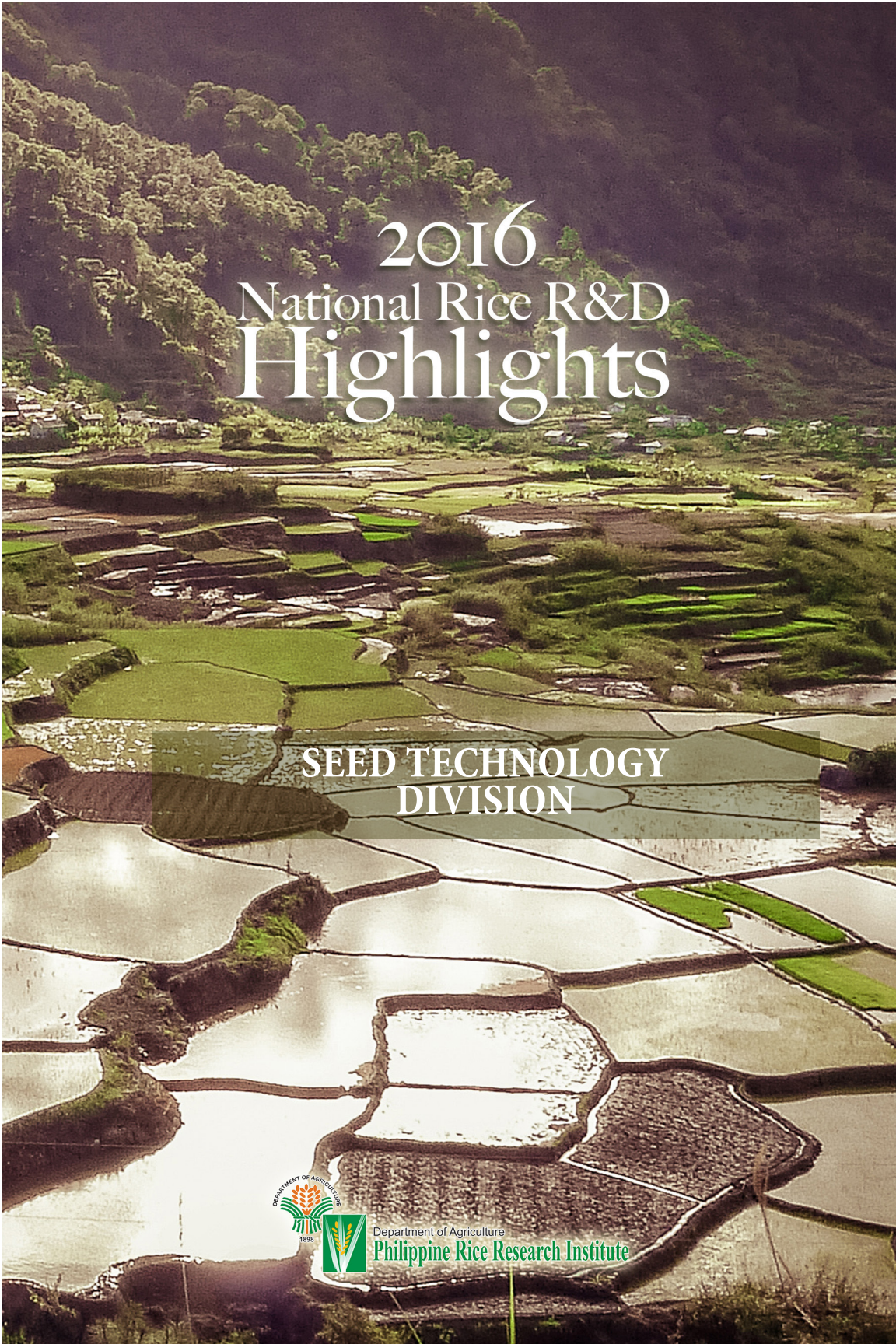

2016 National Rice R&D Highlights

SEED TECHNOLOGY DIVISION

Department of Agriculture
Philippine Rice Research Institute

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Seed Technology Division

Division Head: Susan R. Brena

Executive Summary

The Seed Technology Division leads PhilRice efforts to ensure high quality of seeds and maintain adequate and timely supply of seeds for seed growers and farmers. Supply of good quality seed contributes to good crop stand and performance in the field. For 2016, the Division has three major projects with focus on seed quality assurance in PhilRice seed stocks, improvement of pre-harvest and post-harvest technologies for commercial seed production, and hybrid basic seed production and hybrid seed research.

Good quality seed signifies productive yield. The Division ensures seed quality of PhilRice-produced seeds by implementing three seed quality assurance assessments such as: rigorous field inspection of seed production areas, seed vigor and viability testing of seed stocks, and seed purity testing using grow-out test (GOT) and simple sequence repeat (SSR) markers of inbred, hybrid and parental lines. A total of 306 varieties for both DS2016 and WS2016 (nucleus seeds, breeder seeds, foundation seeds and registered seeds) in 324 field lots were inspected. Despite the high percentage purity in the field at the final inspection, all postharvest operations should be done properly to assure high final seed purity. Seed vigor and seed viability testing were also performed for the breeder seed stocks in warehouse storage produced in DS 2015 including other old seed stocks. In DS2016, 8 BS varieties, FS, and RS were tested for varietal purity after threshing, drying and seed cleaning. In WS2016 varietal purity testing, 23 BS varieties were tested and 6 passed as breeder. For Foundation seed production (FSP) 4 varieties passed while for RS 3 passed after seed cleaning. For seed viability testing of inbred seeds stock from BDD warehouse, WS2014, DS2015, WS2015, and DS2016 BSP, FSP and RSP were tested. Parental lines produced from DS2015 and WS2015 were also tested. Out of 5 FS and 2 CS of WS2014 only 1 and 2 passed viability of 85% seed germination, respectively. All FS varieties of DS2015 did not passed and only 4 FS of WS2015 passed while only 4 RS of WS2015 passed viability testing. On the other hand, all FS seed lots of 2016DS FS passed and parental lines of DS2015 had good germination rates while those produced 2016WS had 0% germination.

Breeder seeds produced in DS2015 were tested twice by accelerated ageing test (AAT) and showed low seed vigor in both testing dates with 2 varieties which failed seed vigor testing. The results show that the seeds deteriorated through time even under cold room condition. For FS produced WS2015, 8 out of 12 passed vigor testing and 6 passed the seedling emergence test while 9 out of 13 RS of WS2015 passed vigor testing. However, seedling emergence test on WS2015 RSP had lower

percent germination compared to vigor testing results. For DS2016 FSP seed stock, 10 varieties were tested for vigor and seedling emergence, only NSIC Rc360 passed vigor test and only NSIC Rc308 passed the seedling emergence test. Parental line seed stocks tested had 95% average germination for vigor testing, however upon testing last November, germination decreased to around 0 - 17 % germination rate.

Seed purity through conventional GOT and using SSR markers determined high genetic purity in hybrids and parental lines tested. Only two seed lots did not pass the standard ≥97% purity required for hybrid parental lines before distribution. To expedite the distribution of parental lines produced in DS2016, 6 seed lots of PRUP TG102 from DSB Negros were analyzed through DNA testing. SSR marker-based seed purity tests were performed. Using RM127 as informative SSR marker, three out of fifteen PRUP TG102 seed lots were found to have impurities, while only one out of sixteen was found in PRUP TG101 seed lots. In WS2016, 39 seed lots of PRUP TG102 and PRUP TG101 from Negros, Isabela and Los Baños were analyzed. Further assessments of seed purity using informative SSR markers (RM1 and RM511) showed the same results as previously identified.

Realization of the full potential of TGMS hybrid seed production depends not solely on the breeding process but also on the technological interventions performed in the field. Through the technologies utilized during pre-harvest and post-harvest stages such as application of phytohormones to enhance synchrony of pollination, optimization of the row ratio and increase in the plant density of the P-lines, seed yield in S x P production can be ameliorated. From the study, exogenous application of phytohormones significantly enhanced plant height of male parents compared to their female partners. The highest height difference between parents and highest yield were observed in plots that received the treatment combination of GA3+Gly+BA+MeJa. Through evaluation of four row ratios for improving S x P seed production of Mestiso 19, the 3:6 and 3:8 P-line to S-line row ratios were found to produce the highest seed yield.

The success of hybrid rice cultivation depends on the efficiency of the hybrid seed production program which enables seed producers to provide high quality seeds at a reasonable price. In the third project, hybrid basic seed research particularly in nucleus and breeder seed production for new recommended hybrids was implemented. Moreover, development of possible alternative to the control plot and strategies for pollen harvest and storage are being undertaken. During the early part of DS 2016, a source population was established for the parent lines of Mestiso 48 and Mestiso 55. From the amount of seeds given, a total of 200 A x B paired crosses for each hybrid were generated. For the newly-released TGMS hybrid PRUP10 (NSIC Rc446H), purification process was conducted last WS 2015 and parental line seeds were sent to Davao del Sur, Davao del Norte, Davao Oriental, South

Cotabato, and Negros Occidental. High yield in the S x P seed production of PRUP10 was only achieved in Davao del Sur with total seed yield of 1,775 kg/ha compared to PhilRice –Isabela and PhilRice – CES. The flowering behavior of new TGMS hybrid PRUP10 was evaluated in Davao del Sur, PhilRice – Negros; PhilRice –Isabela; PhilRice – Los Baños and PhilRice - CES during the wet season.

Two protocols used to evaluate PRUP10's flowering behavior in CES showed that the P-line had flowering duration of 6 – 8 days while the S-lines were observed to flower longer by 2 days. The same duration of flowering and days to heading was observed in the two protocols.
The P-lines of Mestiso 19 and 20 were bred with a purple-base and are already undergoing purification starting WS2016. Nucleus and breeder seed production of CMS (A), maintainer (B) and restorer (R) lines focusing on the purification and production of the parent lines of Mestizo 1 and Mestiso 20 were also performed. Sufficient supply of hybrid parental lines particularly of Mestiso 19 and 20 have been produced in DS2016, and seeds were distributed to various PhilRice stations and hybrid seed growers.

As an alternative to the control plot in TGMS seed production, initial data suggests bagging as an effective method, although additional experiments are needed to substantiate the initial findings. An effective pollen storage strategy is being identified so that viable pollen can be artificially loaded onto stigmas to increase pollination and yield. Initial data indicates that growing pollen grains in media 2 (3% Ca(NO3)2 + 5% Sucrose +1% Agarose +10 % BA) appears to maintain pollen viability even after 1 hr after anthesis. Pollen tube length was consistently high at anthesis. Comparison of different media reveals that pollen tube length grown in medium 2 does not seem to differ between 30 min and 1 hour. Pollen germination declines rapidly regardless of media used thus, to increase the chance of effective pollination, pollen grains must be collected at anthesis and stored immediately in amber glass at -5°C.

The Division is implementing a cohesive set of projects that aims to synergistically enhance hybrid seed production particularly of TGMS hybrids and maintain high seed quality of both inbred and hybrid PhilRice-produced seeds. These are of utmost criticality in realizing the goals of the DA-PhilRice hybrid commercialization program and the national agriculture efforts in general.

I. Seed Quality Assurance in PhilRice Seed Stock

Project Leader: SRBrena

High seed quality is a primary determinant of crop productivity. The term "seed quality" is used to describe a set of characteristics or attributes that determine its value for sowing. Agricultural progress depends on the production and distribution of high quality seeds of high yielding varieties well-adapted to certain regions and conditions. All government programs aiming to attain rice self-sufficiency will be successful if the seeds disseminated for planting by farmers have high physical and genetic purity. Deterioration in seed quality may begin at any point in the plant's development stage starting from fertilization onwards. Steady supply of good quality seeds therefore, depend on good seed production practices (*i.e.* pre-harvest and post-harvest) and also on a good internal seed quality control program. The Seed Technology Division is the PhilRice arm that aims to develop and maintain a system of seed quality assurance to ensure high purity and quality of seeds produced in all the institute's stations.

Internal Field Inspection of Seed Production Areas

EPRico and RCRamos

Seed quality assurance in rice seed begins in the field. As prescribed by several Administrative Orders issued by the Department of Agriculture, inbred and hybrid seed production areas follow set of rules on field inspection. 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 of the entire system of seed certification is under an agency outside of PhilRice.

Activities:

- Field inspection of 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 fields planted to varieties intended for breeder seeds, three areas of 14 x 36 hills were inspected at 20DAT; maximum tillering; onset of flowering; and two weeks before harvest.

- 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.

Results:

- During dry season a total of 142 varieties were monitored and inspected; 107 varieties Nucleus Seed, 8 Breeder Seed (BS), 15 Foundation Seed (FS) and 12 Registered Seed (RS).

- A total of 178 field lots were inspected; 62 field lots planted for Foundation Seed and 116 field lots planted for Registered seed and with a total area of 36.2 ha (12.5 ha for FS and 23.7 ha for RS).

- The average percent purity recorded during final inspection were; 99.95% seed purity in Nucleus seed, 99.94% seed purity in Breeder seed, 99.71% seed purity in Foundation seed and 99.55% seed purity in Registered seed (Table 1).

- Most number of off-type observed was during 20 days after transplanting and off-type was common observed under foundation seed production area (Table 1). Decreasing trend of observed off-type was due to the effort of the rouging team.

