2015 National Rice R&D Highlights

Seed Technology Division

Department of Agriculture
PHILRICE
PHILIPPINE RICE RESEARCH INSTITUTE

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SEED TECHNOLOGY DIVISION

Division Head: SR Brena

Executive Summary

Seed quality assurance is one of the essential components of seed production since this activity raises the quality of the seed produced. Thus, seed quality assurance in rice always begins in the field. This omprises various operations in which seed passes before it reaches the clients. STD 002 is composed of studies on Seed Quality Assurance in PhilRice Seed Stock.

Internal field inspection is being conducted by the division following set of rules as prescribed by several Administrative Orders issued by the Department of Agriculture in order to examine the purity status of breeder (BS), foundation (FS), registered (RS) and nucleus seed production areas of the Plant Breeding and Biotechnology Division and the Business Development Division of the Philippine Rice Research Institute.

I. Seed Quality Assurance in PhilRice Seed Stock

Project Leader: SR Brena

This project focuses on field inspection, varietal purity testing, viability and vigor testing of PhilRice seed stocks. Seed inspection is done as early as 20 days after transplanting (DAT) followed by three inspections at maximum tillering, at the onset of flowering and two weeks before harvest. Field rejection is also done if varieties inspected continue to exhibit off-types despite the rouging done. Report of the field inspection conducted by Seed Tech staff is provided to the respective field workers and follow inspection is done to check on the rouging done after the inspection. Varieties that passed field inspection do not necessary mean that they will pass seed certification by the National Seed Quality Control Services (NSQCS) since field inspection is only half of the entire process chain the seeds passed through before becoming available to farmers. Half of the process consists of threshing, drying, and seed cleaning.

Other than field inspection seed quality monitoring of carry over seed lots of inbred and hybrid seed stock are also conducted under this project. Tests conducted include germination and vigor test. Although germination test is universally accepted, the test measures both strong and weak seedlings because this test consisted of two counting. The first count after seeding is done 5 to 7 days after and this counting measures the highly vigorous seedlings. Fresh, ungerminated seeds in the first count are allowed another 7 days to germinate. Those that will germinate after are considered weak seedlings and number of germinated seedlings is added to the number of germinated seedling in the first count to give the total number of germinated seedlings expressed in percent. A more reliable test to determine the quality of the seed lot is vigor test which can either be expressed as the number of germinated seeds during the first count or to germinate the seeds per lot after ageing (accelerated ageing test).

The success of CMS and TGMS seed production CMS depends on the purity of the parental lines. One of the studies (STD 002-004) in this project assesses the genetic purity of the parental lines. As a general rule, only parental lines with 97% or higher genetic purity is distributed to hybrid seed growers. If there are off-types observed during hybrid seed production, the number is almost neglible. To come up with a more rapid yet accurate test to assess genetic purity, DNA fingerprinting is now being explored. Simple sequence repeats (SSR) molecular markers for parental lines and TGMS hybrids, Mestiso 19 and Mestiso20 have been identified to be polymorphic.

Field inspection of seed production areas
EP Rico, J Managkil, MC Salamanca and SR Brena

Seed quality assurance in rice seed begins in the field. Generally, field inspection starts 20 days after transplanting, at maximum tillering, onset of flowering (most important period to remove off-types), and two weeks before harvest. Although these are prescribed period for inspection, rouging should be done for as long as there are off-types observed in the field. This routine activity is done to assure PhilRice clients of seeds with the high purity. However, despite efforts for seed quality assurance in the field by the Seed Technology division, threshing and the rest of the postharvest operations are controlled by the staff of Business Development Office. Moreover, laboratory certification, one particular aspect in the entire system of seed certification is under an agency outside of PhilRice.

The study was conducted by inspecting nucleus, breeder (BS), foundation (FS) and registered (RS) production areas of PBBD and BDO. The field under each seed class planted per variety was inspected in three replications. For FSP and RSP, 32 x 32 hills were pegged with bamboo sticks. Total number of plants in a pegged area was 1,024. Three-pegged areas per variety were inspected. Off-types considered were plants that exhibited early and late flowering; short and tall plants; and volunteer plants.

Highlights:

- A total of 326 superior cultivars and in a 358 field lots were inspected during 2015 field inspection; 155 varieties and 188 field lots for dry season and 171 varieties and 170 field lots for wet season. Of these varieties inspected during dry season 96 varieties were under nucleus seed production (NSP), 41 varieties were under breeder seed production (BSP), 18 were under foundation seed production (FSP) and 21 were under registered seed production (RSP) and during wet season 91 varieties were under nucleus seed production, 32 varieties were breeder seed production, 14 were under foundation seed production and 13 were under registered seed production.

- Three varieties were rejected under breeder seed production, two (NSIC Rc356 and NSIC Rc9) during dry season and one (NSIC Rc29) during wet. The rejection were due to two different grain type (medium and long), two blade color during panicle initiation stage (pale green and dark green), and two different leaf blade width (broad and slender).

- During dry season final average percent purity were recorded at 99.95% under NSP, 99.92% under BSP, 99.28% under FSP and 99.90% under RSP and during wet season 99.77% under NSP, 99.90% under BSP, 99.90% under FSP and 99.51% under RSP but we assured a100% pure before harvesting.

- Despite the high percentage purity in the field, all postharvest operations should be done properly to assure high percentage passing of the seed lots produced during seed certification by BPI-NSQCS.

Table 1. Genetic purity of inbred varieties planted in 2015 based on field inspection

Production Season	Seed Class	Number of varieties	Total field lot inspected	% Purity during field inspection conducted at				Remarks
				20 DAT	Maximum tillering	Flowering	Before Harvest	
DS 2015	Basic	96	1	99.86	99.97	99.85	99.95	2 varieties were rejected
	Breeder	32	1	99.99	99.98	99.86	99.92	
	Foundation	14	74	99.99	97.31	99.50	99.28	
	Registered	13	114	99.99	99.99	99.77	99.90	
WS 2015	Basic	91	1	99.99	99.70	99.57	99.77	1 variety rejected
	Breeder	41	1	99.97	99.97	99.73	99.90	
	Foundation	18	62	97.40	97.56	99.72	99.99	
	Registered	21	108	99.99	91.74	97.86	99.51	

Seed purity and seed viability testing

SR Brena, EP Rico, J Managkil, and MC Salamanca

In DS 2015, seed purity testing was done in breeder, foundation, and registered seeds produced at the Central Experiment Station. The testing was done in three stages of the postharvest operations: immediately after threshing; after drying, and after seed cleaning. The seeds produced at PhilRice are certified by the National Seed Quality Control Services but the sample submitted to this agency by the seed inspectors is withdrawn from the clean seeds after cleaning just before closing the bags. Philippine Seed Standards, maximum allowable mixture for BS, FS, and RS are 0, 2, and 5 grains for every 500gm sample submitted to NSQCS. To ensure high seed quality standard in PhilRice, STD laboratory seed analyst sampled 500gm sample of each variety for purity testing immediately after threshing, after drying, and after seed cleaning. Though varietal purity test is highly subjective, the test gives an indication of the most likely seed class the variety PhilRice produced will have after seed certification.

Viability testing was conducted on old seed stocks of inbred and hybrid seeds in storage. The test is conducted following the ISTA procedures with four replications per sample and each replicate consisted of 100 seeds. Standard deviation in every seed lot tested was computed after the test.

In this study, BS varieties produced in WS 2014 were also evaluated for genetic purity using grow out test. This was done to assess the seed purity of BS produced at PhilRice. Each variety tested was planted in three replications of 500 hills per replicate. Presence of off-types was assessed at maximum tillering, at the onset of flowering, and at maturity. Off-types observed was expressed in percentage and then seed purity was determined.

The study aims to assess the varietal purity of PhilRice breeder, foundation and registered seeds in DS 2015. Moreover, the study also aims to evaluate the seed viability of remaining seed inbred and hybrid seed stock and distribute only varieties with 85% or higher germinate rate.

Highlights:

- Seed purity of BS varieties produced in WS 2014 was assessed using GOT. Eleven varieties tested in GOT revealed that only NSIC Rc288 is a true breeder seed with 100% seed purity (Table 2). All other varieties that passed as BS were not pure sample. None of the d13 downgraded varieties to FS which were expected to become BS reached 99% seed purity. Highest purity was only 98.54% and lowest was 95.25%. Off-types counted were plants which when not remove during rouging will produce seeds that will cause downgrading or even rejection of the sample submitted for seed certification.

- There were 30 BS varieties tested for varietal purity. After threshing, 86.7% (26) of the varieties evaluated had mixtures. Only four varieties were considered free from off-types. These varieties were NSIC Rc120, 218, 242, and 392. After drying, varietal purity was again assessed and only NSIC Rc218 was free from off-types (data not presented). After seed cleaning, all the varieties evaluated had off-types or none of the varieties passed as breeder based from the result of the varietal purity test done by STD seed analyst.

- Varietal purity of 40 breeder seed varieties produced in WS was assessed after threshing, after drying, and after seed cleaning. Only one variety was found true to type after threshing. Many varieties planted lodged owing to the typhoons experienced during the season.

- The number of off-types present in the FS sample tested for varietal purity increased after seed cleaning. For FS, only in NSIC Rc298 had the least number (2) of off-types counted after seed cleaning and this is the only variety considered as FS based on STD's internal varietal purity test. None of the varieties planted to become registered seeds passed since the number of off-types counted were greater than five grains.

