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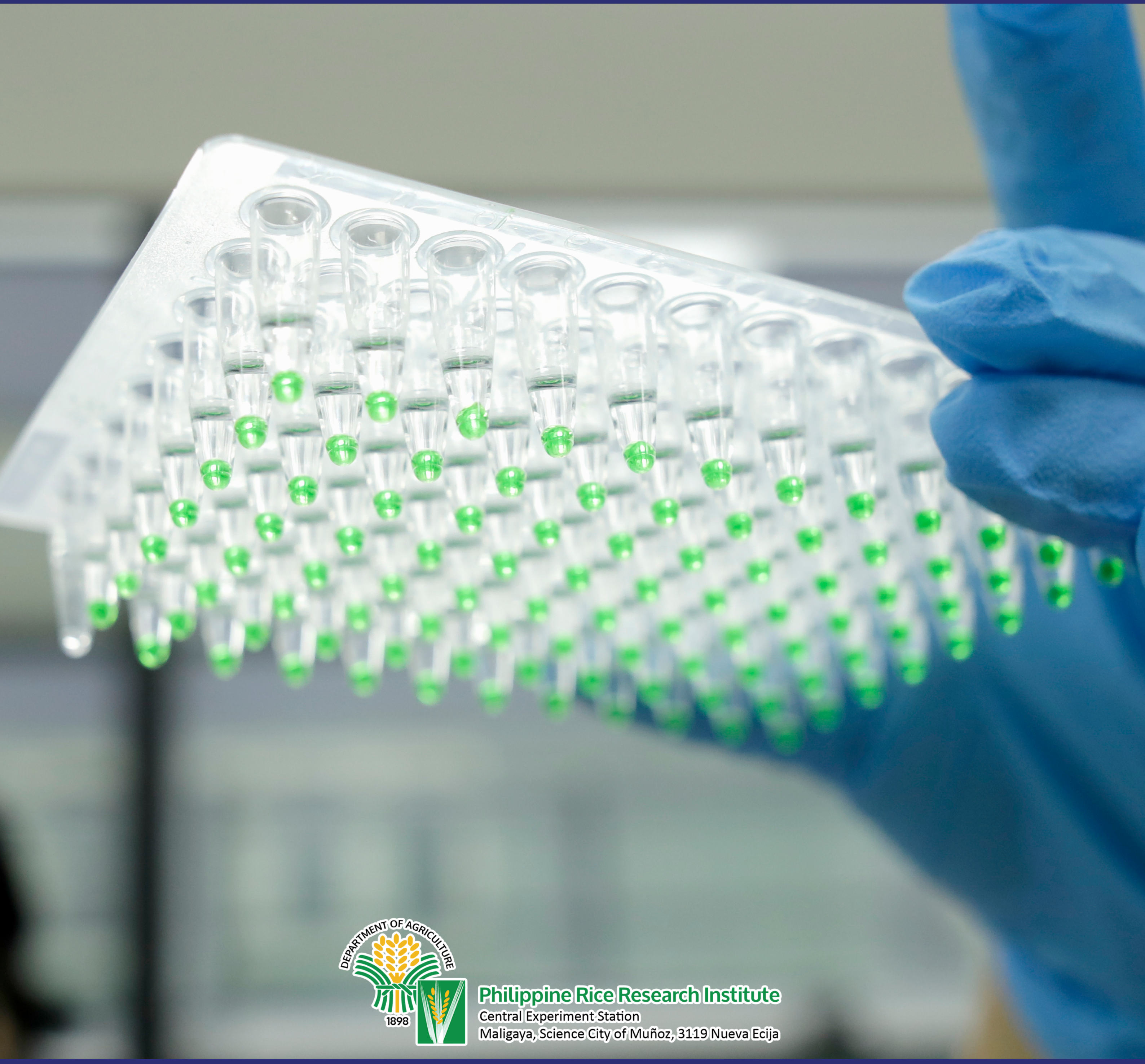

DEPARTMENT OF AGRICULTURE
1898
Philippine Rice Research Institute
Central Experiment Station
Maligaya, Science City of Muñoz, 3119 Nueva Ecija

ABOUT THE COVER

Genetic conservation and characterization in rice is one of the basic principles in crop improvement. This can translate into modern technologies that address climate change, variety release, molecular techniques, rice production and management, grain qualities, malnutrition, and even gender issues.

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FULL PAPER

RICE IMPROVEMENT FOR HIGH TEMPERATURE TOLERANCE IN THE PHILIPPINES

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Abstract

Rice is a flexible crop that can be grown in the uplands, lowlands, and areas prone to drought, saline, flood, cold, and high temperature. However, increasing population, declining area for rice production, and vulnerability to climate change reduce the sustainability of rice production. One of the constraints in rice growing is high temperature or heat stress due to climate change. High temperatures occur in dry and wet seasons, risking the crop's flowering stage. Thus, yield and quality traits need to be improved through breeding new genotypes.

Popular rice varieties in the Philippines have high yields, good grain quality, and resistance to pests and diseases but lack high temperature tolerance. Breeding for heat tolerance is one of the solutions to improve the quality of rice plants. This paper presents the breeding strategies using conventional and biotechnological approaches in developing heat-tolerant rice varieties in the Philippines. NSIC 2020 Rc 600, a result of 10-year work, was released by the National Seed Industry Council as one of the first rice varieties recommended for high temperature areas in the country.

Keywords: *High Temperature, Irrigated Lowland, Quantitative Trait Loci (QTL), Rice Improvement.*

Introduction

Rice encompasses many agro-ecological zones and it is one of the most important crops in the world. Rice is a flexible crop that can be grown in the uplands, lowlands, drought, saline, flood, cold, and high temperature-prone areas. However, increasing population, declining area for rice production, and vulnerability to climate change threatens the sustainability of rice production. Yield and quality traits have to be improved through breeding new genotypes.

High temperature or heat stress is one of the most important constraints in rice production in tropical and sub-tropical regions in Asia, Africa, and Southeast Asia. The severity of the problem in rice growing areas is increasing due to rising temperatures. Global temperatures are estimated to rise between 1.1 - 6.4°C during the next century (IPCC 2007, 2012) and rice production will be threatened. With climate change scenarios as a result of global warming, breeding for heat tolerance is one of the key research areas that may address problems related to temperature increase.

Rice normally grows between 20 - 35°C but with increasing temperature of >35°C, it is also increasingly sensitive especially during reproductive stage. Several studies have indicated that high temperature stress resulted in loss of pollen viability, poor anther dehiscence, and low pollen on stigma (Das et al., 2014); lead to decreased seed setting (Cao et al., 2009); increased phytic acid (Su et al., 2014), high sterility and faster grain development (Satake and Yoshida, 1978); decreased grain size and shape (Cao et al., 2008); decreased kernel weight and amylose, and increased chalkiness (Wu et al., 2016); and shrivelled seeds (Hasanuzzaman et al., 2013). This can result in 10 - 15% decrease in yield.

Rice is grown mainly in tropical and subtropical zones, and a high temperature at flowering can induce floret sterility and can limit grain yield (Osada et al., 1973; Satake and Yoshida, 1978; Matsushima et al., 1982). Horie et al. (1996) suggested that the anticipated high temperature would induce floret sterility and increase the instability of the rice yield even in temperate regions.

The main cause of floret sterility induced by high temperature at flowering is another indehiscence (Satake and Yoshida, 1978; Mackill et al., 1982; Matsui et al., 1997a, b, 2001). Crop scientists have attempted to assess the effects of increasing temperature and high carbon dioxide in the atmosphere on the growth and yield of rice using simulation models (Boote et al., 1994; Horie et al., 1996; Matthews et al., 1997). High temperatures at flowering inhibit swelling of the pollen grains (Matsui et al., 2001), which is the driving force behind anther dehiscence in rice (Matsui et al., 1997a, b). Anthers of high temperature-tolerant cultivars dehisce more easily than those of susceptible cultivars and contribute to pollination under high-temperature conditions (Satake and Yoshida, 1978; Mackill et al., 1982; Matsui et al., 2000, 2001). However, the factors determining the degree of anther dehiscence are unknown.

Popular rice varieties in the Philippines have high yields, good grain quality, and resistance to pests and diseases. However, they lack high temperature tolerance. Breeding for heat-tolerant varieties is one of the priorities for rice plants to cope with high-temperature stress. New rice varieties should possess adaptability to rising temperatures in addition to desirable traits (Manigbas et al., 2014).

