16-1-B-1-2//PSB Rc10/IR69800-5-2-3	6211.68
PR40614-AC1-6-2	Adron 125/PR32220-16-1-B-1-2//PSB Rc10/IR69800-5-2-3	6186.46

Table 22. Total number of selections in all of the GWS populations in 2013 WS.

Generation	Population designation	Cross combination	No. of selections
F_2	GA	MA/MA	3
F_2	AE	AB/AB	27
F_2	GA	MA/MA	3
F_2	G044	NSIC Rc158/NSIC Rc222	16
F_2	G046	PR40853-22-1-2/NSIC Rc158	12
F_2	G047	NSIC Rc222/NSIC Rc226	64
F_2	G051	PR37241-3-1-3-1-1-2/NSIC Rc238	3
F_2	G052	IR09A120/NSIC Rc240	6
F_2	G053	IR10M140/Basmati 370	6
F_3	G032	NSIC Rc158/NSIC Rc222	19
F_3	G035	NSIC Rc226/BW 267-3	3
F_3	G036	IP03/PR37252-2-1-1-1-2-3	17
F_3	G037	IR06M137/Leuang Acc	10
F_3	G039	PR40846/NSIC Rc160	6
F_3	G040	PR40847/PSB Rc82	8
F_3	G042	PR40853/NSIC Rc158	6
F_3	A078	WEED TOL 1/HEX I41	4
F_3	MD14	GB/GB	3
F_3	MD15	GB/GB	2
F_4	G027	ZHONGHUA 1/NSIC Rc222	49
F_4	G028	ZHONGHUA 1/NSIC Rc222??	60
F_4	G030	IR66158-38-3-2-1/NSIC Rc224//NSIC Rc216	33
F_4	G031	IR72967-12-2-3/2*NSIC Rc216	48
F_4	PA	MA/MA	3
F_4	GA	MA/MA	18
F_5	G014	IR66158-38-3-2-1/NSIC Rc160	1
F_5	G015	IR66158-38-3-2-1/NSIC Rc216	4
F_5	G016	IR66158-38-3-2-1/NSIC Rc222	4
F_5	G017	IR66158-38-3-2-1/NSIC Rc224	3
F_5	G018	IR72967-12-2-3/NSIC Rc216	24
F_5	G019	IR72967-12-2-3/NSIC Rc222	18
F_5	G020	IR72967-12-2-3/NSIC Rc224	1
F_5	GB	MB/MB	17
F_5	MD	GB/GB	30
F_6	G007	IR66158-38-3-2-1/NSIC Rc160	11
F_6	G008	IR66158-38-3-2-1/NSIC Rc216	28
F_6	G009	IR66158-38-3-2-1/NSIC Rc222	13
F_6	G010	IR66158-38-3-2-1/NSIC Rc224	3
F_6	G011	IR72967-12-2-3/NSIC Rc216	7
F_6	G023	IR69550/PR30255WH-9A-C1-1-1-2	2
F_6	G024	R1-60/R1-2	10
F_6	G025	PR36823-39-1-8/IR64	7
F_6	G026	PR36244-HY-3-1-4/IR71604-4-1-4-4-4-2-2-2R	32

Figure 22. F_6 breeding lines evaluated in PhilRice Central Experiment Station (2013DS).

Introgression of GLH and RTSV resistance in promising PhilRice breeding lines toward development of improved lines with rice tungro disease resistance

AA dela Cruz, MJC Duque, TF Padolina, and OE Manangkil

Rice tungro disease (RTD) is considered the most serious rice viral disease in the Philippines and in South Asia 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. RTBV is responsible for symptom expression while RTSV alone does not cause any distinctive symptoms except mild stunting in some rice cultivars but it plays the role of a helper virus for vector transmission of RTBV (Hibino 1983; Cabauatan et al., 1993). ARC11554, an Indian rice cultivar, has very strong resistance against RTD. In 1999, Sebastian et al. described the GLH and RTSV resistance in ARC11554 as very tightly linked and mapped in the short arm of rice chromosome 4.

Among the various approaches identified to control and manage RTD, the utilization of virus resistance will be the most effective and practical in the long term (Sta. Cruz et al., 2008). It is crucial for rice plants to have complete protection from RTSV infection to effectively control spread of 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.