- Part of the TGMS hybrids produced in WS 2014 by the Seed to Rice Cooperative (S2R) for PhilRice was not transported to CES and remained in S2R warehouse until May 2015. During the first week of May, the remaining bags were delivered to Davao del Sur for use in the high yielding technology adaption (HYTA) program of the Department of Agriculture. Many of the bags delivered were planted with vigorous growth. However, bags which were not planted were retrieved and tested for viability for delivery to provinces who will be implementing HYTA.

- There were 19 seed lots consisting of 654 bags of TGMS hybrids tested (Table 3). Seed lots with high germination and low standard deviation were SP3M; RB4; RB5; and RB7. Germination values of these seed lots were 96; 95; 95; and 97 while standard deviation values were 1.58; 1.70; 1.78; and 1.47, respectively.

- Six seed lots had below 75% germination with average standard deviation of 3.97. Average germination of the remaining 13 seed lots was 85% with average germination standard deviation of 2.29. Thus, out the 654 TGMS hybrids tested for germination, 61.16% (400 bags) can still be used for planting.

- BS produced in DS 2014 stored in cold room were tested for germination in June 2015. Sixty-one varieties were tested and average germination in 15 bags NSIC Rc104 was the lowest germination (62%). The standard deviation of the germination value obtained was the highest observed among all the varieties tested.

- Germination test of freshly threshed breeder seeds in DS and WS was assessed immediately after threshing and after two weeks to determine the extent of seed dormancy. In DS, 76.7% (23) of the varieties had low percentage germination (1 to 25%). Only NSIC Rc242 had 97% germination immediately after threshing. In WS, 95% of the varieties evaluated, had percentage germination range of 1 to 25%. Only 25% of the varieties tested 14 days after harvest had low germination value (Table 4). The variation in seed dormancy of the varieties tested suggested germination testing after seed cleaning should not be delayed

Table 2. Seed purity assessment of WS 2014 varieties that passed as breeder seeds based on seed certification results.

Variety	Seed class planted	Resulting seed lass	Ave. plant population	Ave. Number of off-types	% off-types observed	% Purity
NSIC Rc146	Nucleus	Breeder	424	7	1.65	98.35
NSIC Rc156	Nucleus	Breeder	460	8	1.74	98.26
NSIC Rc212	Nucleus	Breeder	454	9	1.98	98.02
NSIC Rc272	Nucleus	Breeder	437	10	2.29	97.71
NSIC Rc284	Nucleus	Breeder	467	11	2.36	97.64
NSIC Rc286	Nucleus	Breeder	414	12	2.90	97.10
NSIC Rc288	Nucleus	Breeder	434	0	0	100.00
NSIC Rc328	Nucleus	Breeder	449	14	3.12	96.88
NSIC Rc338	Nucleus	Breeder	442	4	0.90	99.10
NSIC Rc342	Nucleus	Breeder	478	4	0.84	99.16
NSIC Rc346	Nucleus	Breeder	432	16	3.70	96.30

Table 3. Germination test results of retrieved TGMS hybrids.

Seed Lot Number	Number of bags	Average Germination (%)	Standard Deviation	Date Tested
SP-3M	80	91	2.67	June 16, 2015
SP3M	4	96	1.58	June 18, 2015
SP-3M-1	4	93	2.54	June 18, 2015
SP-3M-2	42	73	4.34	June 16, 2015
SP -4M	4	58	3.21	June 18, 2015
SP-4M-1	62	26	3.38	June 18, 2015
SP-5M	60	27	4.05	June 16, 2015
SP-6M	59	50	3.69	June 17, 2015
SP-7M	35	86	2.80	June 16, 2015
SP-7M-1	6	95	2.05	June 18, 2015
SP1-A	34	94	2.13	June 16, 2015
SP- 4B	37	85	2.66	June 16, 2015
SP-3R-2	27	18	5.13	June 17, 2015
SP-5R	58	85	3.28	June 16, 2015
KR1	62	92	2.92	June 16, 2015
RB4	5	95	1.70	June 18, 2015
RB4-1	25	92	2.15	June 18, 2015
RB5	45	95	1.78	June 18, 2015
RB7	5	97	1.47	June 18, 2015

Table 4. Germination test results of breeder seeds produced in 2015 after threshing and 14 days after harvest.

Harvest Season	Number of varieties tested	Number of varieties after threshing with % germination of				Number of varieties after threshing with % germination of			
		1-25	**26-50**	**51-75**	**76-100**	**1-25**	**26-50**	**51-75**	**76-100**
DS 2015	30	23	4	2	1	2	7	6	15
WS 2015	40	38	2			10	17	11	2

Seed vigor testing of buffer stock and carry over seed lots
SR Brena, JM Manangkil, and MC Salamaca

Ideally, it is better not to store seeds because with time viability will decline. However, PhilRice is mandated to produce seeds of released varieties and make them available on time. Making the varieties available would mean that there should always be breeder seed stock ready for foundation seed production. The study concentrates on seed vigor testing of breeder seed stocks in storage which were produced in DS and WS 2014. In vigor assessment, 400 seeds per variety were randomly selected and divided into 4 replicates at 100 seeds per replicate. Each replicate sample is placed on top of a circular chicken wire platform inside wide-mouthed bottle filled with water below. The lid was then tightly closed and placed in a germinator set at 43^{oC} for 3 days. The atmosphere of high relative humidity (RH) and temperature coupled with increase moisture content (MC) of the seeds are factors that cause seed deterioration. After ageing, each replicate sample is subjected to germination test. If the number of germinated seedlings after ageing remains high then the seed lot is considered high vigor seed lots and can be planted even if there is adverse condition in the field with high survival rate.

Highlights:

- Forty-two and 19 BS varieties produced in DS and WS 2014 were tested for seed vigor June 2015 (Table 5). PSB Rc98 exhibited 100% germination after ageing with standard germination of 0.72. On the other hand, only NSIC Rc104 had the lowest seed vigor value (71%). Average vigor value of the remaining 41 varieties was 96% and standard deviation value of 2.53.

- Only 19 remaining BS produced in WS 2014 remained in cold room. The average germination after ageing was 96% and average standard deviation was 2.66.

- Although average germination was highest in BS produced in WS 2014, standard deviation was lower in DS 2014. There were varieties tested whose replicate germination values were far from the mean values obtained. These varieties were NSIC Rc23 and NSIC Rc288 with standard deviation of 5.26 and 5.20, respectively.

- Parental lines tested were produced in PhilRice-LB and Mindoro satellite station. Among the parental lines tested for seed vigor, TG102M from PhilRice-LB had 89% germination after ageing test. All parental lines produced in Mindoro had germination ranged from 81 to 86% but standard deviation of the germination test was already high (Table 6).

- A total of 92 bags of 13 varieties of breeder seed stock produced in WS 2013 stored in cold room were tested for seed viability through laboratory germination; germination after accelerated ageing test; and seedling emergence in the soil. Only five varieties, IR60; NSIC Rc128; NSIC Rc130; NSIC Rc194; NSIC Rc294 high seed quality after two-year storage in cold room (Table 6). Highest seed quality was observed in NSIC Rc128 and NSIC Rc130. Both varieties had higher than 90% normal seedlings even after accelerated ageing test. Moreover, both varieties had also high percentage seedlings that emergence in the soil 14 days after sowing (Table 7).

Table 5. Percentage germination result after accelerated ageing test of DS and WS 2014 BS stock stored in cold room.

Season	Total BS Varieties Tested	Total number of bags tested	Average % germination after ageing test	Average Standard Deviation
DS 2014	42	431	95	2.54
WS 2014	19	146	96	2.66

Table 6. Germination test results of remaining stock of breeder seeds produced in WS 2013.

Variety	Number of bags tested	% Germination in the laboratory	% Germination after accelerated ageing test	% Seedling emergence in the soil	Date Tested
IR60	8	91	92	85	November 5, 2015
PSB Rc28	8	84	72	54	November 5, 2015
NSIC Rc13	6	28	27	23	November 5, 2015
NSIC Rc15	6	23	26	18	November 5, 2015
NSIC Rc122	4	76	76	67	November 5, 2015
NSIC Rc128	7	90	90	91	November 5, 2015
NSIC Rc130	6	94	93	90	November 5, 2015
NSIC Rc142	6	40	35	32	November 5, 2015
NSIC Rc194	13	87	91	87	November 5, 2015
NSIC Rc226	8	84	80	82	November 5, 2015
NSIC Rc272	5	69	76	58	November 5, 2015
NSIC Rc288	7	62	62	65	November 5, 2015
NSIC Rc294	9	88	88	85	November 5, 2015

Table 7. Percentage germination of hybrid parental lines produced in 2014 after AAT.

Parental Line	Seed Source	Production Season	Number of seed lots tested	Number of bags tested	(Vigor Value) % germination after ageing	Average standard deviation
PRUP TG101	PhilRice-LB	DS 2014	3	91	62	3.33
TG102M	PhilRice-LB	DS 2014	1	53	89	3.05
IR68897A	PhilRice-LB	DS 2014	8	65	5	2.80
IR34686R	PhilRice-LB	DS 2014	1	131	86	4.94
TG101M	Mindoro	WS 2014	3	45	85	4.50
TG102M	Mindoro	WS 2014	4	60	82	5.38
IR60189R	Mindoro	WS 2014	2	30	84	4.65
IR71604R	Mindoro	WS 2014	2	30	86	4.89
IR73013R	Mindoro	WS 2014	2	30	81	5.35

**PhilRice –LB parental lines tested in Feb. 2015*
***Mindoro parental lines tested March 2015*

Assessing the seed quality, purity, and genetic identity of hybrid parental lines of public hybrids produced at PhilRice

CH Pablo, LV Guittap, RAG Saludares, and SR Brena

Utilization of hybrid varieties can close the gap between rice supply and demand since it can offer an opportunity to increase yield and can ensure a steady rice supply. It is estimated that 1% impurity in hybrid seed, the yield reduction is 100kg per hectare. Thus, there is a need to assess the genetic purity of seed lots to ensure the farmers could have good quality seeds for higher production volume.