In the Philippines, breeding for high temperature tolerance begins with identifying donor parents and incorporating them to popular varieties (Manigbas and Sebastian, 2007; Redoña et al., 2007). More than 200 varieties from International Rice Research Institute (IRRI) and Philippine Rice Research Institute (PhilRice) germplasm during the hottest period in the dry season (DS), annually from April to May were already identified. Authors also showed that adoption of high temperature-tolerant cultivars is one of the most effective measures to maintain high productivity and stability of rice under the anticipated climate in temperate regions (Horie et al., 1996). Six major QTLs were also identified for high temperature tolerance, which serves as baseline information for marker-assisted selection in the future (Grospe et al., 2016; Manigbas et al., 2018). Two outstanding varieties from India, Nagina 22 (N22) and Dular, and other African varieties were chosen as donors and incorporated to Philippine and other rices cultivars for improvement. This paper presents the breeding strategies using conventional and biotechnological approaches in developing new rice varieties in the Philippines.

Materials and Methods

The development of high temperature-tolerant rice at PhilRice started in 2007 - 2008 dry season (DS) during the screening and selection of donor parents from different germplasm. Hybridization began in 2010 followed by several generation advance, screening and selection of desirable phenotypes, yield trials, mapping populations for QTL analysis and mapping of genes, using Marker Assisted Selection (MAS) through a known marker RM3586 for heat tolerance, pollen fertility, and evaluating agronomic traits for heat tolerance. In this paper, three main components were presented: (a) mainstream breeding and selection, (b) identification of QTLs and mapping of genes, and (c) pollen fertility and yield evaluation. Mainstream breeding and selection under high temperature field conditions were conducted every season at the PhilRice Central Experiment Station (CES) in Nueva Ecija and Southern Cagayan Research Center (SCRC) in Iguig, Cagayan. The select individuals, progeny, lines, and populations developed were used in the succeeding studies on QTL analysis and mapping of genes and pollen fertility and yield evaluation.

A. Mainstream Breeding and Selection

Breeding for high temperature tolerance in rice starts from the selection of the donor parents and hybridization and the succeeding screening, selection, and yield trials. Field screening was conducted in two high temperature locations in PhilRice CES, and in SCRC where temperature can rise up to 39^{o}C during April and May in the DS and September during wet season (WS). Breeding materials were grouped based on flowering dates so that all plants during flowering will coincide with the highest temperature of the month. Selection was based on plant type, resistance to pests and diseases, phenotypic acceptability, tillering ability, sterility and fertility, yield, and grain quality traits. Percent fertility of the grains was determined by counting the number of filled spikelets over the total number of grains in the sampled panicles. The general procedure is illustrated in Figure 1. Temperature and relative humidity were automatically registered every two minutes through MINCER (Micrometeorological Instrument for Near Canopy Environment in Rice) instrument installed in the field from booting stage until maturity.

In certain crosses, F_2 to F_6 populations were grown in the seedling trays (push carts) using the Rapid Generation Advance (single seed descent method) facility at PhilRice (Manigbas and Lambio, 2015). The recombinant inbred lines generated were planted in the field for plant selection and further evaluation.

Table 1 shows the select heat-tolerant breeding lines and their characteristics under field conditions. From the elite breeding lines in Table 1, four were nominated to the National Cooperative Testing (NCT) for heat tolerance, which include PR40330-4-2-7-1-2-1, released in 2020 as NSIC Rc 600. The NCT is implemented by the Rice Technical Working Group (RTWG) of the National Seed Industry Council (NSIC) of the Philippines mandated under the Seed Industry Development Act of 1992 (RA 7308) to conduct field testing and evaluate performance of promising rice lines and hybrids, nominate new

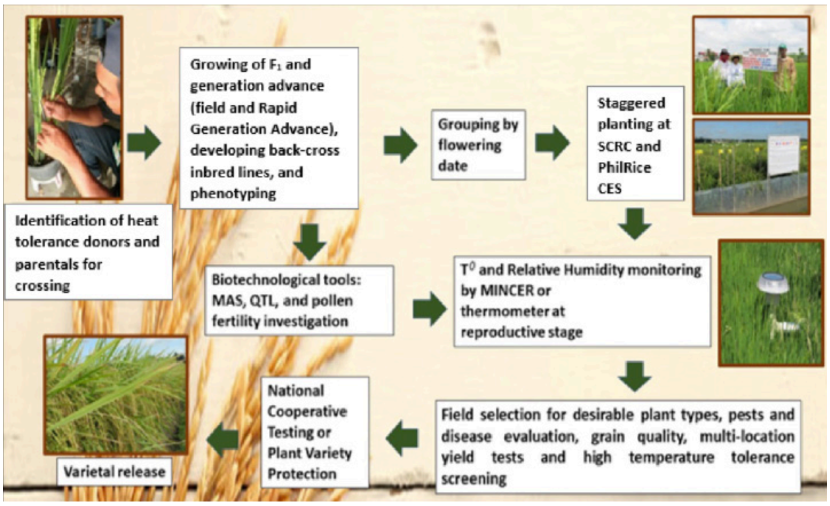

Figure 1. Development of new rice varieties for high temperature tolerance at PhilRice.

Table 1. Select elite breeding lines with heat tolerance and their important characteristics.

Designation	Plant Height (cm)	Mean Yield (t ha^{-1})	Fertility (%)	Blast		Bacterial Leaf Blight		Amylose Content (%)	Class
PR37624-9-3-1-3-3-3-B-1-1-1	112	5.97		3	R	5	I	16.6	Pr
PR40330-4-2-7-1-2-1	101.5	5.81		9	S	5	I	17.9	G3
PR42026-34-1-3-B-2	132	6.75		9	S	9	S	19.7	Pr
PR42116-1-1-1-3-2	114	5.84		9	S	7.67	S	18.7	G2
PR42127-M-1-B-2-B-B-3	111	5.89		5	I	9	S		
PR42129-M-4-B-4-B-B-7	107	5.38		5	I	8.33	S		
PR42130-M-B-B-B-B-B-2	97.3	5.83		7	S	7	S	17	Pr
PR42130-M-1-B-1-2-B-5	101.5	5.12		3	R	7	S	16	G1
PR42130-M-1-B-6-2-B-7	104.5	6.36		5	I	7	S	15.8	G1
PR42130-M-1-B-4-1-B-1	114	5.63		1	R	8.33	S	18.5	Pr
PR42132-M-3-B-B-B-B-9	113	6.22		1	R	8.33	S		
PR42132-M(I)-1-B-8-B-B-6	108	5.53		9	S	5	I	15	Pr
PR42218-7-3-2	107	4.72		9	S	5	I	18.9	G2
PR40780-58-2-2-2-1	114.3	4.19	71.51	6	I				
PR40330-4-2-B-2-1-1	108.7	4.39	71.75	2	R				
PR40330-4-2-B-22-2-3	100.8	4.24	78.22	2	R				
PR42032-26-6-1-B-3	108.1	4.35	61.97	2	R				
PR42028-14-1-1-1-3	106.2	4.3	78.49	2	R				
PR42128-M-4-B-9-3-B-3-B	114.8	4.14	74.12	2	R				
PR42132-M-1-B-B-B-B-17-B	110.3	5.16	63.76	2	R				
PR42130-M-1-B-B-B-B-8-B	108.8	4.54	66.11	2	R				
PR42130-M-1-B-6-3-B-1-B	107.6	4.81	55.38	2	R				
PR42130-M-1-B-8-3-B-1-B	104.6	4.02	55.72	2	R				
PR42130-M-1-B-8-3-B-3-B	101.9	4.83	66.96	2	R	7	S	17.4	Pr
PR42130-M-1-B-8-3-B-5-B	102.3	4.94	25.95	7	S	7	S	18.1	Pr
PR42130-M-1-B-10-3-B-7-B	105.3	5.09	60.91	2	R	7	S	19.5	Pr
PR42132-M-3-B-B-B-B-5-B	115.9	4.37	69.85	7	S	9	S	18.2	Pr
PR42132-M-3-B-B-B-B-6-B	109.5	4.08	66.73	7	S	9	S	18.3	Pr
PR42132-M-3-B-B-B-B-11-B	115.7	4.21	66.72	5	I	7	S	17.9	Pr
PR42132-M-3-B-B-B-B-13-B	110.9	4.2	68.37	9	S	9	S	17.7	Pr

Legend: S=susceptible, I=intermediate resistant, R=resistant, G1=grade 1 grain quality, G2=grade 2 grain quality, Pr=premium grain quality.

improved rice varieties to NSIC, and formulate procedures for varietal evaluation and identification.