- Although 99.95 percentage purity of the varieties under nucleus seed and out of the 107 varieties inspected, three lines were observed having two different height and maturity.

- During wet season a total of 164 varieties were monitored and inspected; 105 varieties Nucleus Seed, 17 Breeder Seed (BS), 27 Foundation Seed (FS) and 15 Registered Seed (RS).

- A total of 146 field lots were inspected; 66 field lots planted for Foundation Seed and 78 field lots planted for Registered seed and with a total area of 46 ha (24.61 ha for FS and 21.39 ha for RS).

- The average percent purity recorded during final inspection were; 100% seed purity in Nucleus seed, 99.98% seed purity in Breeder seed, 99.90% seed purity in Foundation seed and 99.56% seed purity in Registered seed (Table 2).

- Under Nucleus Seed Production six entries lodged during the flowering and at final inspection (two weeks before harvesting) 13 entries were infested by Brown leaf hopper (Hopper Burn)

(Figure 1). All varieties have 100% field purity during final inspection.

- Under Breeder Seed Production 15 varieties were lodged during final inspection. NSIC Rc212 was rejected in the field because the plants were not true to type.

- Under Foundation Seed Production 32 field lots were lodged (Figure 2) and 11 field lots representing 5 varieties with 100% lodged during final field inspection. The lowest seed purity observed was 98.50%.

- Under Registered Seed Production 36 field lots were lodged and 8 varieties were considered 100% lodged during final inspection (Figure 3 and 4). Lowest observed purity was 98.15%, and highest was 99.97%.

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

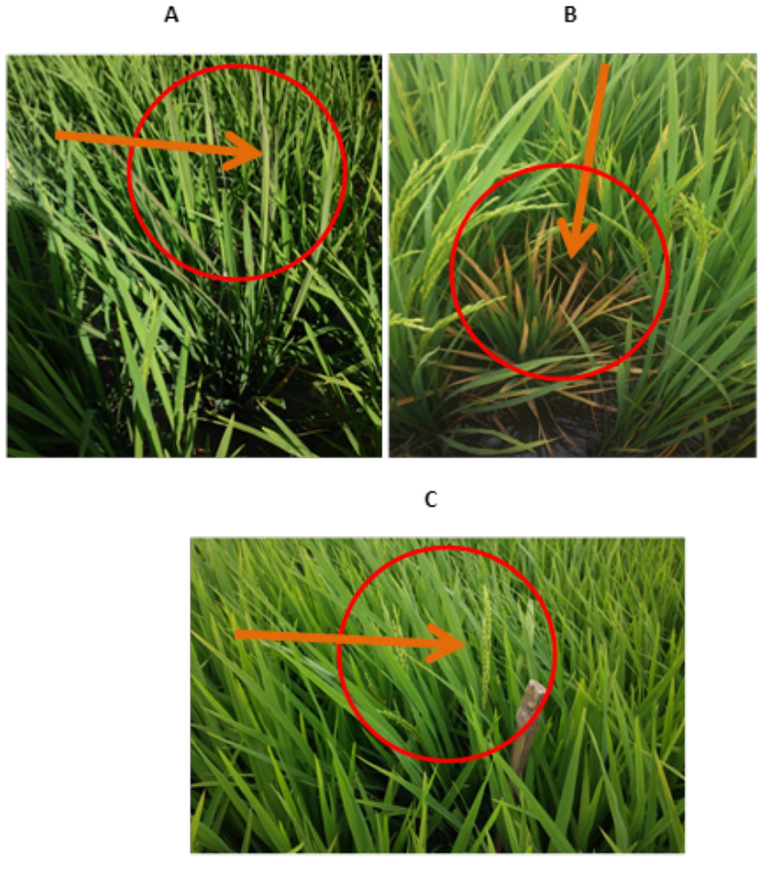


Figure 1. Plants removed during field inspection at maximum tillering, purple –leaf blade (A), diseased plant (B), and early to flower (C).

Table 1. Field purity evaluation of varieties seed produced by BDO and PBBD at different seed classes during 2016 DS inspection.

Seed Class	# of field lot inspected	# of Variety Inspected	Observed off-type during inspection				Percentage purity at Final Inspection
			20 DAT	Maximum Tiller	On-set of Flower	Maturity	
Nucleus Seed	1	107	1	1	1	0	99.95
Breeder Seed	1	8	1	1	1	1	99.94
Foundation Seed	62	15	12	5	3	2	99.71
Register Seed	116	12	10	10	8	4	99.55

Table 2. Field purity evaluation of varieties seed produced by BDO and PBBD at different seed classes during 2016 WS inspection.

Seed Class	# of field lot inspected	# of Variety Inspected	Observed off-type during inspection				Percentage purity at Final Inspection
			20 DAT	Maximum Tiller	On-set of Flower	Maturity	
Nucleus Seed		105	2	2	0	0	100
Breeder Seed		26	2	1	2	1	99.98
Foundation Seed		26	3	1	2	2	99.90
Register Seed		15	11	6	8	2	99.56

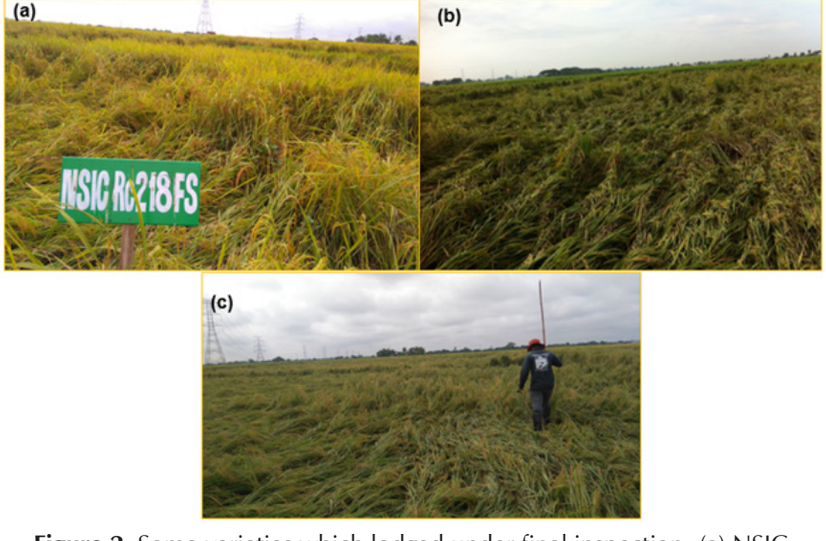


Figure 2. Some varieties which lodged under final inspection: (a) NSIC Rc218 lodged, 80%; (b) NSIC Rc352 lodged, 100%; and (c) NSIC Rc298 lodged, 100%.

Seed Purity and Viability Testing

RCRamos, CPDuruin, VMVMartin, and SRBrena

Rice programs of the Department of Agriculture is anchored on the use of high quality seeds. Since the creation of PhilRice whose mandate is to make available high seed classes of released inbred and public hybrids, multiplication of breeder, foundation, and registered seeds is done in both dry and wet season. The purity of the seeds produced is done in the field prior to harvest through field inspection and after harvest, specifically after threshing, drying, and seed cleaning. Varietal purity testing is routinely done to assure clients of the high quality seeds upon purchase. Furthermore, remaining seed stock of each cropping season is tested for seed viability and only seed lots with 85% or higher germination rates are left in the warehouse for distribution.

Activities:

- In Dry Season 2016, 8 BS varieties were tested for varietal purity after threshing, after drying and after seed cleaning. 500g was provided for varietal purity testing per operation. For foundation and registered seeds production, varietal purity testing were also done after threshing, after drying and after seed cleaning.

- In Wet Season 2016, varietal purity of 23 BS Varieties were tested after threshing but only 19 varieties were tested in after drying and after seed cleaning. For Foundation seed production (FSP) 7 varieties after threshing, 11 varieties after drying and 17 varieties after seed cleaning were tested. For Registered Seed Production (RSP); 4, 7, and 7 varieties after threshing, after drying and after cleaning were tested the varietal purity respectively.

- For seed viability testing of inbred seeds stock from BDD warehouse, WS2014, DS2015, WS2015, and DS2016 BSP, FSP and RSP were tested.

- Parental lines produced from DS2015 and WS2015 were also tested.

Results:

- For DS2016, In 8 BS varieties tested in varietal purity, none of them passed even as registered seeds (Table3). For Foundation Seed Production, only NSIC Rc352 passed as foundation seeds (Table 4). For Registered Seed Production, none of them passed on varietal purity testing as Registered seeds (Table 5).

- For WS2016, in 23 BS varieties tested, only 6 varieties passed as breeder. These were NSIC Rc194, PSB Rc82, NSIC Rc152, NSIC Rc160, NSIC Rc300, and NSIC Rc420 (Table 6). For FS, only NSIC Rc160, NSIC Rc358, NSIC Rc128, and NSIC Rc324 passed as foundation after seed cleaning (Table 7). For RS, only NSIC Rc160, NSIC Rc358, and NSIC RC360 passed as registered after seed cleaning (Table 8).

- 5 FS varieties and 2 CS varieties with many seed lots produced in WS2014 were tested for seed viability. Only NSIC Rc11 with seed lots EA2 and EA3 passed the viability testing of 85% seed germination (Table 9). CS varieties, PSB Rc10 and NSIC Rc240, had low germination rate.

- Among the varieties produced in DS2015 FSP, only NSIC Rc23 did not passed the viability testing (Table 10).

- For the varieties produced in WS2105 FSP, NSIC Rc120, PSB Rc14, NSIC Rc214, and NSIC Rc238 did not passed the viability testing of 85% (Table 11). Also, we tested the seeds produced on WS2015 RSP and NSIC Rc27, NSIC Rc214, NSIC Rc192, and NSIC Rc358 did not passed the viability testing of 85% (Table 12).

- For Dry season 2016 FSP seeds stock produced, all of them passed the viability testing (Table 13).

- Parental lines produced from DS2015 were tested and they had high germination rates (Table 14). However, Parental lines produced from WS 2015 that stored in BDD warehouse had 0% germination rate. Those seeds produced on WS2015 were tested last November 7, 2016.

Table 3. Varietal Purity of DS2016 Breeder Seed Production.

Date Harvested	Variety	After Threshing	After Drying	After Cleaning
6-Apr-16	NSIC Rc158	31	13	6
	NSIC Rc238	76	11	9
	NSIC Rc398	50	49	48
12-Apr-16	NSIC RC160	26	8	10
	NSIC Rc286	41	21	20
	NSIC Rc394	59	50	48
	NSIC Rc396	60	45	11
22-Jun-16	NSIC Rc400	59	22	22

Table 4. Varietal Purity of DS2016 Foundation Seed Production.

Date Harvested	Variety	After Threshing	After Drying	After Cleaning
12-Jun-16	NSIC Rc152	N/A	N/A	1
	NSIC Rc358	36	N/A	13
	NSIC Rc152 (Baloc)	4	N/A	N/A
14-Apr-16	PSB Rc18	26	24	10
	NSIC Rc222	20	21	5
18-Apr-16	NSIC Rc216	13	N/A	5
	NSIC Rc352	4	N/A	2
20-Apr-16	NSIC Rc218	N/A	26	8
22-Apr-16	NSIC Rc160	N/A	N/A	10
	NSIC Rc240	N/A	N/A	10
	NSIC Rc308	81	55	15
	NSIC Rc356	N/A	N/A	12
23-Apr-16	NSIC Rc238	50	N/A	32
26-Apr-16	NSIC Rc300	N/A	N/A	4
28-Apr-16	NSIC Rc360	N/A	N/A	7

Table 5. Varietal Purity of DS2016 Registered Seed Production.

Date Harvested	Variety	After Threshing	After Drying	After Cleaning
12-Jun-16	NSIC Rc152	N/A	N/A	22
	NSIC Rc358	23	N/A	26
	NSIC Rc152 (Baloc)	17	N/A	N/A
14-Apr-16	PSB Rc18	50	45	25
	NSIC Rc222	28	11	9
18-Apr-16	NSIC Rc216	N/A	22	10
	NSIC Rc352	35	N/A	13
20-Apr-16	NSIC Rc218	N/A	30	12
22-Apr-16	NSIC Rc160	N/A	N/A	12
	NSIC Rc240	N/A	N/A	25
	NSIC Rc308	71	24	15
	NSIC Rc356	N/A	N/A	22
23-Apr-16	NSIC Rc238	12	N/A	12
26-Apr-16	NSIC Rc300	N/A	N/A	22
28-Apr-16	NSIC Rc360	N/A	N/A	21

Table 6. Varietal Purity of WS2016 Breeder Seed Production.

Date Harvested	Variety	After Threshing	After Drying	After Cleaning
19-Sep-16	NSIC Rc194	0	6	0
	PSB Rc10	7	10	5
20-Sep-16	PSB Rc82	0	10	0
	NSIC Rc152	0	0	0
	NSIC Rc394	11	4	14
21-Sep-16	NSIC Rc390	9	8	3
23-Sep-16	NSIC Rc238	15	15	10
	NSIC Rc308	0	0	1
	NSIC Rc218	11	10	3
26-Sep-16	NSIC Rc398	0	0	1
	NSIC Rc400	0	9	1
30-Sep-16	NSIC Rc160	0	4	0
	NSIC Rc240	10	14	10
	NSIC Rc298	4	0	2
	NSIC Rc300	0	0	0
	NSIC Rc342	13	N/A	N/A
	NSIC Rc354	2	7	3
	NSIC Rc356	0	N/A	N/A
	NSIC Rc358	0	0	1
	NSIC Rc360	0	10	5
	NSIC Rc396	0	N/A	N/A
	NSIC Rc402	0	N/A	N/A
	NSIC Rc420	0	8	0

Table 7. Varietal Purity of WS2016 Foundation Seed Production.