Genetic purity is the trueness of a plant conforming to the variety's heritable characteristics as described by the breeders. There are 4 factors affecting genetic purity: natural crossing, mechanical admixtures, random drift, mutation and selective influence of pest and diseases (Basra 2002).

Grow-out test (GOT) is one of the methods in assessing genetic purity of crops. It is the morphological examination of the plants on the basis of the observations made in the crop's characteristics with reference to true-to-type sample. GOT is pre-requisite at PhilRice for the determination of seed lot's genetic purity status prior to parental line distribution for use by the hybrid seed growers and researchers. Only parental lines with 97% and higher genetic purity are distributed. At this genetic purity level, off-types observed in the field when the parental lines are planted are very minimal.

Samples were grown in 20 x 20cm grow-out matrix with 500 hills per plot. The experiment was laid out in a randomized complete block design (RCBD) with 3 replications.

Highlights:

- Fifteen seed lots of parental lines (4 TG101-M, 4 TG102-M, 2 IR60189, 2 IR71604, 2 IR73013 and PhilSCAT R-line) produced in Mindoro and 17 seed lots (10 IR58025A, 2 IR68897A and 5 IR73013R) produced by PhilRice Los Baños wet season 2014 were tested.

- Genetic purity through visual evaluation was conducted based on the base color, plant height, days to heading and grain shape.

- All seed lots had 97% and above genetic purity.

- Off-types had purple-colored bases, either taller or shorter as compared to the majority of the population, had different grain characteristics and were early or late to head.

- Among the parental lines tested, 1 seed lot of TG101-M, 1 IR71604R, 1 IR58025A, 1 IR68897A and 2 IR73013R had 100% purity (Table 1 and 2). On the other hand, 2 lots of TG102-M, PhilSCAT R-line and 1 IR73013R had the lowest (97%) genetic purity.

- DNA fingerprinting utilizing simple sequence repeats (SSR) markers is currently undertaken to confirm results.

WS2015

- Seventy six seed lots of parental lines (32 Mestiso 1, 32 Mestiso 20, 5 TG102M and 2 IR34686R) produced in Davao Oriental and thirty eight seed lots (16 PRUP-TG101, 11 PRUP-TG102, 3 TG101M, 4 TG102M, 2 IR71604R and 2 IR3013R) produced by PhilRice-Los Baños were evaluated in the grow-out test during the 2015 wet season.

- From all the seed lots produced by PhilRice-Los Baños, 10 seed lots of PRUP-TG101 came from Lucban, Quezon and 6 came from Tublay, Benguet, 11 seed lots of PRUP-TG102 came from Tublay, Benguet while all the rest (3 TG101M, 4 TG102M, 2 IR71604R and 2 IR3013R) came from Los Baños.

- Genetic purity of the Davao Oriental hybrids and parental had the following values as assessed: 32 seed lots of Mestiso

1 had purity that ranged from 98.23 to 100%, the 32 seed lots of Mestiso 20 had 99.08 to 100%, all of the 5 seed lots of TG102M had 100% and the 2 seed lots of IR34686R had 100% genetic purity.

- All seed lots from the hybrids and parental lines produced in Davao Oriental had 98% and above genetic purity with 1 seed lot of Mestiso 1 had the lowest genetic purity (98.23%).

- From the GOT of the parental lines produced by PhilRice-Los Baños, 11 seed lots of PRUP-TG101 and all seed lots of PRUP-TG102, TG101M, TG102M, IR71604R and IR3013R had 100% genetic purity. One seed lot of PRUP-TG101 had the lowest genetic purity (99.88%).

Table 8. Percent Genetic Purity of Parental Lines from Mindoro harvested December 2014.

Entry	Lot No.	No. of bags	Date of harvest	Treatment	Population	Heading		Total no. of offtype	% Purity
						Early	Late		
TG101-M	Lot I-A	31	1-Dec-14	1	175	1	1	2	98.9
	Lot I-B	28	1-Dec-14	2	142	2	0	2	98.6
	Lot I-C	29	1-Dec-14	3	186	2	0	2	98.9
	Lot I-D	28	1-Dec-14	4	144	0	2	2	98.6
TG102-M	Lot I-A	32	5-Dec-14	5	175	0	0	0	100.0
	Lot I-B	20	5-Dec-14	6	324	0	9	9	97.2
	Lot I-C	27	5-Dec-14	7	264	2	4	6	97.7
	Lot I-D	23	5-Dec-14	8	314	0	4	4	98.7
IR60189R	Lot I-A	56	4-Dec-14	9	356	0	7	7	98.0
	Lot I-B	53	4-Dec-14	10	386	6	4	10	97.4
IR71604R	Lot I-A	40	3-Dec-14	11	403	0	0	0	100.0
	Lot I-B	46	3-Dec-14	12	366	0	2	2	99.5
IR73013R	Lot I-A	45	2-Dec-14	13	421	1	5	6	98.6
	Lot I-B	43	2-Dec-14	14	238	0	3	3	98.7
PhilSCAT R-line	Lot I-A			15	231	1	5	6	97.7

Table 9. Percent Genetic Purity of Parental Lines from Los Baños.

Entry	Lot No.	Treatment	Population	Heading		Total no. of off-type	% Purity
				Early	Late		
IR58025A	M18	1	189	2	0	2	98.9
IR58025A	M19	2	191	3	0	3	98.4
IR58025A	M20	3	159	2	0	2	98.7
IR58025A	M21	4	208	1	1	2	99.0
IR58025A	N18	5	205	3	0	3	98.5
IR58025A	N19	6	214	3	0	3	98.6
IR58025A	N20	7	182	0	1	1	99.5
IR58025A	N21	8	212	0	1	1	99.5
IR58025A	M18	9	67	0	0	0	100.0
IR58025A	N18	10	59	0	1	1	98.3
IR68897A	107-111	11	227	3	0	3	98.7
IR68897A	108-110	12	225	0	0	0	100.0
IR73013R	114-1	13	234	0	0	0	100.0
IR73013R	114-2	14	196	0	4	4	98.0
IR73013R	114-3	15	88	0	0	0	100.0
IR73013R	114-4	16	239	0	1	1	99.6
IR73013R	114-5	17	184	1	3	4	97.8

Utilization of SSR Markers for Seed Purity Testing in TGMS Hybrids of Mestiso 19 and 20

RAG Saludares and SR Brena

With the burgeoning population in the Philippines and decreasing natural resources, rice production needs to step up to achieve rice self-sufficiency. However, high genetic purity is an essential prerequisite for commercialization of any hybrid seeds. In every 1% seed impurity, there is 1kg production decrease. Conventionally, hybrid seed purity is assayed by a grow-out test (GOT). Yet, there is a need for a molecular marker assay to assess genetic purity of hybrid seeds that is both fast and accurate. DNA markers are neutral, less environmentally conditioned and well reproducible. Simple Sequence Repeats (SSRs) has much more polymorphism, co-dominant and large in quantity than most of the other DNA markers. Molecular marker technology in rice has been applied widely in the identification and registration of plant variety and monitoring of the seed purity and the authenticity with high accuracy, high reliability and low cost. The objectives of the study are to investigate microsatellite markers or SSR markers capable of distinguishing rice hybrids and their parental lines and to identify specific primers which can be used for genetic purity testing with the final goal to develop a low-cost, fast, accurate, sensitive and effective DNA fingerprinting method for purity testing of hybrid rice.

Highlights:

- Pure lines of Mestiso 19 and 20 and its parental lines (PRUP TG101, TG101M, PRUP TG102 and TG102M were planted in the basins replicated thrice.

- Leaf samples were collected from each treatment 21 days after seeding at Philippine Rice Research Institute- Seed Technology Division.

- DNA extraction, quantification and amplification via Polymerase Chain Reaction (PCR) and SSR analysis via Polyacrylamide Gel Electrophoresis (PAGE) was conducted at the International Rice Research Institute, Plant Breeding, Genetics and Biotechnology Division, Genotyping Services Laboratory (IRRI, PBGB-GSL).

- Fifty three SSR markers previously identified to be associated with the seed purity tests of both the parentals and its hybrids were initially used for polymorphic screening. However, only 4 markers (RM127, RM1, RM511 and RM71) successfully detected and differentiated the genotypes (Figure 1).

- More marker screening should be done to verify results.

- The limited supply of PRUPTG102 in 2015 resulted in limited S x P seed production areas established. In June 2015, S-line multiplication of PRUPTG102 was established in Don Salvador Benedicto, Negros Occidental. This MFE site was isolated and genetic purity of the material was assured through rigorous rouging.

- However, to ensure confidence in the genetic purity of the S-lines produced and dispatched to hybrid cooperators, seed samples were tested in GOT plots (in progress) and DNA fingerprinting.