B. Identification of QTLs and Mapping of Genes

Identifying and mapping of genes, QTL, and manifesting high temperature tolerance were done simultaneously during breeding and selection of lines and populations. This was done to facilitate MAS and as a way for cloning and characterizing underlying factors useful for genetic improvement. Exploitation of new genes and alleles to predict gene function, isolate homologues, and conduct transgenic experiments were implemented to address high-temperature stress condition. At PhilRice, QTLs were identified using the scheme in Figure 2 and 3 (Grospe et al., 2016).

In 2014 DS, three backcross inbred populations (BC_2F_4) of NSIC Rc 150/ Dular, Gayabyeo/ Nagina22 and Gayabyeo/ Dular were used to study the QTLs governing heat tolerance. The plants were screened under high-temperature condition from heading to maturity. Heading date was recorded as basis for the number of days to maturity. Duration of heading (HD) was determined during the entire stage. The date and time on MINCER and improvised digital clock were synchronized to facilitate phenotype marking. At physiological maturity, plant height, number of productive tillers, panicle length, and number of filled and unfilled grains were recorded. The mean spikelet fertility of the three panicles was used to evaluate high temperature tolerance of the progenies.

Genotyping

Genotyping was conducted at PhilRice's Plant Breeding and Biotechnology laboratory in 2014 using the procedure in Figure 3. Mapping populations were planted in the glasshouse and leaves were collected for DNA extraction. The extracted DNA was purified and quantified before running in the PCR (Polymerase Chain Reaction) machine. PCR products were loaded to the gel using horizontal gel electrophoresis. The gels were then placed to the imager and bands were scored and analyzed.

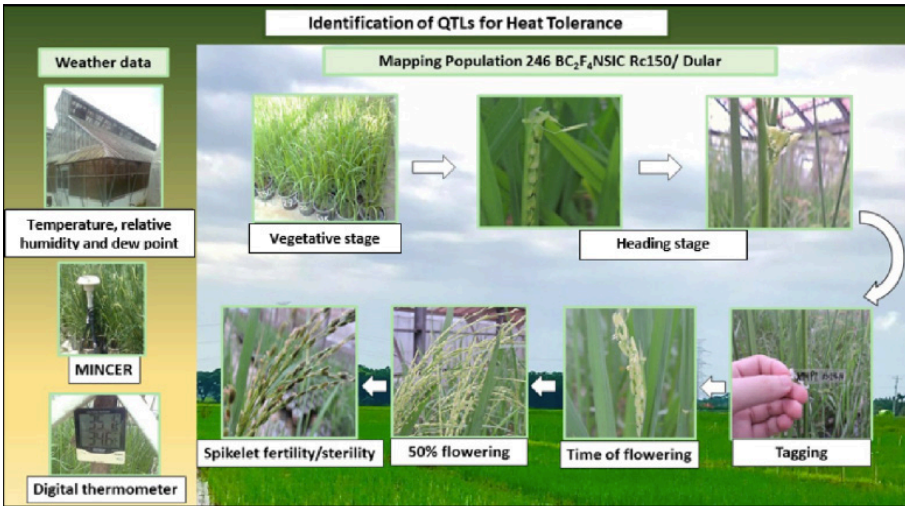

Figure 2. Phenotypic identification of QTLs for heat tolerance at PhilRice.

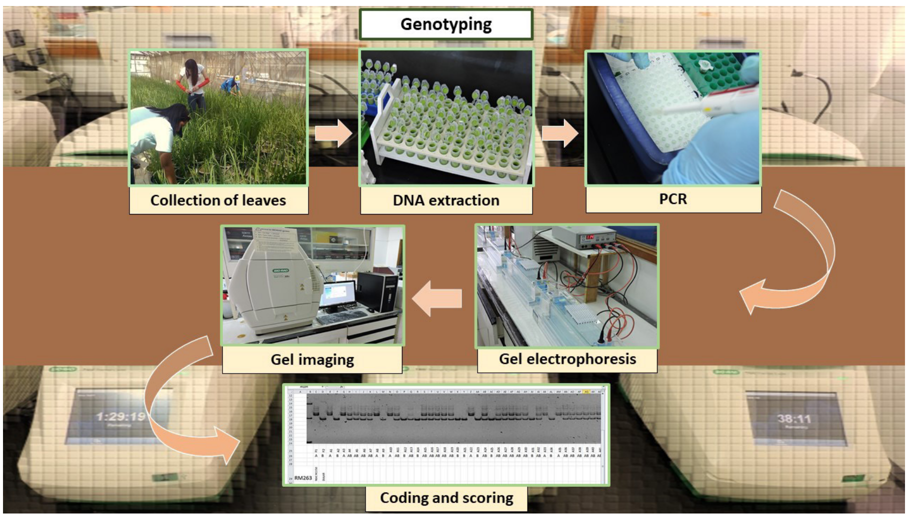

Figure 3. PhilRice's genotyping procedure in the QTL identification for heat tolerance.

C. Pollen Fertility and Yield Evaluation

Pollen fertility of the breeding populations and its correlation to important morphological traits were determined. Pollen fertility of genotypes under high temperature regimes is a plant response to the stress; thus, an important factor in the selection of the breeding lines. The study was conducted from January to May 2014 in two locations representing two temperature regimes: Science City of Muñoz, Nueva Ecija and Los Baños, Laguna. Field experiment in Nueva Ecija (NE-F) and Los Baños (LB) were established using 15 plants per entry and transplanted at one seedling per hill at 20 cm x 20 cm spacing. The experiment was laid out in randomized complete block design (RCBD) with four replications. Three backcross populations (NSIC Rc 160*4/Dular as POP1, Hanareumbyeo*4/N22 as POP2, and Gayabyeo*2/N22 as POP3) with 19 breeding lines each were studied. Checks such as N22 and Dular (tolerant), IR64 and Milyang 23 (intermediate), and IR52 (intolerant) and parents were also included. The entries were grouped based on their days to flower and seeding was timed for the plants to be exposed under high temperatures at reproductive stage. Percent pollen fertility (PPF) was calculated using the protocol as described in the Standard Evaluation System (SES) (IRRI, 2014). Five plants were randomly selected from each entry. Main tiller spikelets were collected and fixed in 70% ethyl alcohol. Pollen grains were stained with 1% iodine potassium iodide (I_2KI) solution. Stained pollens were considered fertile while the unstained were sterile.

Five plants per entry were randomly selected for the measurement of yield components such as the number of panicles per hill, number of filled and unfilled spikelets per panicle, and 1,000-seed weight. Data were analyzed using the Statistical Tool for Agricultural Research version 2.0.1.

Results and Discussion

A. Mainstream Breeding and Selection

More than 65% of the country's rice supply is grown in Central Luzon (Region 3), which is under the threat of high temperature stress in the next decade. Data from Philippine Atmospheric, Geophysical and Astronomical Services Administration (PAGASA) in 2000 - 2007 showed that the mean maximum temperature exceeded 35°C during flowering or reproductive stage of the crop (Figure 4). With the predicted increase in temperature by 2°C in the next 50 years, rice crop production will be affected if no mitigation or adaption are to be implemented. High temperature during reproductive stage to maturity in March to May is at stake. Even during the wet season, the temperature can exceed 35°C during flowering stage, which may result in reduced yield.

Effects of high temperature on rice were evaluated in Cagayan (Region 2) in 2016. The temperature

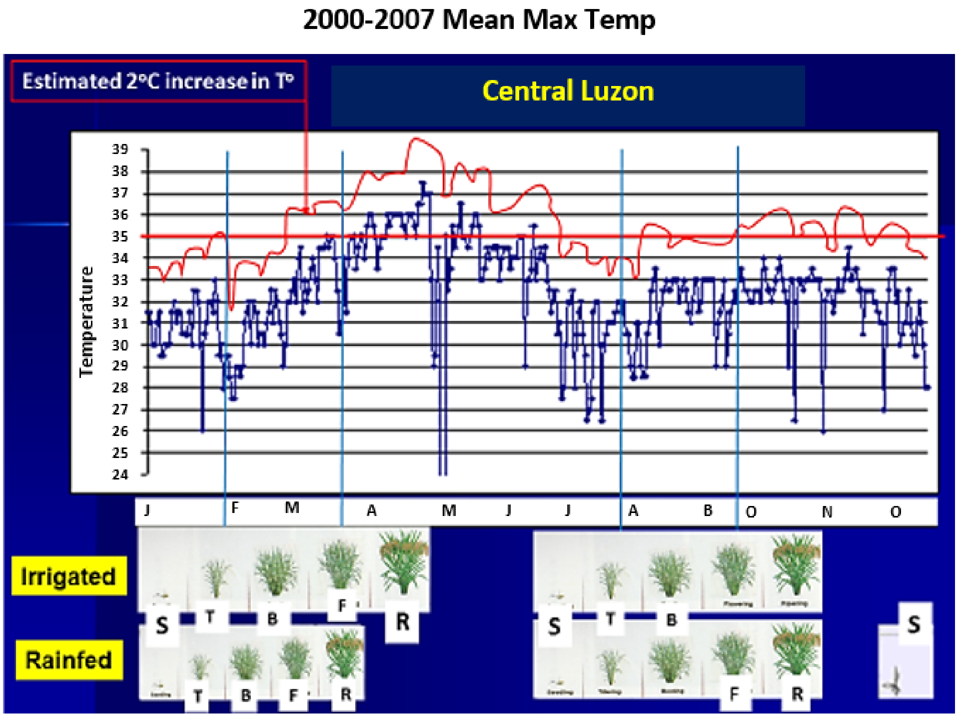

Figure 4. Mean maximum temperature in Central Luzon, Philippines from 2000 to 2007 and estimated increase in temperature (red line) in the next 50 years. The x-axis J, F, M, A, M, J, J, A, S, O, N, and D stands for the months of the year and the stages of the crop under irrigated and rainfed conditions as S=seedling, T=tillering, B=booting, F=flowering, and R=ripening.