Date Harvested	Variety	After Threshing	After Drying	After Cleaning
9-Sep-16	NSIC Rc160	17	5	2
23-Sep-16	PSB Rc10	12	1	11
27-Sep-16	NSIC Rc402	5	8	6
	NSIC Rc358	6	8	1
28-Sep-16	NSIC Rc158	6	13	5
30-Sep-16	NSIC Rc360	-	14	10
3-Oct-16	NSIC Rc152	6	-	-
	NSIC Rc216	-	10	4
	NSIC Rc194	-	-	4
	NSIC Rc128	-	-	2
	NSIC Rc130	-	-	11
	NSIC Rc192	-	-	35
	NSIC Rc342	-	-	0
	PSB Rc14	-	-	6
	NSIC Rc284	-	-	30
5-Oct-16	NSIC Rc298	6	-	-
	NSIC Rc400	-	17	10
6-Oct-16	NSIC Rc214	-	3	5
13-Oct-16	NSIC Rc398	-	23	23
14-Oct-16	NSIC RC396	-	15	-

Table 8. Varietal Purity of WS2016 Registered Seed Production.

Date Harvested	Variety	After Threshing	After Drying	After Cleaning
9-Sep-16	NSIC Rc160	2	2	0
23-Sep-16	PSB Rc10	10	4	6
27-Sep-16	NSIC Rc358	9	15	3
28-Sep-16	NSIC Rc158	-	12	11
30-Sep-16	NSIC Rc360	-	14	5
3-Oct-16	NSIC Rc152	2	-	-
	NSIC Rc216	-	2	10
6-Oct-16	NSIC Rc214	-	5	6

Table 9. Viability Testing of carry over seed lots produced in WS2014.

Variety	Seed Class	Lot No.	Volume (kg)	Viability (%)	Date Tested
PSB Rc14	FS	AA1	170	67	16-Feb-16
		AA2	210	80	16-Feb-16
		AA3	1010	58	23-Feb-16
PSB Rc82	FS	K3	460	65	26-Feb-16
NSIC Rc11	FS	EA1	845	60	17-Feb-16
		EA2	1000	88	19-Feb-16
		EA3	130	91	17-Feb-16
NSIC Rc194	FS	U1	485	49	13-Feb-16
		U3	495	41	26-Feb-16
NSIC Rc212	FS	BA1	575	69	15-Feb-16
		BA2	480	60	13-Feb-16
PSB Rc10	CS	Z1	980	40	3-Mar-16
		Z2	940	54	4-Mar-16
NSIC Rc240	CS	Q1	545	12	2-Mar-16

Table 10. Viability testing of carry over seed lots produced in DS2015.

Variety	Seed Class	Lot No.	Volume (kg)	Viability	Date Tested
PSB Rc14	FS	1	80	99	March 10
NSIC Rc23	FS	3	3360	53	March 10-11
NSIC Rc118	FS	3	1890	97	March 28-30
NSIC Rc214	FS	6	4130	96	March 28-April 5
NSIC Rc240	FS	1	580	98	March 21
NSIC Rc288	FS	1	740	97	March 9
NSIC Rc298	FS	5	3110	99	March 15-21
NSIC Rc388	FS	1	320	87	March 28
NSIC Rc240	CS	3	1060	97	April 4
NSIC Rc298	CS	1	140	97	April 4

Table 11. Viability testing of carry over seed lots produced in WS2015 FSP.

Variety	Seed Class	Number of Seed Lots	Volume (kg)	Viability (%)	Date Tested
NSIC Rc118	FS	2	1850	95	June 27-28
NSIC Rc120	FS	2	1720	23	June 29-30
PSB Rc14	FS	1	440	83	June 30
NSIC Rc214	FS	2	1100	81	July 5 & 7
NSIC Rc11	FS	3	2000	99	July 11 & 13
NSIC Rc238	FS	1	110	76	July 15
NSIC Rc298	FS	2	1520	95	July 14-15
NSIC Rc300	FS	1	360	93	July 15
NSIC Rc360	FS	1	20	90	July 15
NSIC Rc392	FS	3	1100	93	July 15

Table 12. Viability testing of carry over seed lots produced in WS2015 RSP.

Variety	Seed Class	Number of Seed Lots	Volume (kg)	Viability (%)	Date Tested
NSIC Rc23	RS	3	1770	96	July 25-26
NSIC Rc27	RS	1	940	2	July 27
NSIC Rc214	RS	2	1020	75	July 27
NSIC Rc298	RS	3	2140	95	July 27 & Aug 1
NSIC Rc392	RS	3	2380	94	August 1-4
NSIC Rc360	RS	2	1300	89	August 4 & 11
PSB Rc68	RS	2	1760	91	August 11-12
NSIC Rc11	RS	4	3560	95	August 15 & 17
NSIC Rc192	RS	1	80	0	August 18
NSIC Rc118	RS	1	420	92	August 18
NSIC Rc358	RS	1	100	62	August 18
NSIC Rc218	RS	1	220	83	August 18
NSIC Rc226	RS	3	1500	97	August 19

Table 13. Viability testing of carry over seed lots produced in DS2016 FSP.

Variety	Seed Class	Number of Seed Lots	Volume (kg)	Viability (%)	Date Tested
PSB Rc18	FS	2	640	96	September 5
NSIC Rc152	FS	1	280	99	September 5
NSIC Rc222	FS	1	40	99	September 5
NSIC Rc238	FS	2	1070	97	September 5
NSIC Rc300	FS	1	20	99	September 5
NSIC RC308	FS	1	440	99	September 5
NSIC Rc352	FS	2	680	97	September 14
NSIC Rc356	FS	2	970	97	September 15-16
NSIC Rc358	FS	2	90	99	September 5
NSIC RC360	FS	3	1430	88	September 16 & 20

Table 14. Seed Viability of carry over seed lots of Parental Lines produced in DS2015.

Variety	Seed Class	No. of Seed lots	Volume (kg)	Viability (%)	Date Tested
TG101M	BS	1	485	97	April 26
TG102M	BS	1	60	99	April 28
TG101M	FS	1	125	96	April 28
TG102M	FS	1	40	95	April 28
TG102M	RS	5	1095	94	April 15
IR34686R	RS	1	35	95	May 2
IR34686R	CS	1	280	95	May 2

Seed Vigor Testing of Buffer Stock and Carry over Seed Lots

RCRamos, CPDuruin, VMVMartin, and SRBrena

Seed germination is the done under optimum condition in the laboratory but is not a good measure of seed quality. The seed germination indicates only the number of seedlings that remain viable. To assess further the seed quality of buffer stock and carry over seed lots in inbred and hybrids produced by PhilRice, a better quality measure is employed, the seed vigor test. In this test, the seeds are subjected to accelerated ageing test (AAT) in an environment of high temperature, high relative humidity, and moisture content. After the ageing process, the seeds are germinated and result is compared to the control, samples that did not undergo the ageing process. Other than AAT, the number of germinated seeds at first counting can also be considered a good indication of the seed vigor of a seed lot. Another test that will give indication of the field performance is through germinating the seeds in plastic tray with soil as medium. The trays are exposed in racks outside the laboratory where the seedlings experienced extreme heat and rains.

Activities:

- Four hundred seeds from each seed lot were subjected to an environment of high temperature (42C), high relative humidity (100%), and high moisture content for three days. After, the seeds were germinated following the standard germination procedures set by ISTA.

- AAT of hybrid parental lines produced in DS 2015 and BS of inbred kept in cold room was done.

- Seedling emergence test was also conducted to assess the performance of the varieties when planted in the soil. Similarly, 400 seeds per seed lot were planted in four replicates. Each replicate consisted of 100 seeds. Seeds in each replicate were wrapped in paper towels, soaked in water for 24hr, drained, incubated, sown in plastic trays with soil.

Seedlings that emerged were counted after 14 days.

Results:

- Breeder seeds produced in DS2015 were tested twice on January and September through accelerated ageing test (AAT). AAT on September had lower percent germination than ageing test on January; also NSIC Rc11 and NSIC Rc342 did not passed the vigor testing tested last September (Table 15). The results shows that the seeds do deteriorate through time even if it was stored at cold room. However, some seeds tested last January are distributed to the farmers.

- A total of 846 bags with 10,740kg of 12 varieties produced last WS2015 FSP were tested its vigor and seedling emergence. Only 8 varieties passed the vigor testing and only 6 varieties passed the seedling emergence test. Also, the results show that seedling emergence test had lower percent germination rather than vigor testing (Table 16). On WS2015 RSP seed stock, 13 varieties with the total of 910 bags with 17,190 kg. tested its vigor and seedling emergence. Only 9 varieties passed the vigor testing and only 3 varieties passed the seedling emergence test. Same as the WS2015 FSP, seedling emergence test on WS2015 RSP had lower percent germination rather than vigor testing (Table 17).

- On DS2016 FSP seeds stock, 10 varieties with 399 bags or 5,480kg was tested its vigor and seedling emergence. Only NSIC Rc360 did not passed the vigor testing but only NSIC Rc308 passed the seedling emergence test. The results show that there is a decrease of percent germination when tested in the real environment of the field (Table 18).

- Parental lines tested were produced in PhilRice-LB and PhilRice-CES. Parental line tested last April had 95% average germination for vigor testing. However, parental line tested last November only had 0 and 17 % germination test for vigor testing (Table 19).

Table 15. Seed Vigor test of breeder seeds produced in DS2015 kept in cold-room.

Variety	Seed Class	Volume (kg)		Vigor test (%)	
		January	September	January	September
PSB Rc10	BS	50	30	88	88
PSB Rc18	BS	275	180	92	91
PSB Rc68	BS	385	325	96	90
PSB Rc82	BS	50	10	92	86
NSIC Rc11	BS	25	25	93	40
NSIC Rc118	BS	50	50	96	89
NSIC Rc120	BS	100	95	97	88
NSIC Rc192	BS	70	50	97	89
NSIC Rc214	BS	55	20	97	95
NSIC Rc216	BS	220	120	98	93
NSIC Rc218	BS	90	40	98	95
NSIC Rc238	BS	90	55	99	95
NSIC Rc300	BS	140	90	99	96
NSIC Rc308	BS	175	120	99	95
NSIC Rc342	BS	170	165	95	81
NSIC Rc352	BS	100	95	99	86
NSIC Rc392	BS	25	20	97	89
NSIC Rc23	BS	175	-	97	-
NSIC Rc222	BS	125	-	94	-
NSIC Rc226	BS	20	-	98	-
NSIC Rc358	BS	135	-	98	-
NSIC Rc360	BS	60	-	92	-

Table 16. Seed Vigor and Seedling Emergence Test of WS2015 FSP seed stock.

Variety	Seed Class	Number of Seed Lots	Volume (kg)	Vigor (%)	Seedling Emergence (%)	Date Tested
NSIC Rc118	FS	2	1850	89	82	June 27-28
NSIC Rc120	FS	2	1720	16	12	June 29-30
PSB Rc14	FS	1	440	76	75	June 30
NSIC Rc214	FS	2	1100	76	66	July 5 & 7
NSIC Rc11	FS	3	2000	93	91	July 11 & 13
NSIC Rc238	FS	1	110	69	54	July 15
NSIC Rc298	FS	2	1520	92	88	July 14-15
NSIC Rc300	FS	1	360	88	88	July 15
NSIC Rc360	FS	1	20	87	85	July 15
NSIC Rc392	FS	3	1100	86	83	July 15

Table 17. Seed Vigor and Seedling Emergence Test of WS2015 RSP seed stock.

Variety	Seed Class	Number of Seed Lots	Volume (kg)	Vigor (%)	Seedling Emergence (%)	Date Tested
NSIC Rc23	RS	3	1770	92	88	July 25-26
NSIC Rc27	RS	1	940	90	0	July 27
NSIC Rc214	RS	2	1020	70	67	July 27
NSIC Rc298	RS	3	2140	93	88	July 27 & Aug 1
NSIC Rc392	RS	3	2380	93	84	August 1-4
NSIC Rc360	RS	2	1300	88	76	August 4 & 11
PSB Rc68	RS	2	1760	84	84	August 11-12
NSIC Rc11	RS	4	3560	90	84	August 15 & 17
NSIC Rc192	RS	1	80	0	0	August 18
NSIC Rc118	RS	1	420	92	85	August 18
NSIC Rc358	RS	1	100	67	46	August 18
NSIC Rc218	RS	1	220	83	84	August 18
NSIC Rc226	RS	3	1500	93	82	August 19

Table 18. Seed Vigor and Seedling Emergence Test of DS2016 FSP seed stock.