- Total of six lots were produced in DSB, Negros Occidental. Seed lots 1, 2, 4, and 5 were deemed pure sample. Seed lots 1, and 6 had the same electrophoretic pattern and may not be as pure as the other four seed lots.

Table 10. Simple sequence markers (SSR) utilized for marker screening.

SSR Primers	Chromosome no.	Product size (bp)	SSR Primers	Chromosome no.	Product size (bp)
RM1	1	113	RM592	5	270
RM104	1	222	RM111	6	124
RM128	1	157	RM136	6	101
RM220	1	127	RM190	6	124
RM226	1	247	RM204	6	169
RM493	1	211	RM276	6	149
RM499	1	116	RM70	7	170
RM71	2	149	RM234	7	156
RM154	2	183	RM336	7	154
RM250	2	153	RM418	7	283
RM263	2	199	RM455	7	131
RM424	2	239	RM149	8	253
RM475	2	235	RM160	9	131
RM535	2	138	RM201	9	158
RM7	3	180	RM147	10	97
RM168	3	116	RM216	10	146
RM119	4	166	RM228	10	154
RM127	4	223	RM244	10	163
RM177	4	195	RM258	10	148
RM307	4	174	RM333	10	191
RM335	4	104	RM144	11	237
RM153	5	201	RM167	11	128
RM161	5	187	RM202	11	189
RM163	5	124	RM206	11	147
RM164	5	246	RM287	11	118
RM169	5	167	RM247	12	131
			RM511	12	130

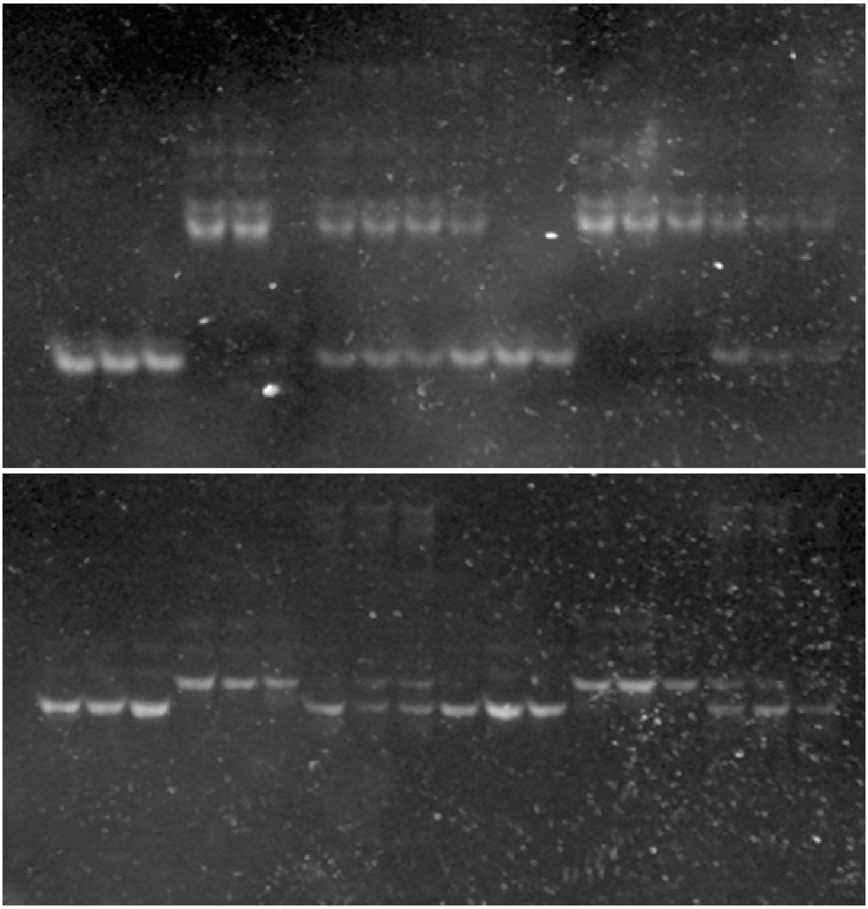

Figure 1. SYBR safe-stained electrophoretic profile of PCR-amplified SSR markers in Mestiso 19 (lanes 7-9) and Mestiso 20 (lanes 16-18) and its parental lines (lanes 1-6 and 10-15, respectively).

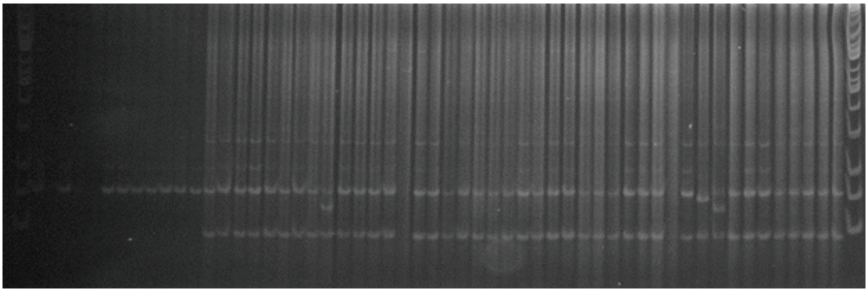

Figure 2. Electrophoretic profile of PCR amplified marker in PRUPTG102 harvested in Don Salvador Benedicto, Negros Occidental in December 2015.

II. Development/Improvement of Preharvest Technologies for Commercial Seed Production

Project Leader: SR Brena

This project deals mainly on hybrid seed production practices that will increase seed yield of released public hybrids intended to be commercialized. Only two TGMS hybrids, Mestiso 19 and 20 are commercialized. Although there were released CMS hybrids such as Mestiso 48, 49, and 55, reproducibility of the parental lines should be studied before these can be used for commercialization.

The TGMS hybrid rice seed production project funded by DBM started in 2012 and is now in progress for 7 production seasons, SxP seed production of Mestiso 19 is discouraging to hybrid seed growers in Davao. In DS 2014, the average seed yield of Mestiso 19 was only 935kg/ha whereas Mestiso 20 was 1885kg/ha. Increasing seed yield in S x P seed production has been a challenge to seed technologist. Thus several studies in this project aim to increase seed yield in Mestiso 19. Increasing seed yield to at least 1.5kg/ha will be high enough to attract more hybrid seed growers to continue partnering with PhilRice in producing F1 seeds of Mestiso 19. Studies in increasing seed yield focus on the use of phytohormones other than gibberellic acid, appropriate row ration, dosage and timing of GA3 application.

Another study in this project screens the response of restorer lines (pollen source) to high temperature. It has been the general observation of STD researchers during dry season in Negros that despite heavy pollen load of Mestiso 20 percentage seed set remained low. Published literatures revealed that pollen desiccates at temperatures higher than 35C. If screened restorer lines can withstand the high temperature during supplemental pollination, then we can probably increase seed yield hybrid seed production.

Enhancing pollen-stigma interaction to improve synchrony of pollination: strategy to break low SxP seed yield of Mestiso 19

REG Ragas, SR Brena, IV Boholano, M Palanog, VP Luciano

At the initial phase of the two-line hybrid rice breeding program, the thermo-sensitive genic male sterile (TGMS) breeding system is considered as economically feasible over cytoplasmic male sterile (CMS) system because of the absence of maintainer and restorer lines in TGMS. In the tropics where day length differences are marginal and where abnormally low temperatures seldom occur, maintaining male sterility in TGMS is easy. Together, these would have lessened the costs of producing hybrid seeds and consequently, reduce its price in the market. However, the consistent low F1 seed set hampers the expected cost savings.

Yield component data from multi-location seed production experiments showed that seed set percentage appears to be the limiting factor in attaining high yields in SxP of Mestiso 19. Seed setting in TGMS line depends upon the extent of outcrossing which is a function of the floral morphology and flowering behavior of TGMS and the male parents (Rahman et al., 2013). Stigma exsertion is especially emphasized as an effective floral characteristic that enhances outcrossing. However, TGMS line or male sterility, in general, often shows incomplete panicle exsertion, which prevents access to about 40% of the spikelets (Yan and Li, 1987; Tian 1991). Because spikelet houses the stigma, the failure of the spikelets to open creates a barrier for stigma exsertion. By reforming these floral characteristics to fit natural outcrossing, improvement on seed set of TGMS hybrid seed production may be possible.

There are important phytohormones that control floral characteristic improvement and are naturally present in a rice plant. Some of these include gibberellic acid (GA3) has an important role in fertility, in addition to allowing panicle exsertion, stamen elongation, and stigma exsertion, they are necessary for the development, release and germination of pollen. Boric acid (BA) is required for normal reproductive processes especially in pollen germination and pollen tube growth. Glycine (Gly) is important in plant growth and development, and is also associated with thermo tolerance in rice spikelets. Methyl jasmonate (MeJa) induces floret opening and stimulates the expansion of floret cells.

The concentration of these phytohormones, however, decreases toward maturity and thus, exogenous application may be important.

The objectives of this study are: (a) to investigate floral traits of parent component lines of Mestiso 19 and (b) determine yield components and their contributions to seed set as affected by exogenous application of phytohormones.

Highlights:

- Plant height difference between male and female parents was significant (p-value=0.00). Compared to control, male plants that received exogenous application of phytohormones grew significantly taller than their female partners (Figure 3). The highest height difference between parents was observed in plots that received a treatment combination of GA3+Gly+BA+MeJa. This would have a positive contribution to the number of dispersed pollen essential for cross-pollination success across rows of female parents.