during the period exceeded 35°C during the flowering period from April to May (Figure 5). Many rice varieties in the farmers' field recorded high sterility of up to 80% (Figure 6). Few varieties can adapt to high temperature stress through early flowering or tolerance. More data points are needed to confirm this initial observation in Cagayan.

National Cooperative Testing (NCT) for Heat Tolerance

Rice breeding lines have to complete the NCT for heat tolerance for three seasons to be recommended as varieties. The NCT for heat tolerance started in 2016 DS in three major sites where high temperature occurs: PhilRice CES, Nueva Ecija; Tuguegarao, Cagayan; and Central Bicol Experiment Station (CBES), Pili, Camarines Sur. Results of the 2016 DS in the NCT showed that among the 22 entries from other Philippine breeding institutions, three elite breeding lines including PR40330-4-2-7-1-2-1 of PhilRice were among the top five entries with 25.9% yield advantage over the high yielding popular check variety, NSIC Rc 222 (NCT, 2016). PR40330-4-2-7-1-2-1 was consistently among the top entries until

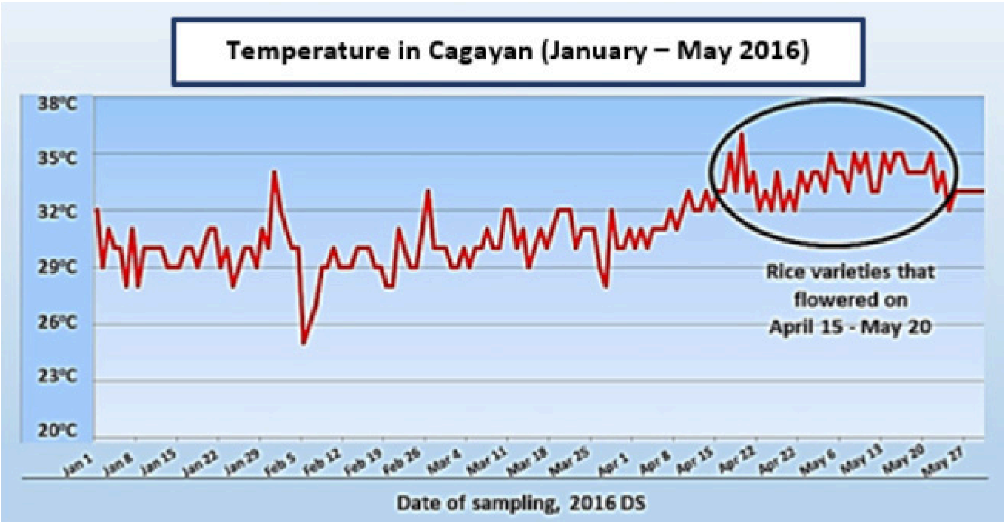

Figure 5. Temperature regime from January to May 2016 in Cagayan, Philippines.

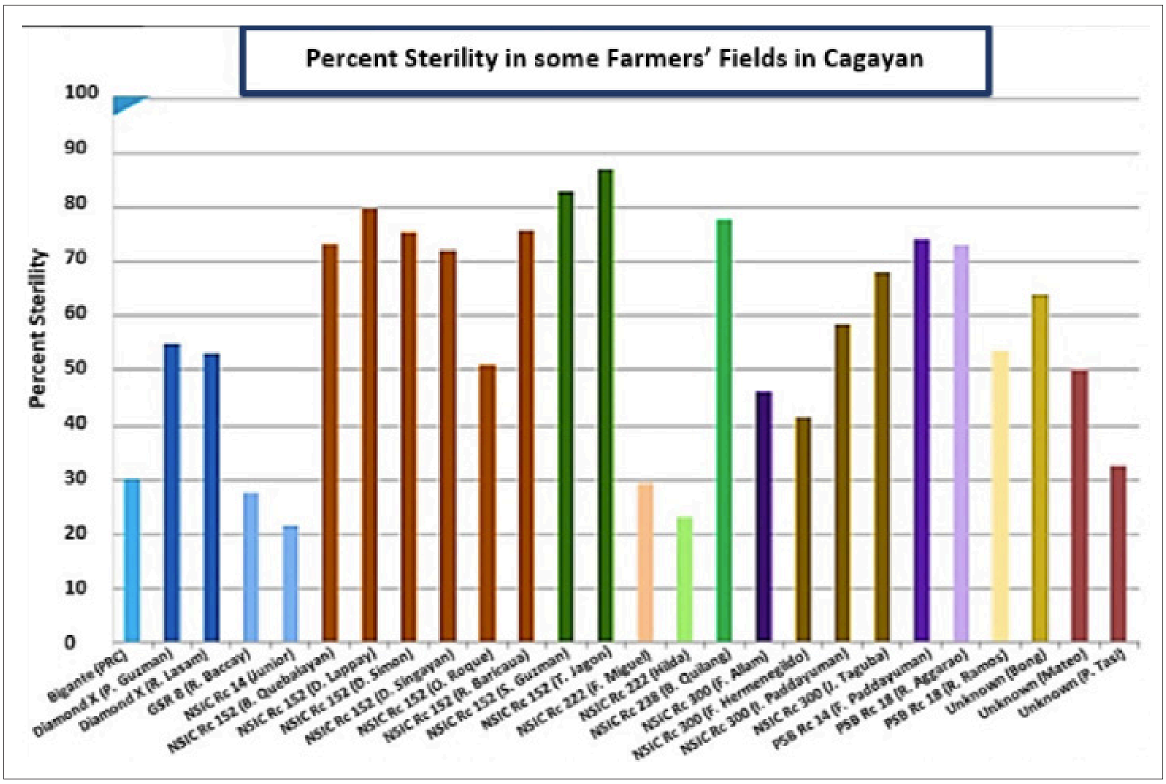

Figure 6. Percent sterility observed in a survey in Cagayan, Philippines.

its release in 2020. Table 2 shows the characteristics of the breeding line. After 10 years of development, PR40330-4-2-7-1-2-1 was released as NSIC 2020 Rc 600 – one of the first heat-tolerant rice varieties in the Philippines (Figure 7).

B. Identifying QTLs and Mapping of Genes

The mapping population had mean fertility of 76.34% ± 0.83 with maximum fertility of up to 98.51% and lowest of 13.07%. Fifty percent of the 124 lines had the highest frequency of 70 - 84% fertility. Twenty-seven percent of the promising lines (67) had fertility greater than 85%. Two lines with fertility lower than 39% were recorded (Figure 8A).

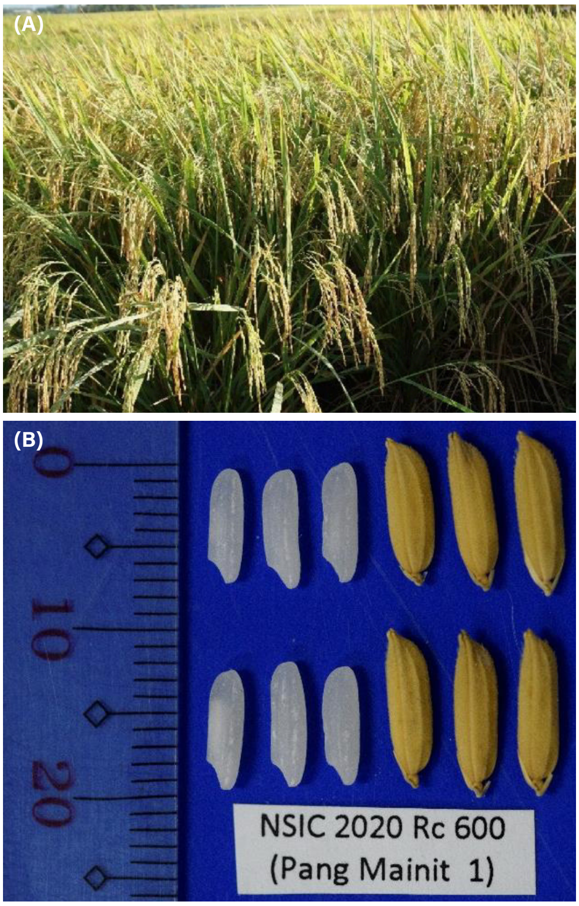

Figure 7. Matured variety (A) and grain size and shape (B) of NSIC 2020 Rc 600.