Variety	Seed Class	Number of Seed Lots	Volume (kg)	Vigor (%)	Seedling Emergence (%)	Date Tested
PSB Rc18	FS	2	640	95	84	September 5
NSIC Rc152	FS	1	280	98	75	September 5
NSIC Rc222	FS	1	40	93	75	September 5
NSIC Rc238	FS	2	1070	92	81	September 5
NSIC Rc300	FS	1	20	95	83	September 5
NSIC RC308	FS	1	440	95	89	September 5
NSIC Rc352	FS	2	680	92	84	September 14
NSIC Rc356	FS	2	970	92	75	September 15-16
NSIC Rc358	FS	2	90	95	72	September 5
NSIC RC360	FS	3	1430	79	64	September 16 & 20

Table 19. Vigor Testing of Parental Lines from PhilRice-LB and Philrice-CES.

Variety	Seed Class	Season Harvested	No. of Seed lots	Volume (kg)	Vigor (%)	Date Tested
TG101M	BS	DS2015	1	485	96	April 26
TG102M	BS	DS2015	1	60	93	April 28
TG101M	FS	DS2015	1	125	97	April 28
TG102M	FS	DS2015	1	40	90	April 28
TG102M	RS	DS2015	5	1095	96	April 15
IR34686R	RS	DS2015	1	35	94	May 2
IR34686R	CS	DS2015	1	280	96	May 2
TG102M	FS	WS2015	4	335	0	November 7-8
TG102M	FS	DS2015	2	165	17	November 9

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

CHDPablo, LVGuittap, and SRBrena

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. 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, minimal off-types are observed in the field when the parental lines are planted.

Activities:

- In 2016 dry season, fourteen seed lots of parental lines (7 IR58025A, 2 IR58025B, 1 IR68897B, 4 IR71604R) produced by PhilRice Los Baños, 6 seed lots (6 PRUP TG102) produced in Negros, 5 seed lots (2 IR71604R, 1 IR58025A, 1 IR68897A and 1 IR34686R) produced by PhilRice-CMU and 2 seed lots (1 IR58025A, 1 IR68897A) during wet season 2015 were tested.

- In the grow-out test during the 2016 wet season, fifteen seed lots of PRUP TG102 produced by PhilRice Los Baños and Negros, sixteen seed lots of PRUP TG101 produced by PhilRice Los Baños and Isabela, nine seed lots (5 IR68897A, 1 IR68897B, IR58025A, 2 IR58025B) produced in Negros, Los Baños and Isabela during dry season 2016 were evaluated.

- Samples were grown in 20cm 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.

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

Results:

Dry season 2016

- The total seed lots tested had 97% or higher genetic purity (Table 20 and 21). Twenty-two out of twenty-seven of the total seed lots tested 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 (Figure 3).

- The genetic purity of the PRUP TG102 seed lots tested in the DS2016 GOT passed the required genetic purity (Table 20).

- Among the parental lines tested, 1 seed lot of IR71604R had 100% purity (Table 21); 3 seed lots of IR58025A,1 IR68897B, 1 PRUP TG-102, 1 IR68897A, 1 IR34686R had 99% purity; and 2 seed lots of IR58025A, 1 seed lot of IR58025B, 3 seed lots of PRUP-TG102, 1 seed lot of IR71604R had 98% purity. On the other hand, 1 seed lot of IR71604R from Los Baños had the lowest 91% purity.

- From the GOT of the parental lines tested, 25 seed lots had 97% or higher seed genetic purity. One seed lot of IR71604R from Los Baños had the lowest genetic purity (91.3%).

Wet season 2016

- In the WS2016 grow-out test, there were forty seed lots tested in the WS2016 grow-out test, nine seed lots (7 PRUP TG102 and 2 IR68897A) came from Negros, fifteen seed lots (8 PRUP TG102, 6 PRUP TG101 and 1 IR58025B) came from Isabela, and sixteen seed lots (11 PRUP TG101, 3 IR68897A, 1 IR68897B, 1 IR58025A and 1 IR58025B) came from Los Baños.

- Nine PRUP TG102 seed lots out of fifteen passed the 97% required genetic purity while fourteen PRUP TG101 seed lots out of sixteen had 97% genetic purity or higher (Table 22 and 23).

- All seed lots of PRUP TG102 from Negros (seed lots DSB P1 – P5) failed to meet the required genetic purity with values ranging from 92-93% purity. An experiment is currently being done to investigate the cause as to why those seeds produced in Don Salvador Benedicto failed the assessment.

- From the GOT of other parental lines (IR68897A, IR68897B, IR58025A, IR58025B) produced by PhilRice-Los Baños, Negros and Isabela, all seed lots passed with 97-99% genetic purity with only 1 seed lot of IR68897A had the lowest genetic purity (96.3%) (Table 24).

Table 20. Percent Genetic Purity of the Tested PRUP TG102 Parental Lines produced in WS2015.

Index #	Variety	Lot No.	Location	Population	Total off-types	Percent % purity
1	PRUP TG102	DSB1	Negros	364	9	97.6
2	PRUP TG102	DSB2	Negros	370	9	97.7
3	PRUP TG102	DSB3	Negros	383	5	98.6
4	PRUP TG102	DSB4	Negros	318	8	97.6
5	PRUP TG102	DSB5	Negros	305	11	96.5
6	PRUP TG102	DSB6	Negros	326	11	96.6

Table 21. Percent Genetic Purity of the Other Tested Parental Lines produced in WS2015.

Index #	Variety	Lot No.	Location	Population	Total off-types	Percent % purity
1	IR58025A	UG 1	Los Baños	471	12	97.4
2	IR58025A	UG 2-1	Los Baños	456	7	98.5
3	IR58025A	UG 2-2	Los Baños	437	4	99.0
4	IR58025A	UG 3-1	Los Baños	399	11	97.3
5	IR58025A	UG 3-2	Los Baños	426	12	97.3
6	IR58025A	UG 1/3-1	Los Baños	462	6	98.7
7	IR58025A	UG 2-1/3-2	Los Baños	451	7	98.4
8	IR58025A	Isabela	Isabela	438	5	98.8
9	IR58025B	UG 2-1	Los Baños	417	11	97.4
10	IR58025B	UG 2-2	Los Baños	409	8	98.0
11	IR68897B	UG 13	Los Baños	441	5	98.9
12	IR68897A	Isabela	Isabela	426	11	97.5
13	IR71604R	UG 8-1	Los Baños	443	39	91.3
14	IR71604R	UG 8-2	Los Baños	459	14	96.9
15	IR71604R	UG 8-3	Los Baños	462	32	93.1
16	IR71604R	UG 8-4	Los Baños	449	12	97.3
17	IR58025A	G9-1	PhilRice CMU	469	9	98.2
18	IR68897A	SB10-1	PhilRice CMU	487	4	99.2
19	IR34686R	F17-1	PhilRice CMU	471	2	99.6
20	IR71604R	F11-1	PhilRice CMU	473	0	100.0
21	IR71604R	F11-2	PhilRice CMU	432	8	98.1

Table 22. Percent Genetic Purity of the Tested PRUP TG102 Parental Lines produced in DS2016.

Index #	Variety	Lot No.	Location	Population	Total off-types	Percent % purity
1	PRUP TG102	DSB P1	Negros	481	31	93.6
2	PRUP TG102	DSB P2	Negros	465	35	92.5
3	PRUP TG102	DSB P3	Negros	457	28	93.9
4	PRUP TG102	DSB P4	Negros	421	31	92.6
5	PRUP TG102	DSB P5	Negros	414	33	92.0
6	PRUP TG102	F4-L1	Isabela	408	13	96.8
7	PRUP TG102	F4-L2	Isabela	388	12	96.9
8	PRUP TG102	F4-L3	Isabela	447	17	96.2
9	PRUP TG102	F4-L4	Isabela	476	16	96.6
10	PRUP TG102	F4-L5	Isabela	411	5	98.8
11	PRUP TG102	F4-L6	Isabela	483	11	97.7
12	PRUP TG102	F4-L7	Isabela	432	7	98.4
13	PRUP TG102	F4-L8	Isabela	374	10	97.3
14	PRUP TG102	DSB-1	Negros	477	10	97.9
15	PRUP TG102	DSB-2	Negros	406	4	99.0

Table 23. Percent Genetic Purity of the Tested PRUP TG101 Parental Lines produced in DS2016.

Index #	Variety	Lot No.	Location	Population	Total off-types	Percent % purity
1	PRUP TG101	F5-L1	Isabela	493	1	99.8
2	PRUP TG101	F5-L2	Isabela	458	2	99.6
3	PRUP TG101	F5-L3	Isabela	464	4	99.2
4	PRUP TG101	F5-L4	Isabela	478	2	99.5
5	PRUP TG101	F5-L5	Isabela	486	11	97.8
6	PRUP TG101	F5-L6	Isabela	468	20	95.7
7	PRUP TG101	KLQ 1-1-1	Los Baños	475	3	99.4
8	PRUP TG101	KLQ 1-1-2	Los Baños	474	1	99.8
9	PRUP TG101	KLQ 1-2-1	Los Baños	381	1	99.8
10	PRUP TG101	KLQ 1-2-1A	Los Baños	467	0	100.0
11	PRUP TG101	KLQ 1-2-2	Los Baños	488	22	95.4
12	PRUP TG101	KLQ 1-2/2A/4	Los Baños	436	1	99.8
13	PRUP TG101	KLQ 1-3-1	Los Baños	485	2	99.7
14	PRUP TG101	KLQ 1-3-2	Los Baños	432	3	99.2
15	PRUP TG101	KLQ 1-4	Los Baños	458	3	99.4
16	PRUP TG101	KLQ 1-5	Los Baños	465	1	99.7

Table 24. Percent Genetic Purity of Other Tested Parental Lines produced in DS2016.

Index #	Variety	Lot #	Location	Population	Total off-types	Percent % purity
1	IR68897A	Lot 1	Negros	455	17	96.3
2	IR68897A	Lot 2	Negros	415	12	97.1
3	IR68897A	UG 2	Los Baños	254	6	97.8
4	IR68897A	UG 2	Los Baños	324	5	98.6
5	IR68897A	UG 12	Los Baños	313	10	96.7
6	IR68897B	UG13	Los Baños	300	8	97.2
7	IR58025A	UG 14	Los Baños	281	4	98.5
8	IR58025B	UG 13	Los Baños	328	3	99.0
9	IR58025B	Isabela	Isabela	238	4	98.3

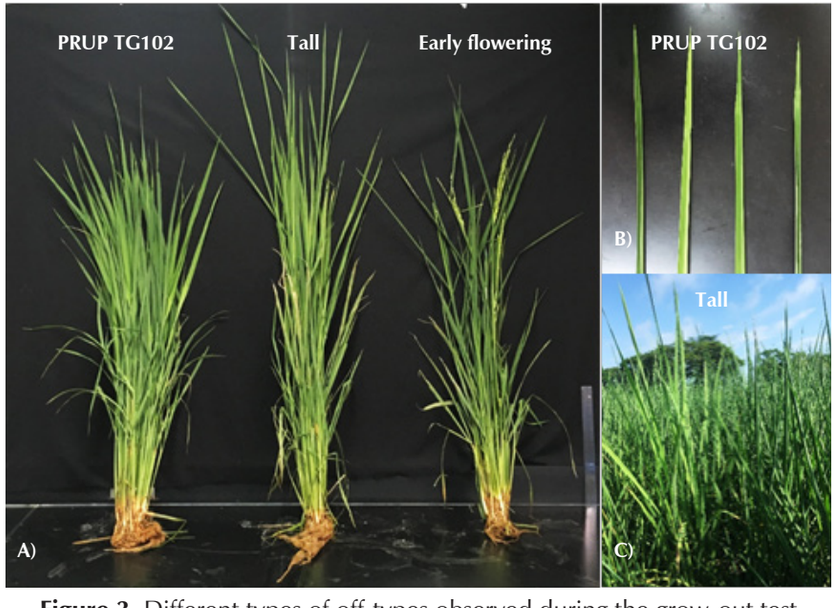


Figure 3. Different types of off-types observed during the grow-out test of hybrid parental lines: a) true-type PRUP TG102 and two commonly observed off-types, tall and early flowering; (b) off-types based on leaf color and width; (c) tall off-type found in the field.

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

CHDPablo and SRBrena

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.

Activities:

- Pure lines of Mestiso 19 and 20 S-lines (PRUP TG101 and PRUP TG102) were planted in the basins replicated thrice. A total of 37 seed lots of PRUP TG102 and PRUP TG101 were tested.
- Leaf samples were collected from each treatment 21 days after seeding at Philippine Rice Research Institute- Seed Technology Division.
- SSR markers previously identified able to identify polymorphisms in the seed lots of both parental lines and hybrids were used in the seed purity assessment of parental lines.
- DNA extraction, quantification and amplification via Polymerase Chain Reaction (PCR) and SSR analysis via Polyacrylamide Gel Electrophoresis (PAGE).

Results:

- To ensure confidence in the genetic purity of parental lines produced and dispatched to hybrid cooperators, seed samples were tested in GOT plots and DNA fingerprinting.