In the past years, PhilRice breeding programs used ARC11554 as donor of RTD resistance but had focused on introgression of a chromosome 4 fragment. Recently, dela Cruz et al. (2013, for publication) verified presence only of GLH resistance in the short arm of rice chromosome 4 while associated the RTSV resistance in ARC11554 to the tsv1 gene on chromosomes 7. Moreover, dela Cruz (2013) associated the RTSV resistance to eIF4Gtsv1 gene, which is coding for a translation initiation factor. The eIF4G gene sequence variant in ARC11554 was found similar to the SNP type in RTSV-resistant Utri Merah 16882, TW16 and in other RTSV-resistant rice genotypes.

In PhilRice, promising breeding lines with either resistance to bacterial leaf blight and or blast, and with acceptable yield, were already developed for irrigated lowland breeding and for direct seeding. Unfortunately, these were susceptible to RTD. We previously identified 2 BC3F6 sister lines (generated from crossing TN1 and ARC11554) from the materials left by Dr. G. O. Romero in Philrice containing the GLH resistance locus and tsv1 according to the set of tightly linked molecular markers verified and developed by dela Cruz et al. (2013). This study, therefore, aims to further improve promising breeding lines for irrigated lowland breeding and direct seedling, along with NSIC Rc298, with RTD resistance by introgression of both GLH resistance locus and tsv1 gene from ARC11554. Ultimately, cultivars with pyramided resistance genes will be developed and will be deployed to endemic areas to provide the best opportunity for effective disease control, and will eventually help alleviate rice productivity particularly in disease hot spot areas.

Highlights:

Activity 1. Evaluation of individual and combined influences of GLH- and RTSV-resistance on infection of rice with RTSV

- The activity was pursued in IRRI in collaboration with Dr. Il-Ryong Choi. F4 materials and corresponding check cultivars (F4-22-103-15-8 and ARC11554, with GLH R locus + tsv1 gene; F4-22-122-2 and TW16, with tsv1 alone; F4-26-301-10-9 and Matatag 6, with GLH R locus alone; F4-22-28-17 and TN1, without any resistance genes) were forced-inoculated with RTSV strain-A.

- In the standard evaluation of resistance to RTSV, 3 viruliferous GLH is being used. In this experiment, higher densities of

viruliferous GLH (5/10/15) were added on individual 14 days after sowing seedlings to distinguish the effects of single genes and their combined effects. GLH was allowed to feed on test plants for 24 hours and was removed afterwards. In mock treatment, non-viruliferous GLH was used. For consistency of the environment, experimental plants were maintained inside the IRRI greenhouse, under natural lighting conditions. At 14 and 21 days after inoculation, ELISA was conducted to index RTSV loads. RCB experimental design with 3 replications and 6 treatments was used.

- The genotypes of the individual test plants were verified using the set of tightly-linked molecular markers for GLH R locus and tsv1.

- ANOVA (Table 23) shows that there was no significant difference among the 3 experimental replications. While a significant difference was observed in the use of 5 versus 15 GLH in inoculating RTSV (Table 24), it did not significantly affect the %RTSV infection of the respective genotypes (Table 23).

- Table 25 shows that the genotypes used in the experiment varied significantly in terms of %RTSV infection. At the extreme ends are the RTSV resistant ARC11554, F4-103, F4-28 and TW16 and RTSV susceptible TN1 and F4-122. Matatag6 and F4-301 containing the only the GLH resistance locus revealed intermediate response but the %RTSV infection in Matatag 6 was lower compared to the %RTSV infection in F4-301.

- The presence of tsv1 gene alone on rice plants conveyed complete protection against RTSV regardless of the number of GLH used to inoculate the virus.

- Results generally demonstrated that the use of resistance to virus-transmitting insects alone may not assure the prevention of virus diseases in rice.

Activity 2. Individual and combined influences of GLH- and RTSV-resistance on infection of rice with RTD (RTSV+RTBV) in the actual tungro hot spot field

- Experiments were established simultaneously in 2 PhilRice stations, Negros and Midsayap. There was high disease incidence in Negros but unfortunately there was none in Midsayap.

- In Negros, many of the experimental plants died due to the unfavorable environmental conditions brought by frequent heavy rainfalls and strong typhoons.