- Seed set of female parents in rows vary. Those that were near to the pollen source appeared to have higher seed set than those in the middle rows (4, 5, and 6). One implication of this result is that the ratio between male and female parents can be adjusted to optimize cross pollination. Essentially, improvements in floral morphology such as dual stigma exsertion were common in plants that received methyl jasmonate (MeJa). As a plant hormone, MeJa induces floret opening and stimulate expansion of floret cells resulting to pistils with larger stigma.

- Percent flowering of male and female parents showed that flowering time and duration were extended through application of phytohormones. Significant effect was seen in all plots treated with gibberellic acid, glycine, boric acid, and methyl jasmonate. Since natural flowering synchrony in both parents is difficult to achieve, extension in the number of flowering days would enhance synchrony.

- In both seasons, the highest yield was consistently observed in plots treated with phytohormones compared to the control. Among all treatments tested, the highest yield was observed in plots treated with GA3 with average yield of 2020.32±65.03 kg/ha, but this was not significantly different to the average yield obtained in plots treated with GA3+Gly+BA+Meja (2017.72±178.45). It appeared that seed set in GA3-treated plots were slightly higher (26.31±5.46) than in GA3+Gly+BA+Meja-treated plots. Control plots gave 485.23±185.62kg/ha yield.

- Careful and rigorous validation of these findings and their cost-effectiveness are currently undertaken.

Table 11. Seed yield and yield components of Mestiso 19 during 2015 DS at PhilRice CES, Maligaya, Nueva Ecija.

Treatment	Productive tiller ($plant^{-1}$)	Total number of spikelets	Seed Set (%)	Panicle exsertion rate (%)	1000 grain weight (g)	Yield ($kg \cdot ha^{-1}$)
Control	7.20	143.30	7.43	76.45	22.38	485.23
GA3	8.30	143.70	26.31	76.77	22.76	2020.32
Gly+BA+ Meja	8.70	149.00	16.00	77.48	21.36	1253.26
GA_3+Gly+ BA+Meja	9.85	146.00	22.01	74.01	22.54	2017.72

Table 12. Seed yield and yield components of Mestiso 19 during 2015 WS at PhilRice CES, Maligaya, Nueva Ecija.

Treatment	Productive tiller (plant^{-1})	Total number of spikelets	Seed set (%)	Panicle exsertion rate (%)	1000 grain weight (g)	Yield (kg·ha^{-1})
Control	6.50	169.91	9.22	81.59	21.18	644.60
GA3	6.90	184.63	10.63	81.68	22.36	870.14
Gly+BA+ Meja	7.40	180.51	13.84	79.24	21.26	1116.73
GA_3+Gly+ BA+Meja	7.28	179.29	12.16	82.98	22.44	1011.44

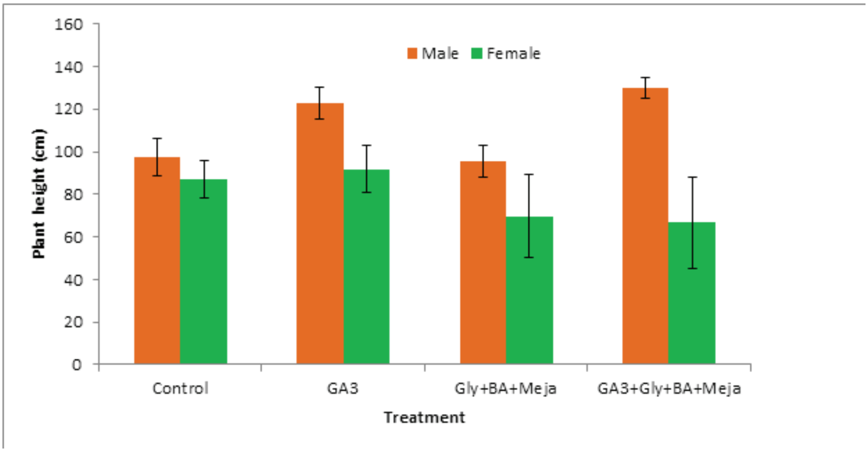

Figure 3. Plant height difference between parental lines as affected by different phytohormones, 2015 DS.

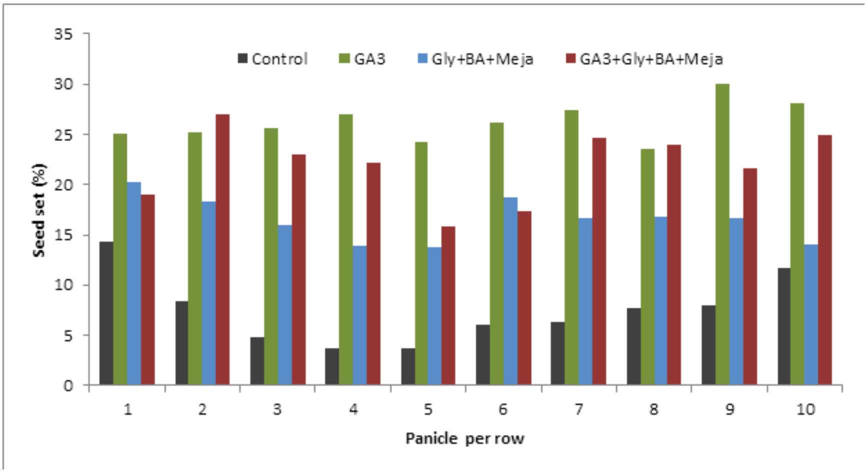

Figure 4. Seed set of panicle per row as affected by different phytohormones, 2015 DS.

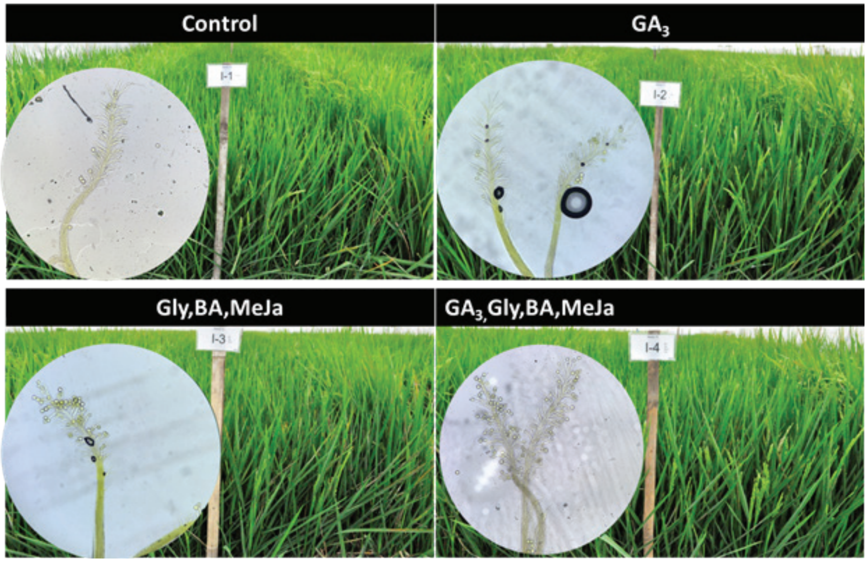

Figure 5. Variability in number of pollen grains attached to the stigma as affected by phytohormones.

Increasing seed yield in SxP seed production by increasing the row ratio and plant density of P line
SR Brena, M Osano-Palanog, and EP Rico

With the initial result obtained on increasing seed yield of Mestiso 19 by altering the row ratio of the seed parent to the pollen parent to 3:8 instead of the standard protocol of 3:10, seed yield increase was achieved in WS 2014 under PhilRice Negros condition. The attainment of higher seed yield in WS relative to DS was affected by temperature. Lower temperature prevailed during supplementary pollination in WS than in DS 2014

It is generally observed that pollen parent of Mestiso 19 less produced less tillers compared to Mestiso 20, regardless of location where SxP seed production is conducted. To increase pollen load that will be supplied to the seed parent (female parent) during supplementary pollination in Mestiso 19, three planting distance were used in establishing the pollen parent in SxP seed production, 15 x 15cm; 20 x 15cm; and 20 x 20cm (control).

The study aims to come up with the best row ratio of the seed parent and the pollen parent and to determine the best planting distance between pollen parent that will increase seed yield in Mestiso 19 seed production. Data presented in the tables below were mean values. Statistical analyses are under process.

Highlights:

- More filled grains were observed in 3:8 row ratio compared to 3:10. Planting distance of 15 x 15cm in P-lines under 3:8 row ratio gave the highest number of filled grains in the S-lines (Table 13).

- Percent seed set was generally higher in 3:8 row ratio, regardless of planting distance used. Although highest seed set was observed in 3:8 with 20x20 planting, there was negligible difference compared to 3:8 with 15x15 planting distance.

- Slightly higher percent seed set in the pollen parent rows was observed in the 3:8 row ratio.

- Computed yield/ha was highest in plots with 3:8 row ratio. Highest yield was noted in 20 x 20cm planting distance followed by 15 x 15cm (Table 14).

Table 13. Percent seed set in SxP seed production of Mestiso 19 in 2015 DS under PhilRice Negros conditions.

Row Ratio (P:S)	P-line Planting Distance (cm)	Filled grains	Unfilled grains	% seed set
S-lines				
3:10	15 x15	67	166	27.26
	20 x 15	77	198	27.17
	20 x 20	81	222	25.68
3:8	15 x15	128	226	36.46
	20 x 15	93	182	33.42
	20 x 20	117	192	36.60
P-lines				
3:10	15 x15	256	118	68.95
	20 x 15	276	127	65.09
	20 x 20	277	124	67.95
3:8	15 x15	262	121	67.43
	20 x 15	244	110	67.78
	20 x 20	281	131	69.50

Table 14. Yield data obtained under SxP seed production using two row ratios of S-lines to P-lines.