- A total of fifteen lots of PRUP TG102 were produced in Don Salvador Benedicto, Negros Occidental and Isabela DS2016. Seed lots DSB P1-P5, F4-L1, F4-L2, F4-L8 and DSB-1 were deemed pure sample (Figure 4). While the seed lots F4-L3 - F4-L7 and DS-2 differed from the expected amplification pattern and thus may not be as pure as the other seed lots.
- A total of sixteen lots of PRUP TG101 were produced in Los Baños and Isabela this DS2016. Impurity was found only in the seed lot KLQ 1-3-1, all the other seed were deemed pure (Figure 5).
- Further assessment of seed purity using SSR markers (RM1, RM127 and RM511) showed the same results as previously identified (Figure 6)
- Based from the results of the grow-out test conducted WS2016, PRUP TG102 seed lots (DSB P1 – P5) produced in Negros were found to be problematic seed lots. This finding was further demonstrated by the seed growers in Davao del Norte in the field. Following the results of the DNA analysis which only detected single band amplifications, the seed contaminants in the seed lots DSB P1 – P5 could be seeds from an inbred variety. These results prompted the researchers to reevaluate the accuracy and robustness of using SSR markers for seed purity testing of hybrid parental lines.
- Currently, an experiment is being done to investigate the cause as to why the seed lots produced in Don Salvador Benedicto, Negros had very low genetic purity and the possibility of what caused the seed admixtures in these seed lots.
- Seed purity of Mestiso 19 and 20 and their parental lines which are produced this WS2016 will be tested using SSR markers.

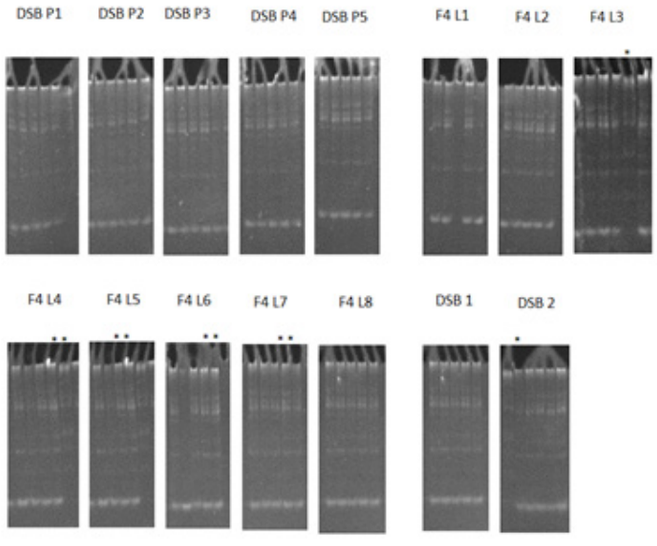


Figure 4. Seed purity assessments of PRUP TG102 seed lots produced in DS2016 using the SSR marker RM127. Asterisks denote impurities/ contaminants.

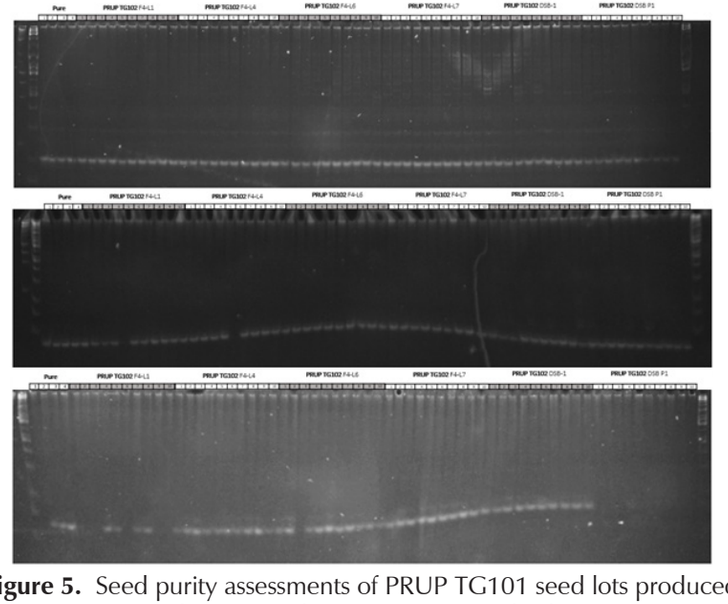


Figure 5. Seed purity assessments of PRUP TG101 seed lots produced in DS2016 using the SSR marker RM127. Asterisks denote impurities/ contaminants.

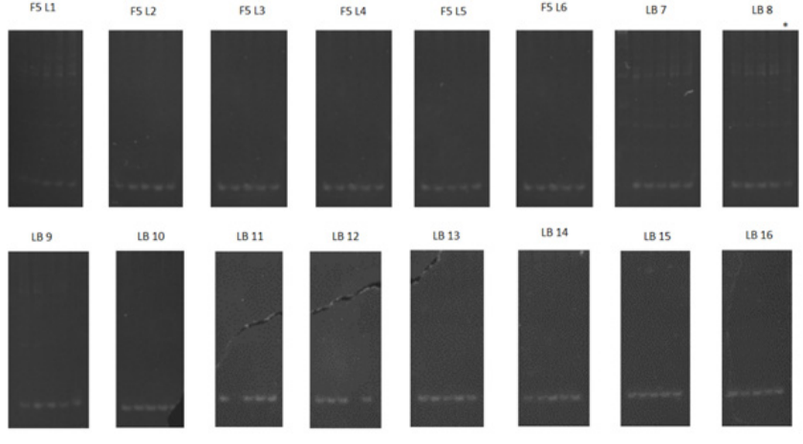


Figure 6. Seed purity assessment of PRUP TG102 seed lots produced in DS2016 using the SSR marker RM127, RM1 and RM511 previously identified to be informative markers.

II. Development/Improvement of pre-harvest and post-harvest technologies for commercial seed production

Project Leader: SRBrena

Two-line hybrid system quickly gained popularity into commercialization. The bottleneck however, remains in limitations in seed production. Floral morphology influences out-crossing which is crucial in hybrid rice seed production. Additionally, the row ratio between S x P contributes in the out-crossing capacity of pollen parent to pollinate the female population. Thereby, characterization of floral morphology that influence cross pollination through exogenous application of phytohormones is functional for the advancement on seed set of TGMS hybrid seed production. Likewise by increasing the density of P line, abundant of pollen guarantee to saturate the female spikelets and efficiently pollinate it. The project aim to increase percent seed set by increasing the population density of the pollen source and reducing the number of S–line rows and to determine the correlation of yield components to floral morphology as affected by exogenous application of phytohormones.

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

AGSFerriol, REGRagas, and SRBrena

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. 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. 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. By reforming these floral characteristics to fit natural outcrossing, improvement on seed set of TGMS hybrid seed production may be possible.

Activities:

- The experimental design was a factorial experiment laid out in a randomized complete block design with plot size of 25 m2 with four replicates. Treatments include four phytohormones applied starting at 5% panicle emergence: Gibberellic acid (GA3), Boric acid, Glycine, and Methyl Jasmonate (MeJA). Water served as control.

- At maturity, total tiller number, number of productive and unproductive tiller, total filled and unfilled grain weights, 1000-grain dry weight and filled and unfilled grain number per panicle were determined. Grain moisture content of the filled grains was measured with digital moisture. Grain yield per treatment plot were calculated.

- For cytological observations of the growth of pollen tubes, florets was sampled ~4 hr after pollination. The samples were fixed in a solution (3:1 ethanol: acetic acid) and were stored at 4° in 70% ethanol until use. The dissected pistils and ovaries were washed twice with distilled water and then incubated in a solution of 8N NaOH for 1 hour. Samples were stained in 0.1% aniline blue in K3PO4 buffer and examined under UV illumination to visualize the callose of pollen tube.

Results:

- 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 7 and 8). 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) (Figures 9 and 10). 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 stimulates 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.

- After one year observation, wet season hybrid seed production gave higher seed set than dry season, given the larger area of land used in wet season (25 sq. m) than in dry season (5 sq. m). Seed set among all yields components contributed to the improvement on yields. In wet season, percentage of seed set in plots treated with GA3+Gly+BA+MeJa had the highest (10.5, DS) and (25.01, WS) while control plots had the lowest (5.42 DS) and (18.69 WS) (Table 25).

- In both seasons, the highest yield was consistently observed in plots treated with GA3+Gly+BA+Meja combination with yields of 1,878.1 and 2,144.56kg·ha-1 in dry and wet season, respectively (Table 25).

- Greater number of pollen grains attached to stigma was observed in plots treated with GA3+Gly+BA+MeJa. Stigma receptivity, the ability of the stigma to support germination of viable, compatible pollen can be enhanced through application of phytohormones combination (Figure 11).

- Floral traits of normal pollen parent, such as anther length (2.56 ± 0.04), pollen count per anther (~24000) and pollen diameter (58.84 ± 0.58) was also observed and studied (Figure 12).

- Traits of normal seed-parent showed that the length of stigma is measured as (2.13 ± 0.02), stigma exsertion is measured to (59.00 ± 5.60) and angle of spikelet opening was narrow as (18.01 ± 0.56) (Figure 13).

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

- Here we review the area, paying attention to earlier approaches but emphasizing recent developments in the search for new management strategies for increasing hybrid seed production with practical applications in researcher's and farmer's field.

Table 25. Seed yield and yield components of Mestiso 19 during dry season and wet season of 2016 at PhilRice CES, Maligaya, Nueva Ecija.

Treatment	Productive Tiller (plant -1)	Total Number of Spikelet	Seed Set (%)	Panicle Exsertion Rate (%)	1000 grain weight (g)	Yield (Kg.ha-1)
Dry season						
Control	5.18±0.82	148.87	5.42 ± 0.08	82.61 ± 2.10	21.2 ± 2.45	467.8±208.16
GA3	6.15±1.27	166.5	8.2 ± 1.81	87.98 ± 0.63	17.8 ± 2.38	820.5±497.59
GA_3+Gly+BA+Meja	5.03±0.26	169.8	6.84 ± 2.41	80.05 ± 4.67	20.54 ± 6.82	621.8± 343.65
Gly+BA+Meja	6.3±0.34	186.7	10.5 ± 1.04	92.5 ± 0.74	30.1 ± 4.91	1878.1± 288.23
Wet season						
Control	6.90 ± 0.59	226.93	18.69 ±2.01	70.8 ± 2.56	66.68 ± 30.90	861.14 ±294.23
GA3	7.0 ± 0.16	231.2	24.88 ± 2.61	86.4 ± 1.17	83.5 ± 8.49	1171.28 ±399.20
GA_3+Gly+BA+Meja	6.70 ± 0.53	214.66	20.86 ± 3.20	65.4 ± 0.67	45.4 ± 12.48	993.22± 166.49
Gly+BA+Meja	9.20 ± 0.15	236.86	25.01 ± 2.47	90.4 ± 2.08	106.02 ± 15.21	2144.56± 467.54

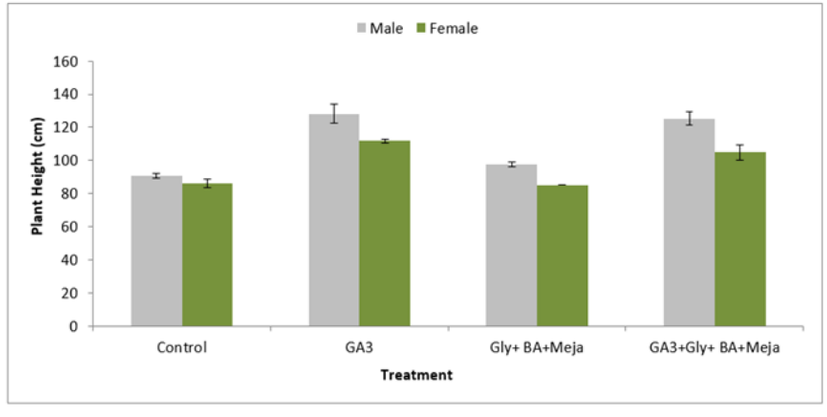


Figure 7. Plant height difference between parental lines as affected by different phytohormones, dry season 2016.

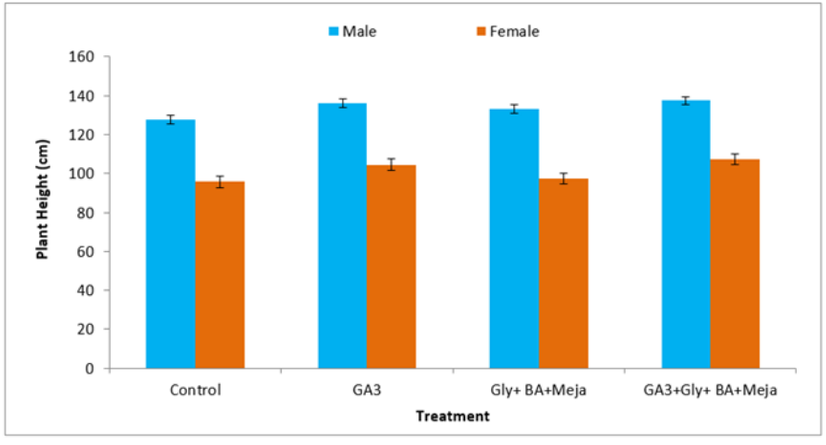


Figure 8. Plant height difference between parental lines as affected by different phytohormones wet season 2016.