BC_2F_4 had mean time flowering at 11:53 AM (11.89 ± 0.06) with earliest time at 09:22 AM (9.37 ± 0.06) and late flowering at 02:46 AM (14.77 ± 0.06) (Figure 8B). The 102 BC_2F_4 lines, which accounted for 41% of the total lines, dehisced from 11 AM to 11:59 PM. Eighty lines (32%) dehisced from 12 PM to 12:59 PM. Twenty-one lines (8%) dehisced from 1 PM to 1:59 PM; 8 lines, 9 AM - 9:59 AM; 29 lines, 10 AM 10:59 AM.; 6 lines, 2 PM - 3 PM. This indicates that 182 lines can tolerated anther dehiscence at high temperature of 35 - 36^{o}C from 11 AM to 1 PM.

Table 2. Characteristics of NSIC 2020 Rc 600 in the NCT as approved by the NSIC.

Characteristics	
Yield range (t ha^{-1})	4.6-7.1
Mean yield (t ha^{-1})	5.7
Maturity (DAS)	110
Spikelet fertility (%)	82
Resistance	
Blast	Intermediate resistance
Bacterial leaf blight	Intermediate resistance
Deadheart (YSB)	Intermediate resistance
White head (WSB, YSB)	Intermediate resistance
Brown leafhopper	Intermediate resistance
Green leafhopper	Intermediate resistance
Grain quality	
Amylose content (%)	20.1 (Intermediate)
Milling recovery (%)	66.9 (Grade 1)
Grain size and shape	6.8 mm long and 3.3 mm width
Acceptability in raw form	93.3

Figure 8C shows the BC_2F_4 lines manifested mean dehiscent high-temperature at 34.8^{o}C ± 0.06 with highest dehiscent threshold at 36.6 and 32.4^{o}C as minimum. The highest frequency was observed in 104 lines (43%) at 35.0 - 35.9^{o}C. There were 24 lines (10% of total lines) dehisced under 36^{o}C. The second largest frequency was observed in 77 lines (31%) at 34.0 - 34.9^{o}C. Thirty-three lines (13%) dehisced at 33.0 - 33.9^{o}C while 8 lines (3%) dehisced at 32.0 - 32.9^{o}C. The results showed that 128 BC_2F_4 lines, composing 52% of the total mapping population, dehisced on temperature greater than 35^{o}C.

The BC_2F_4 lines had mean heading days of 4.57 ± 0.09 with longest heading of 9 days and shortest in 3 days. The 3-day heading duration had the highest frequency with 67 lines followed by 66 BC2F4 lines (54%) (Figure 8D).

QTLs were identified through inclusive composite interval mapping (ICIM) method using IciMapping 4.0 software version. Three major QTLs had a phenotypic variance explained (PVE) value of more than 30%. Others can be considered as minor QTLs. Nine QTLs were detected for high-temperature tolerance in chromosomes 1, 3, 4, 5 and 10 (Table 3). *q*HTfert3, which is a major QTL for fertility, was located in chromosome 3 with 57.4% PVE; *q*HTdt3 QTL for time of dehiscence on chromosome 3 with 31.7% PVE; and *q*HThd3 for heading date on chromosome 3 with 30.9% PVE. This can be used as decision guide for breeding using MAS and fine mapping of the novel genes for high-temperature tolerance (Grospe et al., 2016).

A similar study also showed the interaction of QTLs with other QTL in different chromosomes

(Figure 9). QTLs included were *q*HTfert1 - *q*HTfert3 with 14.0% epistasis coefficient (interaction variation), *q*HTfert4 - *q*HTfert3 with 14.3%, *q*HTtof10 - *q*HTfert3 with 13.9%, *q*HTtof10 - *q*HTdt3 with 5.0% and *q*HTdt5 – *q*HTdt3 with 13.2%. It was observed that there was one QTL interacting on two QTLs. These QTLs included *q*HTfert3 (*q*HTfert1; 14.05%, *q*HTfert4; 14.3%), and *q*HTdt3 (*q*HTtof10; 13.6%, *q*HTdt5; 13.2%). QTLs, *q*HThd3 - *q*HTfert3, were also located on the same chromosome interacting with one another.

Polygenic interaction among the identified QTLs described putative polygenic mechanism. It also depicted multifactorial inheritance that includes interaction with high-temperature environment, which provides an indication for wider spectrum of gene-interactions.

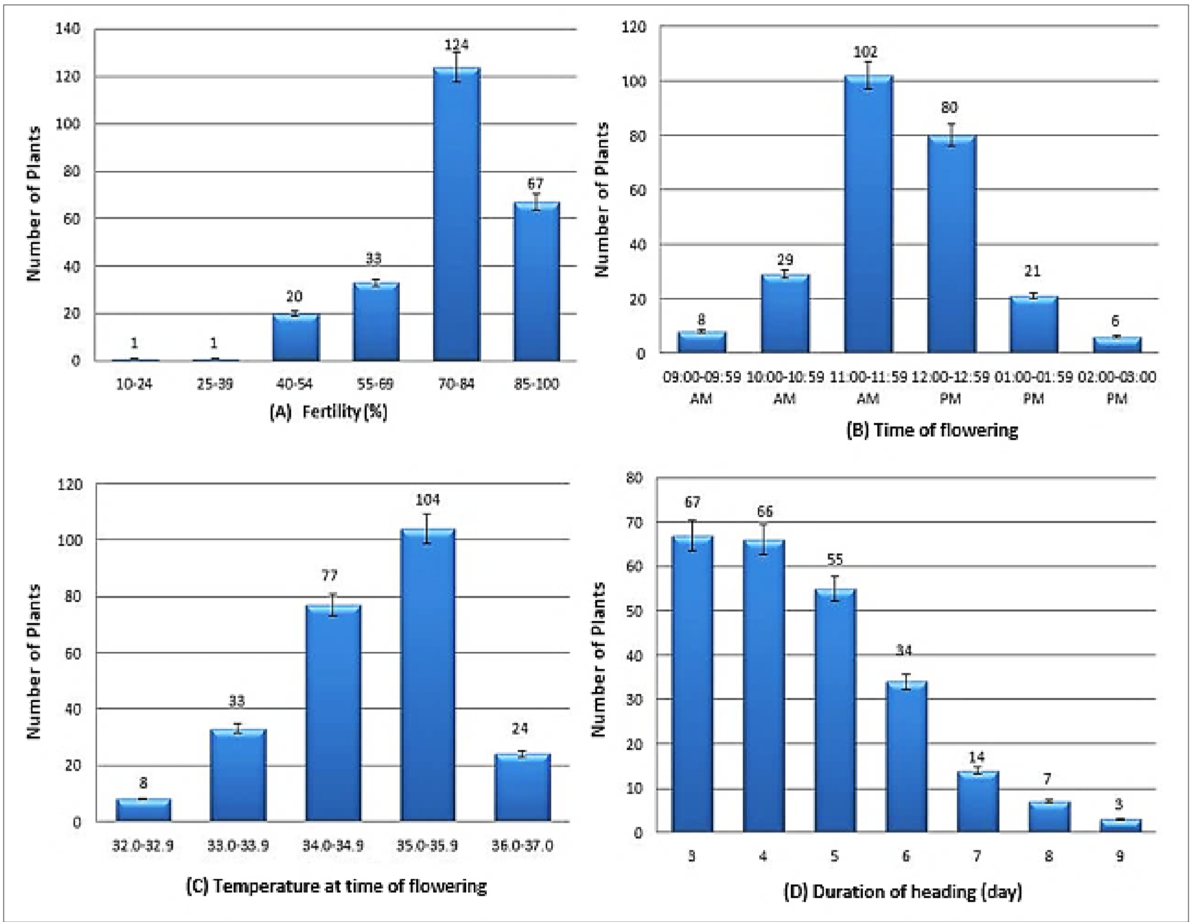

Figure 8. Number of plants in the mapping population as affected high temperature stress on percent fertility (A), time of flowering (B), temperature at time of flowering (C), and duration of heading (D).