Row Ratio	Planting Distance in P-lines	Plot yield (gm)	Computed yield/ha
S-lines			
3:10	15x15	149.7	1357.1
	20x15	143.8	1310.7
	15x15	126.5	1141.8
3:8	15x15	147.2	1326.7
	20x15	125.8	1144.0
	20x20	158.0	1433.1
P-lines			
3:10	15x15	111.6	3358.4
	20x15	108.0	3258.3
	15x15	117.0	3515.9
3:8	15x15	100.3	2999.7
	20x15	108.7	3283.8
	15x15	111.3	3363.7

Effect of temperature on growth, grain yield, pollen development and grain quality of selected restorer lines and pollen parents

LV Guittap and SR Brena

One of the most effective ways of mitigating the effect of climate change is the deployment of high temperature-tolerant cultivars. In breeding stress tolerant crops, information on the effect of temperature to rice growth and development is necessary. It is important to look at other aspects on how temperature affects rice such as the pollen behavior, spikelet sterility and fertility and grain quality to further understand the mechanism of heat stress. Conducting a field experiment to determine the effect of temperature would provide a broader understanding of the complex nature of temperature effect on growth performance. Furthermore, it is also important to determine what specific stage or stages are most vulnerable to the stress. This would be helpful in recommending cultural management practices to reduce if not eliminate the effect of heat stress. In hybrid seed production for example, flowering behavior and agro-morphological characteristics are important. Quantifying the effect of temperature on pollen sterility and days to flowering for synchronization would be helpful in attaining high seed yield. This would also aid in understanding pollen behavior.

The study was conducted mainly to identify the influence of temperature on flowering behavior and pollen growth and development of different restorer lines and pollen lines. Also other important agro-morphological characteristics will be identified.

Highlights:

- Six (6) restorer lines (R-lines) and two (2) pollen parents (P-lines) were tested in Los Banos (LB) and Central Experiment Station at Maligaya (CES). The R-lines lines include IR34686R, IR60819R, and IR71604R, the restorer lines of three popular hybrids Mestizo 1, Mestiso 3 and Mestiso 7. Other R-lines were IR60912R, SRT3R, and IR73385R, the male parents of Mestiso 21, Mestiso 25, and Mestiso 26 respectively. Pollen parents tested in the study were TG101M and TG102M which are the P-lines of two popular TGMS-based hybrids Mestiso 19 and Mestiso 20. Restorer lines with their respective hybrids are summarized in Table 15.

- Temperature data at Los Banos were gathered at PAG-ASA Agromet Station at UPLB. Maximum temperature did not reach more than 35 C during the critical months in 2014 and 2015. In the case of CES, data were gathered with three data points 9:00AM, 11:00AM and 2:00PM. The maximum temperature was recorded in May 2014 at 34.84 oC (Table 16 &17).

- The flowering data were already reported. Microscopic evaluation are currently being done are to assess the staining of the lines affected by temperature and to determine the surface appearance of the pollen. Five plants were randomly selected from each entry and then fixed with 70% alcohol. Sampling was done in three different positions of the panicles, top, middle and bottom.

- Surface appearance was evaluated also under microscope. Four (4) Classifications were based on the Hybrid Rice Breeding Manual (IRRI, 1997) namely unstained withered sterile, unstained spherical sterile, stained round sterile, and stained round fertile.

- Pollen samples were also collected on the onset of flowering. Evaluation for pollen fertility and other pollen characteristics will be done under a microscope using IKI solution as stain to determine the effect of temperature to pollen. The samples will be observed in three fields and will be scored using the SES published by IRRI.

Table 15. List of restorer lines tested with respective hybrids and days to 50% heading.

Lines	Hybrid involved	Days to 50% flowering			
		Los Banos		Nueva Ecija	
		DS	WS	DS	WS
IR34686R	PSB Rc72H	105	105	105	105
IR60819R	NSIC Rc116H	95	97	93	94
IR71604R	NSIC Rc136H	89	90	87	87
IR73385R	NSIC Rc230H	95	95	92	92
SRT3R	NSIC Rc232H	90	91	87	88
IR60912R	NSIC Rc206H	95	95	93	94
TG101M	NSIC Rc202H	86	87	88	89
TG102M	NSIC Rc204H	96	97	98	98

Table 16. Temperature at Los Banos during the experiment (source: Agromet Los Banos).

Month	Temperature (C)		
	Minimum	Maximum	Average
January 2014	20.5	28.7	24.6
February 2014	20.7	30.5	25.6
March 2014	22.2	31.7	27.0
April 2014	23.7	34.6	29.1
February 2015	21.0	29.5	25.3
March 2015	21.8	31.1	26.5
April 2015	23.4	32.9	28.2
May 2015	24.1	34.2	29.2

Table 17. Temperature at CES during the experiment.

Month	Temperature (oC)		
	9:00AM	11:00AM	2:00PM
March	28.22	30.11	31.71
April	28.63	30.70	32.33
May	31.14	33.56	34.84

Optimizing Seeding Interval, Row Ratio, and Timing of GA3 Application for Increased Seed Yield of M20 in PhilRice Negros

BUT Salazar

Seed production in two-line hybrids is faster since maintainer line is no longer required compared with three-line hybrids (Virmani et al., 2003). In other countries like China, two-line hybrids have 10% more yields compared to three-lines. In the Philippines, PhilRice Negros (having favourable temperature for two-line hybrid seed production) has been producing F1 seeds of M20, a major two-line hybrid variety commercially released in the country, yet increasing the seed yield to 1t/ha remains a major challenge. Initial data gathered from the station's seed production area WS 2012 and 2013 showed that average spikelet fertility ranged from 25 to 30%. Though this is very close to the ideal seed setting rate (during the fertile phase) of >30% (Virmani et al., 2003), seed yield was only 0.43 and 0.30t/ha, respectively. These figures are far from China's 2.5 to 3.0t/ha average seed yield for two-line hybrids (Yuan, et al. _; Lu et al., 1998). Though it is inappropriate to compare seed yields from two countries considering the differences in the parentals, agro-climatic conditions, and cultural management practices, among other factors, it is worthwhile to identify which factors influenced the seed yield obtained in the station. Through this, improvements on the practices can be done.

Generally, this study aims to increase seed yield of M20 in PhilRice Negros by 10% by 2016 through optimization of seeding interval, the row ratio and timing of GA3 application. The study has 3 substudies; sub-study 1. Synchronizing flowering of NSIC Rc204H parentals through optimum seeding interval; sub-study 2. Determining the appropriate row ratio for increased seed yield of M20 at PhilRice Negros; and sub-study 3. Determining the optimum timing of GA3 application for increased seed yield of M20 at PhilRice Negros. On-going data processing for the result of WS 2015.

Highlights:

- Generally, yield is highest in plants sown following the effective accumulated temperature (EAT), followed by transplanting when leaf age (LA) is 4.5 to 5, then the conventional which uses the differences in growth duration (GD) of the parentals (Table 18). Furthermore, more filled grains were observed from EAT, followed by LA, and least were from GD treatments.

- Grain yield from EAT treatment was significantly higher by 230 kg compared with LA, and 370kg more than those harvested from GD plants. On the other hand, the 3:8 row ratio gave the highest yield with 1.53t/ha, which is significantly higher than the rest of the treatments (Table 19).

- As you increase the number of S lines, e.g. 3:8 and 3:10, the number of filled grains decreases, while the number of unfilled grains increases. The opposite is observed in the 2:4 and 2:6 treatments.

- In sub-study 3, plants are generally taller when GA3 are sprayed in three splits compared to two splits regardless of timing of application (Tables 20 and 21). While productive tillers and length of the panicles are not significantly different, the number of filled grains and spikelet fertility showed notable differences across treatments.

- Generally, spikelet fertility is higher in plant sprayed with GA3 in three splits at 5 to 10% heading, than the rest of the treatments. Compared to control, grain yield is highest when GA3 is split 3times and sprayed at earlier heading. Grain yield is also higher when GA3 is split into two, and sprayed at a latter heading (35%). When spraying at earlier heading of S (5 to 10%), applying GA3 in three splits led to higher yield compared to two splits. However, spraying GA3 in two splits at later heading (35%) yielded more than three splits.

Table 18. Yield and yield components under different seeding interval, DS 2015.

Treatment	Plant height (cm)	Prod tiller	Panicle length (cm)	Panicle exerted (cm)	Filled (no.)	Unfilled (no.)	Spikelet fertility (%)	Yield (tha^{-1})
LA	100.31	11	22.73	19.77	36.67 a	87	29.7	1.25 b
GD	96.38	11	22.00	18.9	29.53 b	81	26.83	1.11 b
EAT	101.72	10	22.73	20.03	40.07 a	80	33.5	1.48 a
ANOVA	ns	ns	ns	ns	*	ns	ns	*
CV (%)	4.71	10.14	4.13	2.97	8.00	12.18	10.48	6.66

*ɔt significant; *significant at 5%.*

Table 19. Yield and yield components under different row ratio, DS 2015.