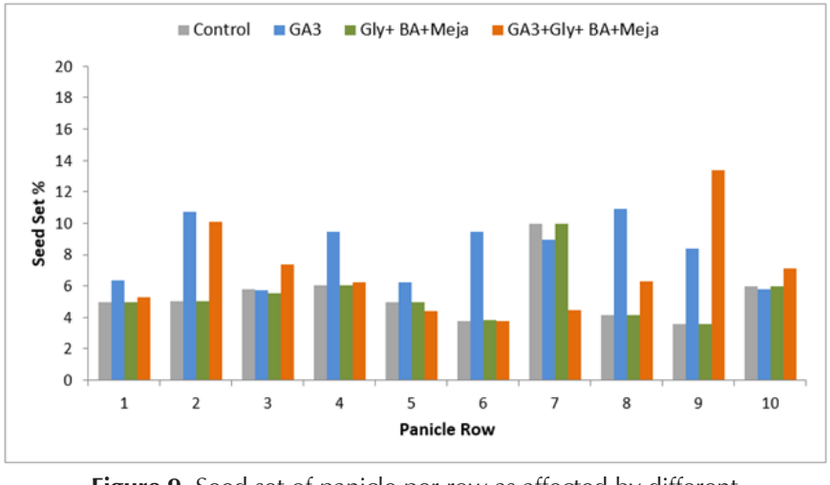


Figure 9. Seed set of panicle per row as affected by different phytohormones, dry season 2016.

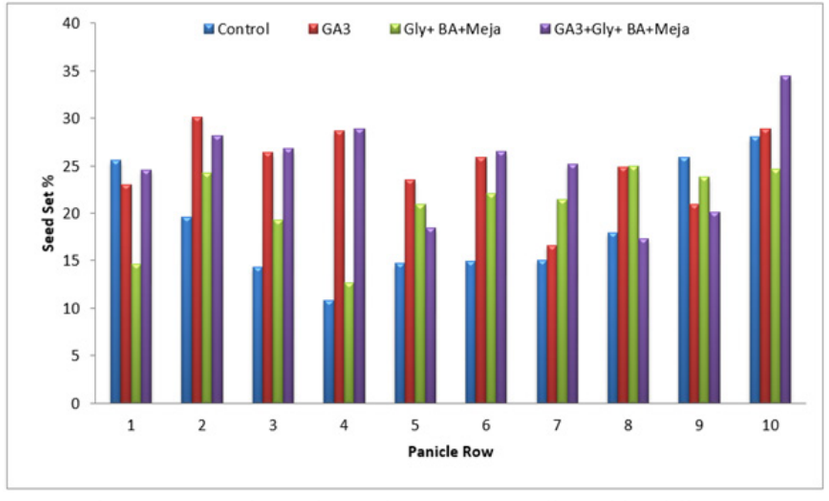


Figure 10. Seed set of panicle per row as affected by different phytohormones, wet season 2016.

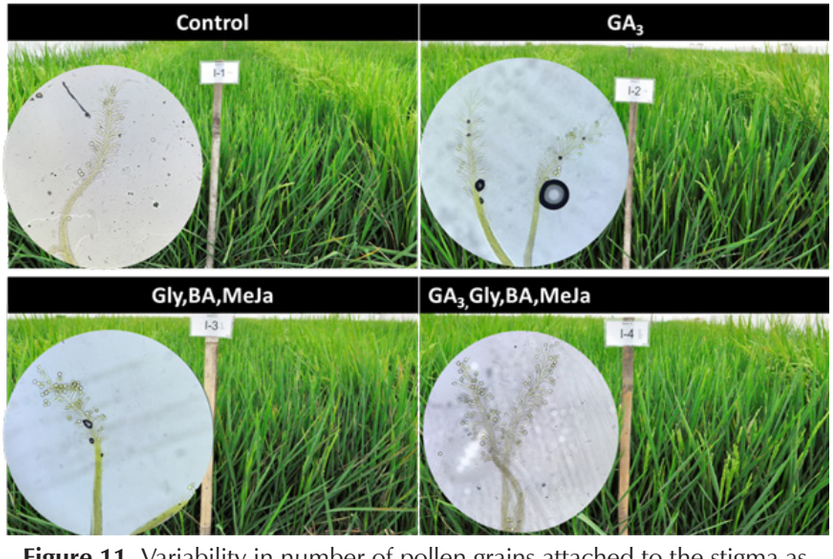


Figure 11. Variability in number of pollen grains attached to the stigma as affected by phytohormones.

Figure 12. Floral traits of pollen parent (P-line) showing anther and pollen characteritics.

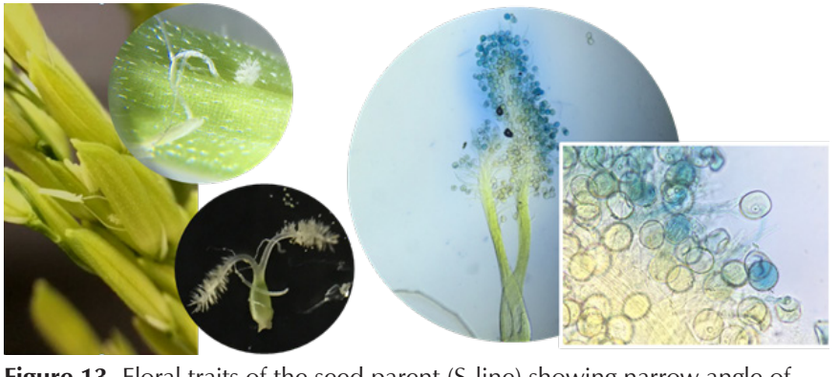


Figure 13. Floral traits of the seed parent (S-line) showing narrow angle of spikelet opening resulting to partial stigma exsertion.

Increasing seed yield in SxP seed production of NSIC Rc202H (Mestizo 19) by increasing plant density of the pollen parent

MOPalanog, EPRico, and SRBrena

A seed yield of at least 1 t/ha is considered to be the profitability threshold in the Philippine hybrid seed production. However, F1 production of Mestiso 19 is very low which discourages farmers in adopting the technology. Currently, the row ratio in S x P seed production is 3:10 with only three rows of pollen donors that will potentially pollinate 10 rows of TGMS lines (S-lines) resulting in high number of unpollinated S-lines. By increasing the plant density of P line, pollen will be abundant enough to saturate the stigma and pollinate it. Thus this study aims to increase percent seed set by increasing the population density of the pollen source and reducing the number of S –lines rows.

Activities:

- S x P seed production of Mestiso 19 was evaluated using four row ratios, 3:6; 3:8; 3:10, and 3:12 of pollen parents to sterile parents. P and S–lines were planted in 15 x 15 cm planting distance. The experiment was laid in Randomized Complete Block Design (RCBD) with three replications. Field experiment was conducted in PhilRice-Negros.

- The S –lines were transplanted 19 days after sowing and P – lines were transplanted at the same time at 22 days (P1); 19 days (P2); and 16 days (P3) after sowing, respectively.

- GA3 was applied at 200 g/ha. Application was done in two splits. Sixty percent (120 gm/ha) was applied at 20 – 30% heading stage and the remaining 80 g was applied at 40 – 60% heading or 2 days after the first application. Additional application of 30g/ha for three consecutive days was done during flowering stage to prolong stigma receptivity.

- Supplementary pollination was done from 9am to 2pm daily using rope during the peak of anthesis and was continuously performed until P line ceased to flower.

- Control plots were established parallel to S x P seed production to determine the possibility of self-pollination in S-lines. Seedlings were transplanted in 15 x 15 cm planting distance with one seedling/hill planting density and received similar cultural management as with S-lines in SxP.

- Yield and yield components were obtained from S-line rows while only yield component data were gathered for P-line

rows. Each S-line row was sampled for seed setting. Analysis of variance and mean comparison for significant difference for grain yield and yield component was done using Statistical Tool for Agricultural Research (STAR).

Results:

- Analysis of variance revealed a highly significant (Pr>0.0022) yield variation among treatments indicating a highly variable yield response in various row ratios. Among the row ratios, 3:6 obtained the highest mean yield of 3851 t/ha followed by 3:8 ratio with 3803 kg/ha with no significant yield difference between the two treatment ratios. The former two ratios yielded significantly higher than 3:10 (2117 kg/ha) and 3:12 (1932 kg/ha) ratios with no significant yield difference between the latter ratios. Result of mean comparison among row ratios using Duncan's Multiple Range Test (DMRT) is presented in Table 26.

- Higher grain yield in rows was observed in 3:6 and 3:8 which ca be attributed to the capability of pollens from P-line rows to saturate the fewer S-line rows thereby increasing the percentage of pollination of S-line flowers resulting in increased seed setting or spikelet fertility. Moreover, the heavier pollen load of P-line of M19 (REGRagas, unpublished) make it difficult to pollinate the S-line rows at the inner plot.

- Seed setting of S-line rows is highly correlated with grain yield hence the higher the seed-setting the higher the grain yield of S-lines. Apparently, S-lines adjacent to P rows tend to have higher seed set than rows towards the inner rows regardless of the row ratio (Figure 14).

Table 26. Grain yield response of various S:P row ratios with mean comparison using Duncan's Multiple Range Test (DMRT).

Row Ratio (S:P)	Mean Yield (kg/ha)
3:6	3852a
3:8	3803a
3:10	2116b
3:12	1932b

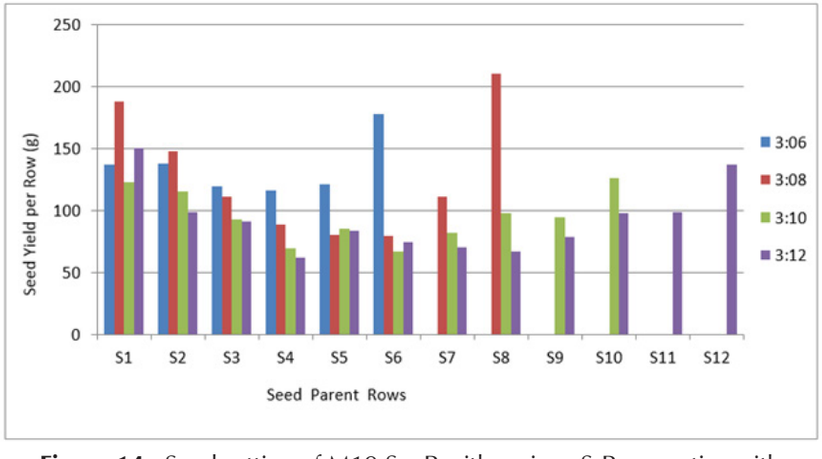


Figure 14. Seed setting of M19 S x P with various S:P row ratios with planting distance of 15x15 between rows for both S-lines and P-lines.

III. Hybrid Basic Seed Production and hybrid seed research

Project Leader: SRBrena

The successful commercialization of hybrid rice is linked to the development of hybrid rice seed technology, which is directly dependent on production of good quality seeds and timely supply of genetically pure seeds to seed growers and farmers. The commercialization and distribution of genetically pure seeds is a crucial factor for the complete heterotic expression of rice hybrids in order for the large-scale adoption of this seed technology. The genetic purity of the parents must be maintained to produce quality hybrid seeds in commercial quantities at an economical price and available every time they are required. This project also aims to ensure essential pollination through pollen collection and long-time storage and guarantee the pure, true-to-type and high quality seed that is essential for the successful implementation of government's hybrid rice commercialization program.

Hybrid nucleus and breeder seed production studies for newly released hybrids

LV Guittap WB Abonitalla SR Brena MT Talavera, and TM Masajo

Hybrid rice technology has proved to be effective in increasing production of rice in the country and elsewhere. To date, the National Seed Industry Council (NSIC) has released more than 70 hybrid rice varieties, 24 of which are public hybrids developed by IRRI, PhilRice, and UPLB. Currently, four public hybrids (Mestizo 1, Mestiso 7, Mestiso 19, and Mestiso 20) are popular and widely grown by farmers as a result of the DA-PhilRice Hybrid Commercialization Project. There is an urgent need to identify new hybrid varieties to replace the currently grown hybrids in the future. Two CMS-based and a TGMS-based hybrid have been identified as potential replacement for the currently grown varieties given that they satisfy the requirements for commercialization. These hybrids are Mestiso 48 (NSIC 2013 Rc318H), Mestiso 55 (NSIC 2014 Rc368H) and PRUP10. 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.

Activities:

- Parent lines of Mestiso 48, Mestiso 55 and PRUP10 were evaluated during the season. Paired cross generation was done for the A- and B-line of Mestiso 48 and Mestiso 55. Seed purification for the P-line of PRUP10 was also initiated.

- The component lines of the three hybrids were established in an evaluation nursery. Data on days to flowering and other agro-morphological characteristics were collected. The information collected were used to developed a protocol for the seed production of these hybrids

- Seed familiarity kits were packaged and distributed to seed grower cooperators.

Results:

- In 2015 wet season, original seeds of Mestiso 48 and Mestiso 55 parentals were requested from PhilRice CES. From the seeds received, a germplasm file was processed and packed for storage to serve as future reference and original seed stock. During the early part of 2016 dry season, a source population was established for the parent lines of Mestiso 48 and Mestiso 55. From the amount of seeds given, a total of 200 AXB paired crosses for each hybrid were generated.