Table 3. Different QTLs identified for heat tolerance in chromosomes 1, 3, 4, 5, and 10.

Designated QTL name	QTL Position (cm)	Left Marker	Right Marker	PVE (%)	LOD	DPE	Action
*q*HTfert1	332.8	RM9	RM7318	18.6	8.99	B	PD
*q*HTfert3	987.9	RM16238	RM3586	57.4	9.18	B	PD
*q*HTfert4	757.6	RM348	RM559	10.2	8.46	A	PD
*q*HTtof10	81.2	RM25213	RM6142	16.3	8.95	A	PD
*q*HTdt3	963.9	RM16238	RM3586	31.7	7.83	A	PD
*q*HTdt4	201.6	RM16742	RM3308	9.4	8.15	A	PD
*q*HTdt5	458.4	RM178	RM480	9.2	7.18	A	OD
*q*HTdt10	78.2	RM25213	RM6142	7.1	6.69	A	PD
*q*HThd3	846.9	RM16102	RM16238	30.9	6.54	A	OD

PVE - phenotypic variance explained, DPE – direction of phenotypic effect, A – NSIC Rc 150, B – Dular, PD – partial dominance, OD – overdominance.

MAS was also applied in the selection for high temperature tolerance using known marker RM3586 (Zhang et al., 2008). Table 4 shows the breeding lines selected using this marker.

PCR results of breeding lines were gathered, after which rice were planted for further screening and selection under high temperature conditions. They were evaluated based on the selection criteria mentioned earlier. Fertility/sterility percentage was also compared with the molecular data. Results show that there was a high degree of association between the molecular and phenotypic data of the intermediate tolerant and heterozygous individuals but not for the susceptible and tolerant types (Figure 10).

It was observed that other populations have different association between genotype and phenotype (Figure 11).

C. Pollen Fertility and Yield Evaluation

The maximum temperature during the reproductive stage under the field (LB and NE-F) and screen house (NE-GH) were plotted against the percent pollen fertility (PPF) in each of the breeding lines (Figure 12). The results generally showed a negative response of PPF on increasing maximum temperatures at a temperature range from 34°C to 39°C. Some lines had 80% pollen fertility at maximum temperature greater than 38°C, an indication of their

Table 4. Select elite breeding lines identified as tolerant and heterozygous using RM3586.

Designation	50% days to flower	Sterility (%)	Genotype Data
PR44500-A3-3-2-2-2-B	80	30	Heterozygous
PR44498-A2-1-3-3-3-B	78	11	Tolerant
PR44498-A3-1-1-1-3-B	78	18	Tolerant
PR44498-B1-6-2-2-2-B	78	12	Tolerant
PR44499-A10-3-2-2-2-B	76	18	Heterozygous
PR44499-A1-2-1-2-5-B	76	19	Tolerant
PR44499-A13-1-2-1-1-B	76	17	Heterozygous
PR44499-A14-1-1-1-3-B	76	11	Tolerant
PR44499-A14-5-2-3-6-B	76	12	Tolerant
PR44499-A14-6-2-2-6-B	75	12	Tolerant
PR44499-A15-1-1-3-6-B	76	12	Heterozygous
PR44499-A15-9-2-3-3-B	88	6	Heterozygous
PR44499-A16-1-3-3-2-B	74	5	Tolerant
PR44499-A4-4-2-3-4-B	89	7	Tolerant
PR44499-A5-6-1-1-1-B	76	14	Tolerant
PR44499-A5-6-1-2-6-B	77	10	Tolerant
PR44499-A8-5-3-1-1-B	76	18	Heterozygous
PR44499-A9-5-2-2-5-B	786	10	Heterozygous
PR44499-B11-2-2-3-3-B	74	15	Tolerant
PR44499-B12-5-1-3-1-B	76	33	Heterozygous
PR44499-B4-4-3-1-2-B	72	10	Tolerant
PR44499-B5-5-3-1-1-B	72	19	Tolerant
PR44500-A8-2-1-2-2-B	80	20	Heterozygous
PR44500-A8-2-1-2-5-B	100	30	Heterozygous
PR44500-B13-10-2-2-3-B	100	23	Heterozygous
PR44500-B15-5-1-1-5-B	78	16	Tolerant
PR44500-B17-2-1-2-3-B	76	10	Tolerant
PR44501-A15-16-1-1-5-B	78	12	Tolerant
PR44501-A4-3-3-1-5-B	76	42	Heterozygous
PR44505-A14-15-1-2-3-B	100	31	Heterozygous
PR44499-B15-4-2-1-5-B	85	20	Heterozygous
PR44499-B15-4-2-3-6-B	73	27	Heterozygous
PR44500-B9-2-3-1-2-B	73	21	Heterozygous
PR44500-B9-2-3-1-3-B	73	20	Heterozygous

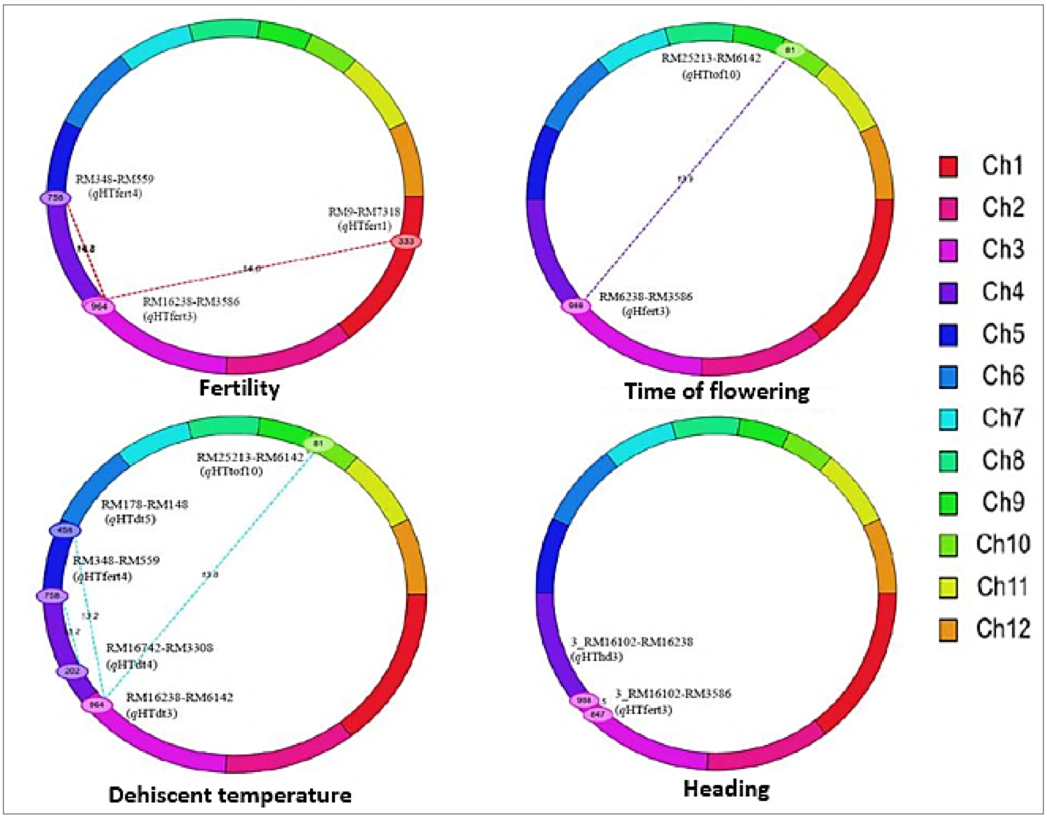

Figure 9. Interaction of QTLs identified with other QTLs in chromosome 1-12 of rice.

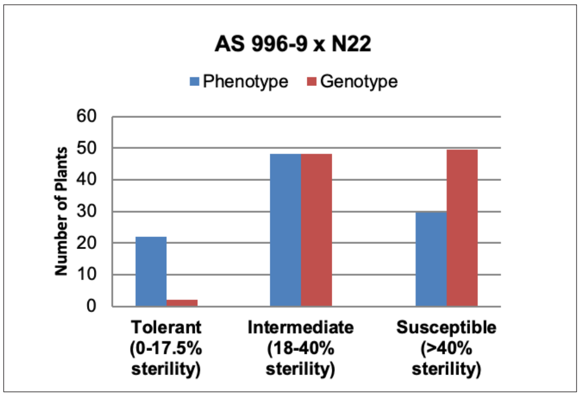

Figure 10. Number of plants in AS996/N22 mapping population that conforms with the molecular data (genotype) and were observed in the field (phenotype) using % sterility as a parameter.