Treatment	Plant height (cm)	Prod tiller	Panicle length (cm)	Panicle exerted (cm)	Filled (no.)	Unfilled (no.)	Spikelet fertility (%)	Yield (tha^{-1})
2:4	96.87	8.20	22.67	20.07	48.00	75.00	39.70	1.15 bc
2:6	98.87	8.70	22.80	19.50	45.00	80.00	37.23	1.14 c
3:8	92.03	8.83	22.57	19.10	41.00	78.00	34.03	1.53 a
3:10	93.67	9.07	22.43	18.80	37.00	88.00	30.47	1.32 b
ANOVA	ns	ns	ns	ns	ns	ns	ns	**
CV (%)	3.78	10.32	1.96	3.02	10.17	9.07	10.62	6.85

Table 20. Difference in height before and after treatment application, DS 2015.

Timing and ratio of GA_3 application	Change in height (difference in height before and after GA_3 application, cm)			
	S	P3	P2	P1
2:6:2 at 5-10% S	24.17ab	62.27ab	53.90ab	61.23ab
2:8 at 5-10% S	24.73a	75.07a	61.10a	80a
control	8.23c	15.23c	15.10c	18.93c
2:8 at 35% S	12.33bc	59.67ab	53.13ab	57.50ab
2:6:2 at 35% S	23.8ab	52.77b	42.87b	44.93b
ANOVA	*	**	**	**
CV (%)	13	10.85	16.6	15.83

*icant at 5%; **significant at 1%.*

Table 21. Yield and yield components under different timing and ratio of GA3 application, DS 2015.

Treatment	Plant height (cm)	Prod tiller	Panicle length	Panicle exerted	Filled grains	Unfilled grains	Spikelet fertility	Yield (tha^{-1})
2:6:2 at 5-10% S	102.45a	11.06	22.02	19.92a	66.37a	96.28	41.03a	1.68a
2:8 at 5-10% S	99.25a	10.67	22.37	19.74a	56.32ab	95.17	38.07a	1.34b
control	76.89c	9.93	21.92	17.63b	32.74c	101.72	24.72c	0.71c
2:8 at 35% S	89.32b	10.53	22.24	19.43a	47.84b	103.45	31.81b	1.59a
2:6:2 at 35% S	98.92a	10.40	21.80	19.37a	46.95b	94.56	33.82b	1.34b
ANOVA	**	ns	ns	**	**	ns	**	**
CV (%)	5.08	6.46	2.10	3.08	12.03	10.30	5.14	6.78

III. Hybrid Basic Seed Production and Hybrid Seed Research

Project Leader: SR Brena

Public hybrid breeding is done by institutions such PhilRice, IRRI, UPLB, and PhilScat. Once a new public hybrid variety is released, the parental lines are purified and once pure parental lines are available, production of nucleus and breeder seeds (BS) begin. At present, nucleus and BS production are focused on the two TGMS hybrids, Mestiso 19 and 20. PhilRice released Mestiso 29 (CMS) in 2011 but this hybrid was not commercialized because of poor eating quality of cooked rice when cooled and the F1 plants lodged often. In 2013, three CMS hybrids were released, Mestiso 47, 48, and 49. Among these three hybrids, only parental line multiplication of Mestiso 48 was conducted.

Once the parental lines are available, development of seed production protocols are developed to be distributed together with seed kit to hybrid cooperators for seed production trials. From the seed kit provided, days to heading, flowering, flowering duration, and time of flowering and closing are gathered. These parameters provide basis for synchronization of flowering once the hybrid has commercial value.

To date, the study on seed production capacity of hybrids should be conducted before the variety is released in order to determine ahead the materials with commercial value. Once the variety is released, parental lines are already available for hybrid seed growers who will partner with PhilRice in seed production. Parental lines of any particular hybrid is ready for distribution after the materials are tested for genetic purity using GOT. Following this scheme of making hybrid parental lines and its F1 seeds become available on time will make the hybrid seed production project of PhilRice sustainable.

Nucleus and breeder seed production studies for new recommended hybrid varieties

LV Guittap, WB Abonitalla, SH Escamos and TM Masajo

The hybrid rice technology has proved to be effective in increasing production of rice in the country and elsewhere. To date, 67 hybrids have been released by the National Seed Industry Council (NSIC) of which about 23 were developed by IRRI, PhilRice, and UPLB, are considered public hybrids. Four of these hybrids are popular and are widely grown by farmers. Several newly released hybrids have been identified as potential replacement for the currently grown varieties. These hybrids are Mestiso 32 (NSIC 2011 Rc250H) and Mestiso 55 (NSIC 2014 Rc368H). Upon release of a hybrid variety, seed production of parents and F1 should follow to popularize and commercialize the hybrid. Likewise, protocols on basic and F1 seed production methods for the new hybrids should be studied and established in order to give proper recommendations to hybrid seed growers. The project had the following objectives: a.) Check purity and genetic identity of component (parental) lines in NSIC 2011 Rc250H (Mestiso 32) and NSIC 2014 Rc368H (Mestiso 55); b.) Characterize the two new NSIC hybrids released and the component parents based on agro-morphological and grain characters; c.) Develop protocol on the method of basic seed production of the parents and the F1 seeds of Mestiso 32 and Mestiso 55; d.) Field test the seed production protocols developed for Mestiso 32, and Mestiso 55 and e.) Do initial seed increase of the parents of Mestiso 32, and Mestiso 55 to anticipate popularization and commercialization.

Highlights:

- In collaboration with the hybrid commercialization group of PhilRice, this study focused on two CMS-based hybrids which are in line and being considered as hybrids for commercialization. Component lines of Mestiso 32 and Mestiso 55 approved by NSIC on 2011 and 2014 respectively were included in the study.

- The component lines of NSIC 2011 Rc250H or Mestiso 32 are IR68897A, IR68897B and IR73013R. Small quantities of breeder seeds for each line are already available since 2014 wet season. The seeds were used for seed production studies for several sites like CMU and Isabela. For this season, seed production activities were conducted to multiply the seeds in anticipation for large scale planting.

    a. Head-to-row evaluation nursery was established during the season. About 500 panicles which were selected in 2014 wet season were planted in head-to-row arrangement. The entries were evaluated based on uniformity and trueness. Also days to 50% flowering and other agro-morphological parameters were noted. After evaluation, around 299 entries were selected and bulked. From these, 252kg of seeds were harvested as nucleus seeds.

    b. Seeds of the A-line and B-line were also produced. Since A-line and maintainer line are also the same with existing hybrids being commercialized, they were included in breeder seed production. Around 75kg A-line with corresponding B-line harvested were allotted to the Mestiso 32 hybrid. These seeds will be utilized in packaging seed familiarity kits and for further studies in stations involved.

c. Breeder seeds of IR73013R (R-line of Mestiso 32) was also produced during the season. A total 683 kg seeds were processed and packaged for seed certification.

d. Currently, 90kg breeder seeds of IR73013R is stored at Los Banos. Seeds for 1-hectare foundation seed production establishment for A, B, and R-lines were given to CMU for 2015 wet season planting.

e. For 2015 wet season, seed purification process will be done to address the sterility problem observed in Mestiso 32 hybrid.

- In the early part of 2015 DS, component lines of Mestiso 55 were requested from CES. The parental lines are as follows: IR79128A, IR79128B and PR31559R. From the seeds received, a germplasm file was processed and packed for storage to serve as future reference and original seed stock.

- An observation nursery was established for A, B and R-line of Mestiso 32 and 55 to monitor the flowering behavior and to characterize the parentals. The information generated will be compiled and use as basis in formulating seed production protocols. Based on the dry season data (Table 22), days to 50% heading (DTH) of IR73013R is 104 days. On the other hand, DTH of IR68897A and B (Mestiso 32) is 79 days. For Mestiso 55 component lines, initial observations showed that R-line, PR31559R flowered 80 days after seeding. This is a rare case in CMS-lines since the restorer line flowered earlier compared to the A-line (IR79128A) at 89 days. Generally in CMS parental lines, A-lines usually flowers earlier compared to restorer lines. Further testing is needed to confirm this data.

- Other hybrid being considered for release is PRUP 10. The TGMS parent of the hybrid is PRUP TG101 while the pollen parent is SN 758. During the season, original seeds of SN 758 were forwarded to the group. The seeds were processed and samples were stored as seed file and original seed stock for future reference. NBSP staff with the help of TGMS group at LB selected 670 panicles from the source population. The panicles will be established in head-to-row to purify the pollen parent. The female parent on the other hand was not requested since there are available seeds in the storage.

Table 22. Flowering and plant height of Mestiso 32 and Mestiso 55 component lines at LB condition. 2015 DS.

Component line	Days to 50% heading	Plant height (cm)
Mestiso 32		
IR68897A	78	76
IR68897B	78	76
IR73013R	104	122
Mestiso 55		
IR79128A	89	113
IR79128B	89	122
PR31559R	80	120

Hybrid and nucleus and breeder seed production

LV Guittap, WB Abonitalla, SR Brena, SH Escamos and TM Masajo

Successful commercial exploitation of hybrids in highly autogamous cereal crops like rice depends on the extent of superiority of hybrids over existing popular inbred varieties and the ease at which F1 seeds could be economically produced. It would take good-performing hybrids and an organized and efficient system of seed production and distribution to popularize and commercialize hybrid varieties. Like all hybrids involving inbred parental lines, genetic purity of the parents must be maintained to produce quality hybrid seeds in commercial quantities every time required. Pure, true-to-type and high quality seed is essential for the successful implementation of government's hybrid rice commercialization program. This project at PhilRice Los Baños was assigned the responsibility to produce and distribute basic seeds of released public hybrids. These are the hybrids bred by IRRI and PhilRice, tested in the NCT and released as varieties by the National Seed Industry Council (NSIC). The project is jointly implemented by PhilRice Los Baños in collaboration with UPLB and IRRI.