- PRUP 10, a TGMS-based two-line hybrid bred by PhilRice and UPLB was approved by NCT last May 2016. Purification process of PRUP TG101 was conducted last wet season. For the pollen parent, SN 758, nucleus seeds were already produced in the early part of the season.

- Seed production kits were packaged and distributed during the season. A package of kit can plant 0.25 ha SXP F1 seed production. Recipient of the kits were seed cooperators in Davao Oriental and PhilRice Stations in Isabela, Negros, Midsayap and in Nueva Ecija. The group at Los Banos with the assistance of the TGMS Breeding researchers already established a seed production protocol for this specific hybrid which is now being used as basis for further studies and optimization.

- S x P F1 seed production was also established during the season. A total of 250kg F1 hybrid of PRUP10 was harvested. The seeds were distributed to different locations across the country for further testing.

- In order to further improve the efficiency of hybrid seed production, the TGMS Breeding group at Los Banos developed purple-version of the pollen parents of Mestiso 19 (TG101M-P) and Mestiso 20 (TG102M-P). Around 1000 panicles each of the pollen parents were given to the project for purification in the wet season. Also, an on-going activity is being conducted to purify the parent lines of Mestiso 32.

Identification of best location and time of the year/season optimum for seed production and quality
SRBRena, MOPalanog, and EPRico

PhilRice has released new hybrids, Mestiso 47, 48, and 55. These are all cytoplasmic male sterile (CMS) hybrids. Although these are registered and released by the National Seed Industry Council, seed production has not yet been explored on a commercial scale owing to the problem of Mestiso 48 when A-line are multiplied for foundation seeds. Mestiso 55 on the other hand is released but for is recommended for planting only during the dry season. The only released hybrid tried in this study was PRUP10 now given the NSIC number, NSIC RC446H. This hybrid is the third TGMS hybrid bred by PhilRice and UPLB. This new TGMS hybrid uses PRUPTG101 as the seed parent, the same seed parent as Mestiso 19 but uses SN758 as the pollen parent. In dry season, parental lines of this hybrid was sent to Davao del Sur, Davao del Norte, Davao Oriental, South Cotabato, and Negros Occidental. The parental lines were planted in South Cotabato but was affected by drought. Parental line sent to other places were not planted owing to lack of water brought about by the long dry season experienced in from March to September in some places.

In wet season, Different set of parental lines were sent to Mr. Alfredo Crisostomo of Davao del Sur, PhilRice – Isabela; PhilRice – Negros; PhilRice – Los Baños; and PhilRice- CES. Following the standard protocol provider by the breeder, seed production was set up.

Results:

- In Davao del Sur, 0.2ha was planted to S x P seed production of NSIC Rc446H following protocol number 1 where differential seeding for P-lines were 1, 4, 7 days.

- Based on the observation of the cooperator, S x P seed production in Davao del Sur can be done only during wet season because long cold spell is experienced during dry season. Likewise, S x P seed production was successfully tried in PhilRice – Isabela and PhilRice – CES.

- High seed yield was only achieved in Davao del Sur. The total seed yield obtained was 335kg or 1,775kg/ha. This yield level was higher than the seed yield obtained in Mestiso 19 during the post NCT trials conducted after its release.

- Yield obtained in PhilRice –Isabela and PhilRice –CES was lower than Davao del Sur owing to two typhoons experience during the season when the plants were at the flowering stage. Yield obtained in CES was only 95.69kg.ha.

- Heights of the P–lines and the S-lines at CES even without GA3 were 114.4cm and 100cm, respectively. The height difference between the parental lines showed that the inherent height of the parental lines is ideal for pollination.

Flowering behavior and seed production capacity of hybrid parental lines in different locations and seasons
SRBRena, MOPalanog, and EPRico

Despite of the several public hybrids released by NSIC, only NSIC Rc446H parental lines were planted in S x P seed production in five locations. The flowering behavior was evaluated in Davao del Sur, PhilRice – Negros; PhilRice –Isabela; and PhilRice – Los Baños during the wet season. Parental lines sent during dry season cropping were either planted but suffered drought or were not planted owing to lack of water. Only parental lines planted during the wet season were characterized. Two protocols were used in PhilRice –CES. For protocol 1, the target date of sowing the S –lines was 7 days and the differential seeding for the three sets of P –lines are 1, 4, and 7 days. For protocol 2, the target date for sowing the S –lines is 4 days but the same differential seeding was used for the P –lines.

Results:

- Parental lines planted in CES showed that the flowering duration of the P –lines lasted for 6 to 8 days only while the S-lines were observed to flower longer by 2 days.

- Days to heading for the P –lines was 96 days and for the S –lines was 99 days from the day of sowing.

- The same duration of flowering was observed in the two protocols used (Table 27).

Table 27. Agro-morphological characteristics of NSIC Rc446H parental lines.

Parental lines	Plant Height (cm)	Number of productive tillers	Days to Heading	Duration of flowering (days)
P-line	114.4	21	96	6-8
S-line (Protocol 1)	100.4	11	99	8-10
S-line (Protocol 2)	100.4	11	99	8-10

Nucleus and breeder seed production

LV Guittap, WB Abonitalla, SR Brena, MT Talavera, 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 PhilRice, UPLB and IRRI tested in the National Cooperative Testing (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.

Activities:

- For the CMS based hybrids, populations of CMS (A), maintainer (B) and restorer (R) lines raised from seeds known to be highly pure were used as source of individual A and B plants to be lifted for pair crossing. Only true-to-type A and B individuals were taken for crossing inside a screenhouse. Sterility of A is confirmed both by pollen examination and observing natural seed set.

- Evaluation nurseries were grown to look at the individually reconstructed A lines for agro-morphological traits and sterility behavior by way of seed set and by pollen examination. Corresponding seed increase nurseries was established to further look at the A and B lines and to raise nucleus seed. Only selected A and B individual pairs were allowed to contribute to the crop of nucleus seeds.

- For the production of basic seeds of the TGMS lines, original breeder seeds were planted in the male fertile environment to induce seed development. True-to-type plants were selected and scored for spikelet fertility. Part of seeds from selected plants will be grown in single progeny rows while remaining seeds will be stored. Entries that are uniform and completely sterile were selected and the corresponding balance seeds of such entries will be bulked. Bulked seeds were planted at the male fertile environment (MFE) for seed increase and harvests were considered as the nucleus seeds of the TGMS lines which will then be used in the breeder seed production also at MFE.

- Harvested hybrid parental breeder seeds were processed, packed, stored and distributed by PhilRice Los Baños to seed grower cooperatives and PhilRice stations. To assure good quality of stored breeder seed stock, regular monitoring of seed viability is being done with the assistance of PhilRice-CES Seed Technology Division. Likewise, a seed inventory system will be developed to keep track of the seed flow and status of all nucleus and breeder seeds in store.

Results:

- The project focused on the purification, production, and distribution of the parent lines of Mestizo 1 (PSB Rc72H) and Mestiso 20 (NSIC Rc204H) during the season in support to the DA-PhilRice TGMS Hybrid Commercialization Program. During the dry season, IR58025A X B (Mestizo 1) paired-were generated. Pollen samples were collected from 750 A-line individuals. The A-line plants were evaluated in the laboratory under a microscope to determine the extent of sterility. A total of 362 (48%) individuals were found to be completely sterile (CS) and lifted for crossing with corresponding B-lines (maintainer lines). The 362 A-line individuals were processed and will be evaluated for sterility, uniformity, trueness and other agro-morphological parameters during the wet season.

- A total of 300 kg IR58025A was harvested and processed during period. The average yield for the A x B seed production plots ranged from 700 to 900 kg/ha during the dry season. Corresponding amount of maintainer line (IR58025B) was harvested during the season. Sufficient amount of R-line is being maintained in storage. The group also produced as total of 300kg IR68897A with corresponding amount of maintainer line.

- Breeder seed production for the S-line of Mestiso 20, PRUP TG102 was undertaken at MFE site in Benguet. Unfortunately, the area was affected by severe drought during the flowering stage in mid-April. The seeds were discarded due to high percentage of spikelet sterility resulting to poor seed set. The pollen parent of Mestiso 20, TG102M was not multiplied since there are sufficient amount stored at Los Banos.

- An added responsibility was given to the project in support to the DA-PhilRice TGMS hybrid commercialization program.

Requirements for S-line foundation seeds for the nationwide SxP seed production was produced under the management of the group. An MFE site located at Quezon were planted with PRUP TG101 in the dry season. A total of 1,200 kg of seed were produced during the period.

- As of July 11, 2016, a total of 75kg breeder seeds of PRUP TG102 (Mestiso 20) were dispatched to PhilRice Stations in Isabela, Negros and CMU for foundation seed production. Also, 75kg of breeder seeds of Mestizo 1 were established at different PhilRice Stations in Luzon and Mindanao.

- 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.

Development of Possible Alternative for the Control Plot in TGMS Seed Production

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

The establishment of control plot (CP) is an added feature in the seed certification of TGMS SXP hybrid seed production. CP generally estimates the degree of selfing in SXP seed production. Although this method is very effective in determining seed selfing, several difficulties on the establishment of CP were identified. Some seed grower considered it a burden because of the additional space and isolation requirements. On the part of seed inspectors, inspection process is very challenging because of the vulnerability of the CP to outside interventions. In order to address these limitations, there is a need to develop a more efficient substitute for the CP. Methods tried were "bagging" of S-line plants in the SXP plots and "lifting" from the field to the screenhouse or to an area isolated from pollen contaminants.

Activities:

- A 0.25ha SXP seed production plots of Mestiso 19 was established in the field following the standard protocol in TGMS hybrid seed production. Planting interval of the parentals was based on the information gathered at Los Banos condition. Row ratio for the parent lines was 3:10 (P-line : S-line). As indicated in the current certification standard for TGMS two-line hybrids, a 40 m^2 control plot was planted following the specifications stated in Administrative Order 8 series of 2012 (AO 8 s. 2012). The requirements include the plant spacing, isolation, and field management.

- The 0.25 hectare plot was divided into 2 lots established in two different planting dates. An area of 0.125 ha can accomodate 10 blocks of SXP composed of 10 rows x 320 hills per row (3200 s-line plants). For each block, 100 random plants were bagged with glaccine bags at the onset of flowering.

- Using the same seed production plots, 200 s-lines randomly selected were lifted from the field. The plants were planted into pales and maintained inside the screenhouse and isolated from possible pollen contamination.

Results:

- For the dry season, only Mestiso 19 (PRUP TG101xTG101M) seed production plot was planted due to area and isolation limitations. The experiment covered two planting dates with two week- interval in January. A control plot was established for each of the planting dates using the standard protocol of AO 8 s. 2012.

- Randomly selected panicle from an S-line plant was marked to represent one plant sample. A total of 1,900 panicles were bagged during the period. The seed set of each panicle was determined by counting the number of filled grains. For the SXP plot established on JAN-I, 6 samples were recorded to have seed set ranging from 1 to 6 seeds per panicle. JAN-II planting date on the other hand, had 20 panicle samples with seed set from 1 to 16 seeds. Damaged and/or empty samples were discarded.

- The average S x P yield of Mestiso planted during the season was 835kg/ha. Selfed seeds in the control plot were 5.0 to 7.2g.

- Initial data shows that bagging is an effective method in preventing the s-line plants from being pollinated since the percentage of plants pollinated was only 1.6%. It was also observed that the planting date with higher amount of selfed seeds in the control had more panicles with seed set. For the lifted plants, different trend was observed. No seed set was recorded for the lifted plants established in JAN-II.

- Temperature data during the critical stage of flowering will be collected to reinforce the result of seed setting. SXP seed production plot of Mestiso 20 will also be established in the wet season. Additional planting dates for Mestiso will be

established to generate more data points to compare the new methods with control plot.

Strategies for pollen harvest and storage in view of increasing pollination and yield in hybrid seed production of Mestiso 19

REGRagas, AGSFerriol, and SRBrena

Yield advantage of hybrids over inbred has led to increase demands of hybrid seeds. Mestiso 19 (NSIC Rc202H) is a two-line public-bred hybrid that gains popularity across regions of the Philippines due to its high yields (reported national average reached 8±1 t·ha-1). The bottleneck, however, remains in the production of its seeds. Hybrid seeds are produced by cross-pollination between a male-sterile (female) line and a fertile line (male). Insufficient pollination has been found to be one of the most important factors responsible for low yields in many field crops. Pollen, the male gametophyte of the flowering rice plant, has an important role in cross-pollination. Upon landing on the stigma for pollination, the pollen grain produces a long pollen tube to deliver sperm to the ovule for fertilization. To achieve successful cross-pollination, it is important that flowering synchrony happens between male and female parents. Pollen, the male gametophyte of the flowering rice plant, has an important role in cross-pollination. Upon landing on the stigma for pollination, the pollen grain produces a long pollen tube to deliver sperm to the ovule for fertilization. To achieve successful cross-pollination, it is important that flowering synchrony happens between male and female parents. This study will develop a simple procedure for pollen collection, dehydration, and long-term storage to increase hybrid seed production efficiency.

Activities:

- Parent line of Mestiso 19 (PRUP TG101 and TG101M) was used in the experiments under irrigated condition. At anthesis, a mature, actively flowering tiller was bagged in a paper sack and shaken to release as much pollen. Rice pollen was collected daily by shaking the rice panicles contained in a paper bag. Collected pollen was air dried at room temperature and sifted using a mesh size of 0.125mm to remove anthers and contaminants.