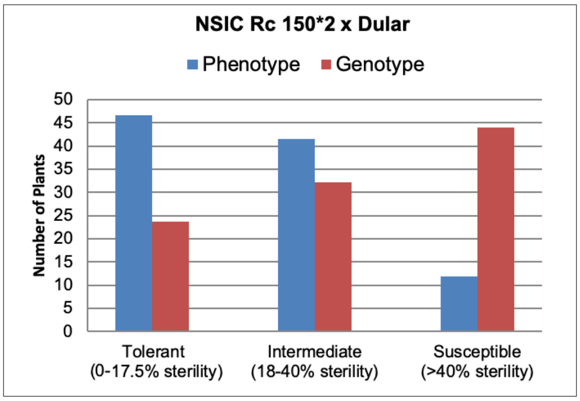

Figure 11. Number of plants in NSIC Rc 150/Dular mapping population that conforms with the molecular data (genotype) and were observed in the field (phenotype) using % sterility as a parameter.

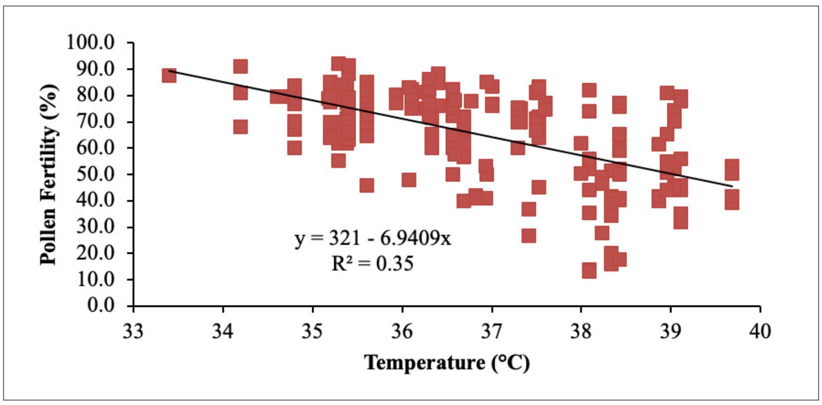

Figure 12. The relationship between pollen fertility (%) and maximum temperature dynamics during the reproductive stage.

potential to tolerate high temperatures. The lines showed reduced PPF by 37% in NE-GH relative to LB. This reduction was attributed to an increase of more than 2°C in the maximum temperature in NE-GH. About 5% decrease in PPF was also observed in NE-GH relative to NE-F when the difference in maximum temperatures increased to 1.8ºC (Table 5). Progenies of the cross between Hanareumbyeo/N22 had the highest mean pollen fertility (54.6%) among populations under NE-GH. In LB and NE-F, progenies of Gayabyeo/N22 had the highest PPF at 75.1 and 80.2%, respectively.

There was a positive correlation between pollen fertility (%) and plant grain yield (g m^{-2}) of the selected lines (Figure 13). It was noted that entries with the highest pollen fertility were not necessarily the top yielders except for PR42222-3-1, which showed consistent good pollen fertility and yield. Many of the selected lines that exhibited 80% pollen fertility had grain yield of more than 500 g m^{-2}. Lines with less than 60% fertility generally yielded around 300 g m^{-2}.

The pollen fertility and yield evaluation results were very useful and has become one of the recommended criteria for the NCT heat tolerance evaluation of the breeding lines.

Table 5. The percent pollen fertility of three Back-cross Inbred Lines populations under different maximum temperature regimes.

Genotype	Cross/Parent	LB	NE-F	NE-GH
POP1	NSIC Rc160*4/Dular	72.4 ± 9.6^{de}	67.5 ± 11.7^{bc}	41.7 ± 16.2^{d}
POP2	Hanareumbyeo*4/N22	76.5 ± 6.5^{bcd}	68.0 ± 8.9^{bc}	54.6 ± 16.8^{bc}
POP3	Gayabyeo*2/N22	80.5 ± 5.6^{abc}	75.5 ± 7.8^{ab}	48.9 ± 15.6^{cd}
NSIC Rc160	Parent	84.0^{abc}	85.0^{a}	41.0^{d}
Hanareumbyeo	Parent	75.0^{cd}	75.0^{ab}	62.0_{b}
Gayabyeo	Parent	85.0^{ab}	80.3^{a}	26.8^{e}
N22 (03911)	Tolerant	87.5^{a}	80.0^{a}	83.0^{a}
Dular	Tolerant	79.8^{abcd}	79.0^{a}	77.8^{a}
IR64	Intermediate	77.8^{bcd}	77.2^{ab}	78.3_{a}
Milyang 23	Intermediate	76.8^{bcd}	74.8^{ab}	57.7_{b}
IR52	Susceptible	64.8^{e}	60.0^{c}	45.3_{d}

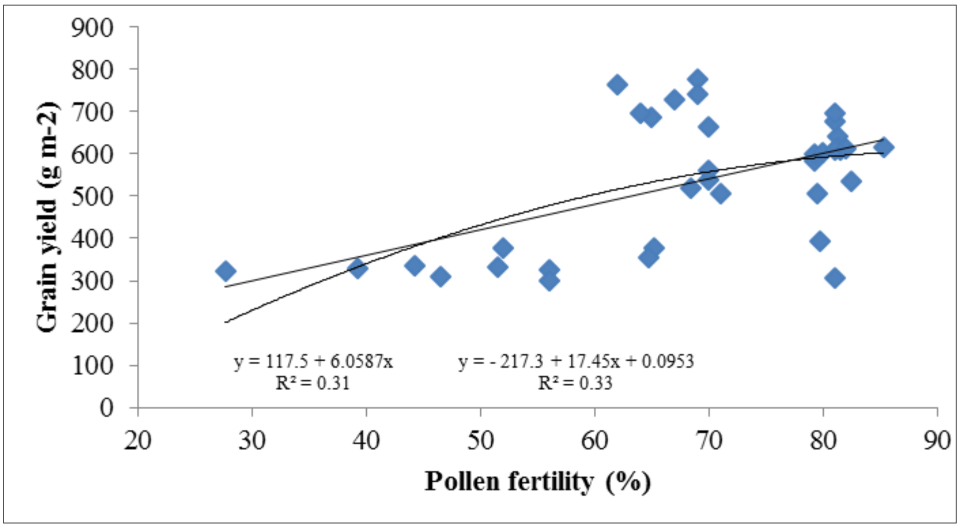

Figure 13. Relationship between pollen fertility and plant grain yield obtained from selected rice genotypes that flowered under maximum temperature of 35 - 39°C.

Conclusion

Reports show that high temperature is affecting rice – the Philippine's staple food. As such, rice breeding and improvement has to continue to produce new varieties that can adapt to climate change. Selection for multiple abiotic traits is currently a priority because rice is not only affected by high temperature but also by other stresses such as drought, salinity, and submergence. Continuous studies should be done in developing markers and QTL identification for heat tolerance and other important traits in different mapping populations. Field screening and analyses of data based on phenotypic correlation and multiple linear regression are conducted to determine highly reliable QTLs, specifically in finding associated traits to fertility. On pollen fertility, fertility was reduced at temperatures between 35.4 - 38.4°C. Some genotypes exhibited 80% pollen fertility beyond 38°C, which shows potential to tolerate high temperatures. Results of pollen fertility study was useful in evaluating genotypes for high temperature tolerance; thus, pollen fertility data was recommended and incorporated in the NCT guidelines. NSIC Rc 600 was released in 2020 as a result of this study, which is one of the two rice varieties recommended for high temperature areas in the Philippines.