Highlights:

- Four (4) public released hybrids bred by PhilRice, UPLB and IRRI: PSB Rc72H (Mestizo 1), NSIC Rc136H), NSIC Rc136H (Mestiso 7), NSIC Rc202H (Mestiso 19), and NSIC Rc204H (Mestiso 20) are involved in this study. These hybrids are currently being planted in commercial scale particularly the thermo-genetic male sterile (TGMS-based) hybrids Mestiso 19 and 20. The component lines of these hybrids are purified, maintained, multiplied and distributed to support the government commercialization program.

- For this period, AxB paired-crosses of CMS-hybrids Mestizo 1 and Mestiso 7 were generated (Table 19a.). Pollen samples were collected from 2,038 A-line plants in the AxB seed production plots. The A-line plants were evaluated in the laboratory under a microscope to determine the extent of sterility. A total of 1,005 were found to be completely sterile (CS) and were considered for crossing with corresponding B-lines (maintainer lines).

a. For IR58025A, the female parent of Mestizo 1, around 1,138 A-line individuals were tagged, sampled and evaluated. After evaluation, about 43% or 491 plants were found to be CS and were crossed with respective maintainer lines. Out of the 491 crosses, 316 pairs were effective (crosses that produced sufficient amount of seeds). On the other hand, the A-line of Mestiso 7 IR68897A which is also the CMS-line of other released hybrids such as Mestiso 29 and Mestiso 32 generated 514 crosses from 900 plants evaluated. A total of 287 crosses were considered effective for the particular A-line.

b. The total 603 effective crosses generated from IR58025AXB (316) and IR68897AXB (287) were labeled correspondingly. At least 30 seeds from each of the A-line entries were collected and seeded for evaluation this coming 2015 wet season. Basis for evaluation will be sterility, uniformity, trueness and other agro-morphological parameters.

- Seed production plots at male fertile environment (MFE) site located in Tublay, Benguet were considered to be the source population of TGMS plants to be selected to generate nucleus seeds of S-lines of Mestiso 19 and Mestiso 20. More than 1000 individual plants from each of the S-lines were collected (Table 19b.).

a. A total of 2,267 individual plants were selected from the source population for the S-line of Mestiso 19 (PRUP TG101) and Mestiso 20 (PRUP TG102). Out of the total plants 1,045 were PRUP TG101 and about 1,222 were PRUP TG102. The plants were tagged and harvested individually. Upon drying in the oven, the panicles were threshed and labeled correspondingly. For each entry, 40 seeds were separated and will be seeded for evaluation in sterility, uniformity, trueness and for other agro-morphological characteristics at the male sterile environment (MSE)

- Breeder seed production of hybrid parental lines was done in Los Banos for the CMS hybrids and Tublay, Benguet for the TGMS parents. Amount produced for each line is summarized in Table 23.

a. During the period, a total of 1,216kg A-line were produced. For the A-line of Mestizo 1, (IR58025A) about 615kg A-line seeds were harvested. From a total area of 0.68ha, the IR58025AxB seed production plots yielded 904kg//ha on the average with a yield ranging from 800 to 1080kg/ha. On the other hand for IR68897A, a total of 601kg were harvested and processed for certification. As for the maintainer or B-lines, only IR58025B was harvested since sufficient IR68897B were available in the storage.

b. Restorer line of Mestizo 1 (IR34686R) and Mestiso 7 (IR71604R) were also multiplied during the season. A total of 415kg IR71604R and 370kg IR34686R were produced and were already submitted to the NSQCS for certification.

c. Pollen parents of Mestiso 19 and Mestiso 20 which are TG101M and TG102M respectively were also produced at Los Banos. A total of 838kg of P-lines were produced in which 454kg were TG101M and 384kg TG102M.

d. During the dry season, breeder seed production for S-lines was undertaken at Tublay, Benguet. The TGMS-lines of Mestiso 19 and 20 were planted in the area. About 254kg PRUP TG101, the S-line of Mestiso 19 was produced. On the other hand, PRUP TG102 (S-line of Mestiso 20) produced about 270kg.

- An added responsibility was given to the project with the DAs TGMS hybrid commercialization program. Requirements for S-line foundation seeds for the nationwide SxP seed production was produced under the management of NBSP. An MFE site located at Lucban, Quezon were planted with PRUP TG101 during latter part of December. A total of 1,763 kg of seed were produced during the period. The seeds were submitted for foundation seed certification.

- Amount of hybrid parents produced and distributed in the period from January to June 2015 is shown in Table 20. Breeder seeds of CMS-based hybrid parentals distributed were mainly for foundation seed production. The S- and P-lines dispatched were for S-line foundation seed production in MFE sites and P-lines in MSE area. Most of the breeder seeds of IR58025A and IR68897A seeds produced this season however were used for AxR F1 seed production for the 2015 wet season. Sufficient amount of breeder seeds of hybrid parentals of public released hybrids are kept in the cold rooms at Seed Processing and Storage Facility at Los Banos. They are distributed to accredited hybrid seed growers on request.

Table 23. Summary of activities in the generation of paired crosses. 2015 DS.

Parental lines	Pollen evaluation		No. of A-line plants evaluated	A-line plants selected for paired-crossing (%)	No. of crosses made	No of effective crosses
	CS	HS and below				
IR58025AxB	491	647	1138	43	491	316
IR68897AxB	514	386	900	57	514	287
Total	**1005**	**1033**	**2038**		**1005**	**603**

Table 24. Summary of activities in plant selection at MFE. 2015DS.

Parental lines	No of plants tagged	No of plants harvested	No of plants processed
PRUP TG101	1100	1100	1045
PRUP TG102	1400	1400	1222
Total	**2500**	**2500**	**2267**

Table 25. Summary of amount (kg) of breeder and foundation seeds produced and distributed during the 2015 dry season at PhilRice Los Baños.

Hybrid/Parental	Amount of breeder seeds produced (kg)	Amount of foundation seeds produced (kg)	Amount of Seeds Dispatched (kg)		
			AXB/S	AXR	Research
Mestizo 1					
IR58025A	615	-	60	300	
IR58025B	138	-	24		
IR34686R	370	-	20		
Mestiso 7					
IR68897A	601	-	45	300	
IR68897B	-	-	18		
IR71604R	415	-	15		
Mestiso 19					
PRUP TG 101	254	1763	15		4
TG 101M	454	-	5		2
Mestiso 20					
PRUP TG 102	279	-	15		4
TG 102M	384	-	5		2
TOTAL					
A-line	**1216**	**-**	**105**	**600**	**-**
B-line	**138**	**-**	**42**	**-**	**-**
R-line	**785**	**-**	**35**	**-**	**-**
S-line	**533**	**1763**	**30**	**-**	**8**
P-line	**838**	**-**	**10**	**-**	**4**

Identification of the best location and time of the year/season optimum for seed production and quality

SR Brena and LV Guittap

SxP seed production of Mestio 19 and 20 was tried in other location to make seed available in various location on time. The current program on TGMS hybrid rice seed production project is concentrated mainly in Davao Oriental but utilization of the hybrids is mainly in Luzon. Identifying SxP seed production site outside Mindanao would benefit farmers. Other done SxP seed production, male ferlie environment was identified in Bicol and S-line seed production was established in May to augment the seed requirement of PRUPTG102 requirement in Mindanao.

SxP seed production was established in DS 2015 in BIARC, San Agustin, Pili, Camarines Sur. One fourth hectare was planted with parental lines of Mestiso 19 following the standard protocol of 3:10 ratio of the pollen parent to the seed parent. The area was only established in May since TGMS hybrid seed production must not experience cold spell that prevail during the months of January to March.

Highlights:

- In DS 2015, no problem was observed in the field from transplanting to maximum tillering. However, no yield was attained because of failure in flowering synchronization. Water became limiting because of the late establishment of the SxP plot.

- SxP seed production was tried in other locations in WS 2015 owing to lack of parental lines.

- The S-line multiplication established in Bicol failed because the prevailing temperature during production is higher than the requirement for S-line multiplication.

Flowering behavior and seed production capacity of hybrid parental in different locations and seasons

SR Brena and LV Guittap

Parental lines of a promising TGMS hybrid was established to determine the flowering behavior of the parental lines when planted in different locations. The study also aims to determine the susceptibility of the parental lines to diseases. The parental lines of this promising hybrid is PRUPTG101 (the same female parent as Mestiso 19) and SN758. The parental lines were planted in CES; Agusan; Bicol; and Negros.

Highlights:

- Female and male parental lines were soaked at the same time and transplanted after 21 days to establish the best differential seeding to be used in each location.

- In the case of Agusan flowering behavior of both parental lines was almost observed at the same time and 3 day differential seeding can be used similar to the seed production protocol of Mestiso 19. Blooming habit of both parental lines in Agusan 83 days after soaking was shown below (Figure 6).

- In Negros and CES, the same differential seeding interval can be used.

- Male parent was susceptible to tungro and this was observed in Negros and Bicol. In PhilRice – Bicol, the field was flowed under due to tungro incidence.

Figure 6. Flowering 85 days after soaking.

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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