- Some collected pollen grains were subjected to in vitro germination testing in different media (Table 28). The remaining pollen grains were placed in storage containers (amber glass paper envelope and zip lock) and were to different storage treatments: 24, 48 and 72h of refrigeration at 28°C, 5°C, -5°C. The in vitro germination tests were performed using different culture media (Table 28). After 2 to 3h, pollen grains were stained in a 0.005% aniline blue solution [0.005% aniline blue in 0.15M K2HPO4 (pH 8.6)] and were counted under a fluorescence microscope. Twenty different fields of vision, each with atleast 30 grains were examined per treatment. Pollen grains were considered germinated when the pollen tube length is equal or greater than the diameter of the pollen grain.

- To assess the extent of pollination and tube growth, rice flowers were emasculated and artificially pollinated by hand. The samples were fixed in a solution (3:1 ethanol: acetic acid) and stored at 4° in 70% ethanol until use. The dissected pistils and ovaries were washed twice with distilled water and then incubated in a solution of 8N NaOH for 1 hour. Samples were stained in 0.1% aniline blue in K3PO4 buffer and examined under UV illumination to visualize the callose of pollen tube. The numbers of pollen tubes and the extent of their growth through the length of the style will be assessed.

Results:

- Pollen viability decreases after anthesis, but growing the grains in media 2 (3% Ca(NO3)2 + 5% Sucrose +1% Agarose +10 % Boric acid) appears to maintain pollen viability even after 1 hour after anthesis. (Figure 15A)

- Analysis of variance revealed that only Media and Collection time x Media is significantly correlated with germination, and pollen tube growth in collection time variates. However, Media appeared to be significantly correlated to germination and pollen tube length, collection time showed to be significantly correlated to viability abs germination, while collection time x media showed to be significantly correlated to pollen tube length (Table 29).

- Pollen germination decreases over time regardless of media used. To increase the chance of effective pollination, pollen grains must be collected at anthesis and stored immediately in amber glass under cold storage at negative 5°C (Figure 16).

- Pollen tube length was consistently high at anthesis. Comparison of different media reveals that pollen tube lengths from medium 2 do not seem to differ between 30 min and 1 hour (Figure 15).
- Highest viability of pollen grains at more than 50% is only

achieved after 24 hours of storage. Pollen decreased its viability and germination beyond 24 hours (Figure 16).

- Among the storage materials, amber glass was observed the best packaging to preserve the shelf life of pollen grains (Figure 16).

Table 28. List of media used for pollen viability test with their components and individual concentrations.

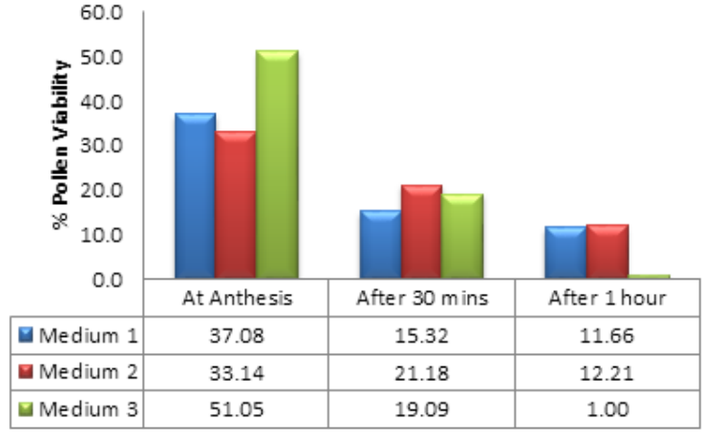
Culture Medium Code	Components			
1	10% PEG	20% sucrose	0.05% $CaCl_2$	0.01%Boric acid
2	3%$Ca(NO_3)_2$	5% Sucrose	1% Agarose	10 % Boric acid
3	4 % Calcium nitrate	14% Sucrose	1% Potassium Nitrate	1% Boric acid

- Additional optimization procedures in both collection time and storage conditions must be performed in replicates for another season to determine the most effective method of pollen harvest important for hybrid seed production.

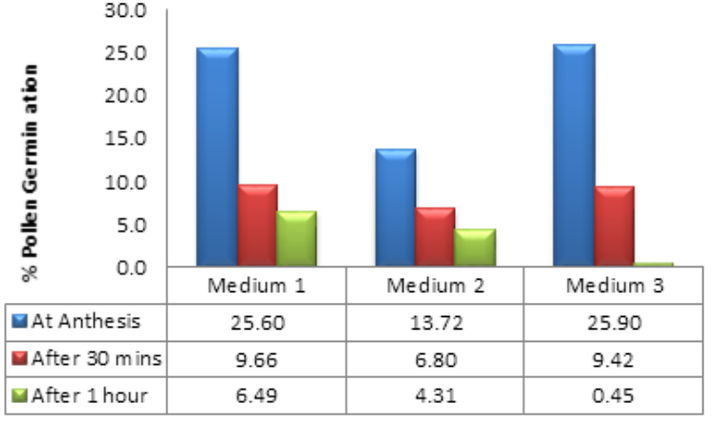
Table 29. Analysis of Variance of different treatments under varying collection times and storage conditions.

VARIATE	Media	Media x Rep	Collection Time	Collection time x Media
Collection Time				
Viability	0.0557	0.1259	0.1298	0.1129
Germination	0.0138	0.0335	0.5731	0.0008
Pollen tube length	0.0019	0.7362	0.5288	0.0349
Storage Condition				
Viability	0.8664	0.7172	0.0219	0.3561
Germination	0.0700	0.8292	0.0165	0.266
Pollen tube length	0.0076	0.4221	0.4345	0.0339

A)



B)



C)

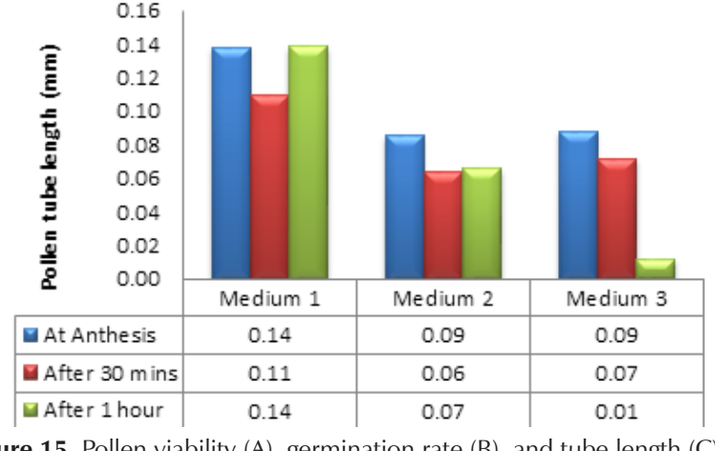
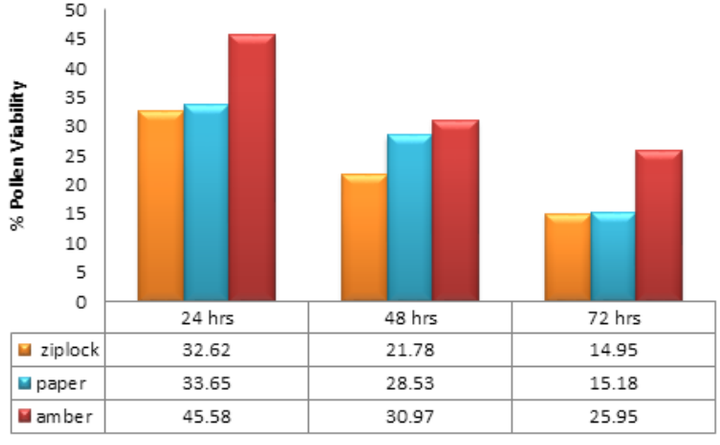


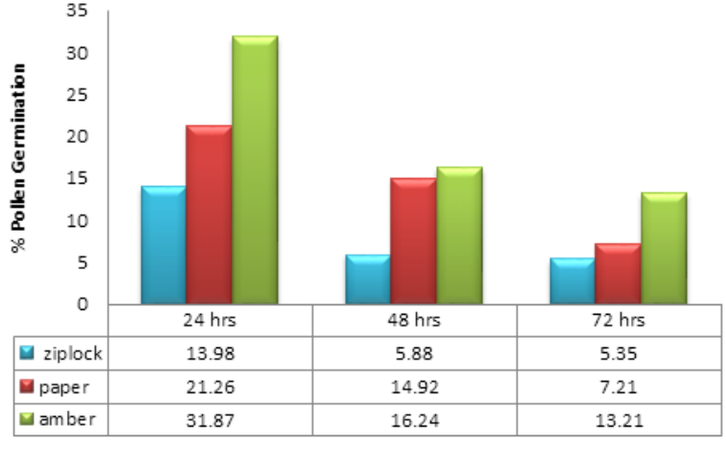
Figure 15. Pollen viability (A), germination rate (B), and tube length (C) as affected by different media and time of collection.

A)



	24 hrs	48 hrs	72 hrs
ziplock	32.62	21.78	14.95
paper	33.65	28.53	15.18
amber	45.58	30.97	25.95

B)



	24 hrs	48 hrs	72 hrs
ziplock	13.98	5.88	5.35
paper	21.26	14.92	7.21
amber	31.87	16.24	13.21

Figure 16. Pollen viability (A) and germination rate (B) as affected by different media and time of collection.

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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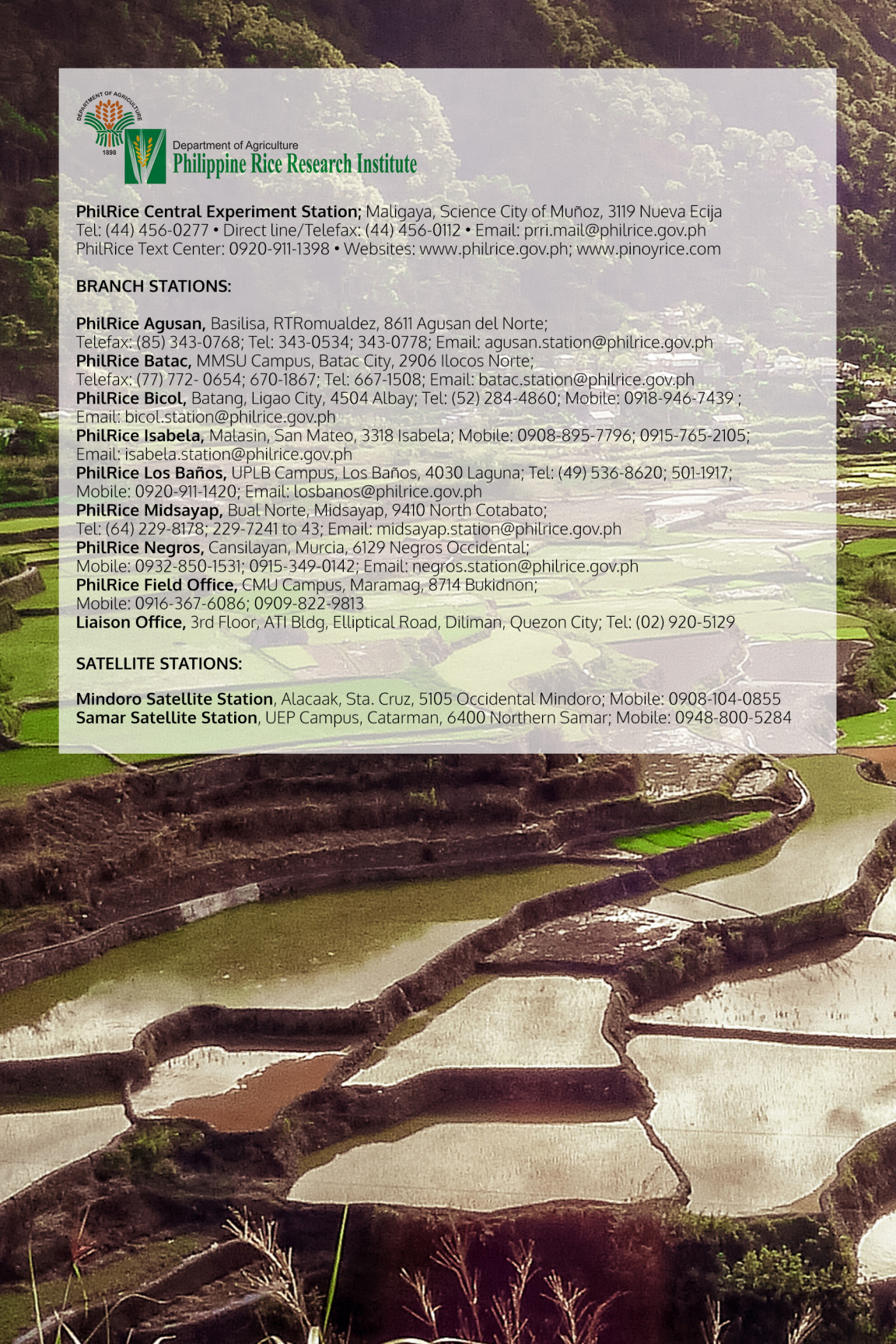

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