- Table 26 shows that among the F4 plants that survived, F4-22-103 had the highest % plant survival (34.19%), followed by lines with only tsv1 gene such as F4-22-28-17 (21.94%), F4-22-154 (17.42%), F4-22-28-36 (15.48%) and the line carrying the GLH resistance locus alone only has 10.97%.

- Interestingly, F4-103 exhibited very low % RTSV infection, although some plants were infected with RTBV (Table 26). Likewise, F4-103 recorded the lowest %RTBV infection and %RTSV+RTBV infection.

- Data gathered from the Negros trial may not be ideal for statistical analysis since many entries were lost but it is highly recommended that this experiment be repeated in about 3 hot spot areas this coming wet season to establish strongly the individual and combined influences of GLH and RTSV resistance genes in rice infected with RTD. At the same time, forced-tube inoculation of RTBV+RTSV on individual plants in a controlled environment will further support the field experiment data, thus, it is also hereby recommended.

Activity 3. Introgression of GLH and RTSV resistance in promising PhilRice breeding lines

- The 1st batch of parentals to be used in the generation of F1s was established by staggered planting of recipient lines (3 lines for irrigated lowland breeding, 3 lines for direct seedling and NSIC Rc298) and donor lines (2 BC3F6 carrying the GLH resistance locus and the tsv1 gene). Establishment of materials started in December 2013 and will go on until February 2014.

Table 23. ANOVA showing the interactions among replications, genotypes, number of GLH used, and GxE.

Source	Df	SS	MS	F	P-value	
Reps	2	208.33	104.17	1.06	0.36	ns
Genotypes	7	125065.28	17866.47	181.63	0.00	**
GLH	2	700.00	350.00	3.56	0.04	*
Genotypes * GLH	14	1988.89	142.06	1.44	0.17	ns
Error	46	4525.00	98.37			
Total	71	132487.50				

Table 24. Comparison among number of GLH used to inoculate RTSV alone (Tukeys HSD).

GLH	Mean	
5GLH	32.08	a
10GLH	37.08	ab
15GLH	39.58	b

**Means with the same letter are not significantly different*

Table 25. Comparison among genotypes in terms of % RTSV infection (Tukeys HSD).

Genotypes	Mean*	
ARC11554	0.00	a
F_4-103	0.00	a
F_4-28	0.00	a
TW16	0.00	a
Matatag	27.78	b
F_4-301	65.56	c
F_4-122	97.78	d
TN1	98.89	d

**Means with the same letter are not significantly different*

Table 26. Individual and combined influences of GLH and RTSV resistance genes in rice infected with RTD.

	Genotypes	GLH R locus	*tsv1*	otal	% Plant Survival among F4 lines	RTBV alone	%RTBV	RTSV alone	%RTSV	RTBV+ RTSV	%RTBV +RTSV
F4 lines											
1	F_4-154	-	+	27	17.42	7	25.93	2	7.41	1	3.7
2	F_4-103	+	+	53	34.19	5	9.43	1	1.89	1	1.89
3	F_4-22-28-17	-	+	34	21.94	10	29.41	4	11.76	4	11.76
4	F_4-22-28-36	-	+	24	15.48	9	37.5	5	20.83	3	12.5
5	F_4-26-301	+	-	17	10.97	3	17.65	1	5.88	1	5.88
	Total			155	100.00						
Check cultivars											
6	ARC11554	+	+	20		0	0	0	0	0	0
7	Matatag 6	+	-	20		0	0	0	0	1	5
8	TN1	-	-	2		0	0	1	50	1	50
9	TW16	-	+	20		0	0	0	0	0	0

*for check cultivars, agronomic traits and ELISA data were gathered only on 20 plants except for TN1 wherein only 2 plants survived.

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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Department of Agriculture

PHILRICE

PHILIPPINE RICE RESEARCH INSTITUTE

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

We accomplish this mission through research and development work in our central and seven branch stations, coordinating with a network that comprises 57 agencies and 70 seed centers strategically located nationwide.

To help farmers achieve holistic development, we will pursue the following goals in 2010-2020: attaining and sustaining rice self-suffiency; reducing poverty and malnutrition; and achieving competitiveness through agricultural science and technology.

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

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