Acknowledgment

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UNCOVERING NATURALLY OCCURING BACTERIAL LEAF BLIGHT RESISTANCE GENE AMONG MAINTAINER LINES AND DEVELOPMENT OF RESISTANT LINES

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Abstract

Maintainer or B line plays an important role in obtaining seed and yield stability of cytoplasmic male sterile (CMS) or A line for seed multiplication and production of F_1 rice hybrids. Maintainer line is used to maintain the sterility of A line, which shares identical gene and similar morphology with the B line. To develop economical and commercial A line, development of maintainer line with excellent trait such as pest and disease resistance is needed. Infection process of bacterial leaf blight (BLB) is due to various factors, which include the entry of bacteria on naturally or mechanically wounded host plant. Flag leaf clipping is a common practice during A line and F_1 production. However, flag leaf clipping before pollination creates wounds on plants' leaves, which triggers infections from bacteria *Xanthomonas oryzae* pv. *oryzae* (*Xoo*). As such, development of a resistant maintainer line is needed to avoid BLB infection. In this study, 50 maintainer lines were screened for naturally occurring BLB resistance genes and used the identified resistant lines for breeding maintainer lines for stacked genes. Four functional markers, *Xa4*, *Xa5*, *Xa*7, and *Xa*21, were used for gene confirmation. Phenotyping results showed that some of these maintainer lines exhibited resistant reaction to 13 *Xoo* races and have similar resistance reaction to IRBB lines or the resistant checks. Three maintainer lines with naturally occurring BLB resistance genes (*Xa4+Xa7; Xa4+xa5)* and IRBB62 (*Xa4+Xa7+Xa21)* were selected as *Xa* gene donors to produce breeding population. Nine lines showed homogenous population after F_6 generation. Confirmed *Xa* genes were *Xa*4, *Xa4+xa5, Xa4+Xa7*, and *Xa4+xa5+Xa7.* Initial evaluation of nine developed maintainer lines using PXO79 isolates showed intermediate and resistant reaction. Moreover, grain quality characteristics of four breeding lines were comparable to the features of IR58055B, maintainer of PSB Rc 72H (Mestiso 1) widely used as parent lines for developing three-line hybrids. The improved lines widened the current maintainer germplasm pool for BLB resistance and facilitate the development of CMS lines with BLB resistance and multiple traits for grain quality.

Keywords: *Bacterial Blight, Xa Gene, Maintainer Line, F_1 Hybrids, MAS, Rice.*

Introduction

The bacterial leaf blight or BLB, which is caused by *Xanthomonas oryzae* pv. *oryzae* (*Xoo*), is considered the most threatening bacterial disease in rice-growing countries (Chukwu et al, 2019; Leung et al, 2004; Swing, 1990; Mew, 1987). Breeding for resistant varieties is the most economical and effective means to control the disease. Environmental factors such as high relative humidity, optimum temperature (>30°C), and sunshine duration cause BLB infection. In addition, wounds caused by either natural (rainstorm, winds); mechanical (leaf cutting); or natural openings such as stomata, hydathodes, and nectaries, facilitates disease development (Huang and De Cleene, 1989).

Three parentals — CMS, maintainer (B line), and restorer lines (R line) are needed in the development of three-line F_1 hybrids. Flag leaf clipping is a common practice in A line (A x B) and F_1 seeds (A x R) production. Flag leaf cutting method is a technique in which the leaves are cut by scythe at a level just above the flag leaf joint of the main tiller. This facilitates the transfer of pollen parent (maintainer or restorer) to the seed parent (A line). However, this practice oftentimes injures to the plants resulting in disease development. The resistance of three-line hybrids is hereditary. Thus, parent lines should first undergo proper pest and disease evaluation before these can be used in developing F_1 hybrids. The susceptibility of five maintainer lines (IR58025B, IR62829B, LianB, and BOB) used for developing three-line hybrids in Philippine Rice Research Institute, Central Experiment Station (PhilRice CES) was reported by Borines (2001). This susceptibility was also observed in some hybrid parental and hybrids, which constrained the Hybrid Rice Program (HRP) in the Philippines (De Leon et al., 2003). Virmani and Kumar (2004) recommended the use of BLB resistant parent lines whether it contain dominant or recessive genes as these can produce a more durable F_1 hybrids. To address the susceptibility of hybrid varieties promoted by the HRP, researchers conducted BLB resistance breeding by introgression of BLB resistance to hybrid parent lines. Three maintainer lines IR58025B, IR68888B, and IR68897B of Mestizo 1, Mestiso 2, and Mestiso 3, respectively, were improved for bacterial blight resistance (Agarcio et al., 2007; Borines et al., 2008; Tabanao et al., 2013). The development of improved maintainer lines with multiple resistance was successful; however, converting them into CMS lines particularly when using wild abortive (WA) cytoplasm was quite challenging. This may attribute to the genotypic background of BLB resistance gene donor and the backcross method used in the introgression of resistance gene. The product of backcross method recovered almost the genotype of donor or recurrent parent (Mumm, 2007). The BLB resistance donor of the developed maintainer is IRBB62, a pyramided line developed by International Rice Research Institute (IRRI) and contains *Xa4*, *Xa7*, and *Xa21* gene. IR24 was used as the recurrent parent in developing IRBB62, a known strong restorer line with two dominant fertility restorer genes, *Rf3* and *Rf4*. This fertility restorer genes are reportedly liable for restoring fertility of CMS line with WA cytoplasm. This reaction is due to the presence of recessive (*rf*) gene in the nucleus of CMS and dominant (*Rf*) gene in IRBB62.

CMS and maintainer have similar morphology except that CMS has cytoplasmic sterile gene with nuclear recessive gene (*S, rf*) while maintainer has cytoplasmic fertile gene with nuclear recessive gene (*N, rf*). Thus, to have effective CMS line, development of maintainer line with desirable traits should be bred first. Breeding of BLB resistant maintainer lines without fertility residual effect when converted to CMS line is a big challenge to hybrid rice breeders particularly if the BLB resistance source is limited with the IRBB lines. The search for an alternative approach is urgent to stabilize the yield of three-line hybrids. This study explored the naturally occurring BLB resistance gene among maintainer germplasm of three-line hybrid rice breeding program and the use of forward breeding and marker aided selection (MAS) to incorporate resistance gene for the development of new source of maintainer line with BLB resistance.

Materials and Methods

Plant Materials and Xoo Isolates

Fifty maintainer lines from IRRI, PhilRice, and abroad were screened for their reaction to BLB using 13 Philippine *Xanthomonas oryzae pv. oryzae* (*Xoo*) strains: PXO61 (Race 1), PXO86 (Race 2), PXO71 (Race 4), PXO79 (Race3b), PXO340 (Race 3c), PXO99 (Race 6), PXO112 (Race 5), PXO145 (Race 7), PXO280 (Race 8), PXO339 (Race 9a), PXO349 (Race 9b), PXO347 (Race 9c), and PXO341 (Race 10). Resistant (IRBB61 and IRBB66) and susceptible lines (IR24) were used for phenotyping and genotyping check. Materials from other countries were obtained from the technical collaboration of PhilRice with R&D institutions including Yunnan Agricultural University, Jiangxi Academy of Agricultural Sciences, Guangxi Academy of Agricultural Sciences, Fujian Agricultural University, and Yangzhou University. Materials used for BLB assessment and *Xa* gene confirmation were sown and grown in a screenhouse at the PhilRice CES during 2009 wet season (WS). Generated F_1 and breeding population selection was established in an experimental field from 2010 dry season (DS) until 2012.

Marker Aided Selection (MAS), Breeding Population Development and Selection

Leaf Collection

After 21 days, single seedlings were transplanted using 13 hills per maintainer genotype at 20 cm x 20 cm spacing. Leaf samples were collected 20 days after transplanting for genotyping. Each collected leaf was placed in glassine bag and immediately stored in ice box to prevent DNA degradation. All entries were quick freeze in liquid nitrogen and grind to a fine powder using mortar and pestle. DNA were isolated and extracted using the method of Dellaporta et al. (1993). The process is described below.

DNA Extraction

The collected leaf sample of individual entries was quick freeze in liquid nitrogen and grind to a fine powder using mortar and pestle. The powder was then transferred to a 50 mL tube. Fifteen mL of extraction buffer and 10 mL of 20% sodium dodecyl sulfate (SDS) was added to each tube and mixed vigorously by shaking then incubated in a water bath at 65°C for 10 min. Each tube was filled with 5 mL 5 M potassium acetate, which was vigorously shake before incubating in a water bath at 65°C for 20 min. After incubation, tubes were spun at 25,000 rpm for 20 min. The supernatant was poured, added with 10 mL isopropanol, mixed, then incubated at -20°C for 30 min. The tubes were then spun for 20,000 rpm for another 15 min to pellet the DNA. The supernatant was removed by pouring to dry the pellet while the tubes were inverted into paper towel for 10 min. The DNA pellet was dissolved by adding 0.7 mL of 50 mM Tris, which was transferred to new Eppendorf tube and centrifuge for another 10 min to remove insoluble debris. After the supernatant was transferred to new Eppendorf tube, 75 µL 3M sodium acetate and 500 µl isopropanol were added. After mixing, the DNA was pellet by centrifuge for 30 seconds, washed with 80% ethanol, then added with 100 µL 10 mM Tris.

Molecular Markers and PCR Analysis

The functional markers used in this study were derived from the studies on the development of molecular markers for the selection of resistant genes: *Xa*4 (Zfiang et al., 1998), *Xa*5 (Blair and McCouch, 1997), *Xa*7 (Porter et al., 2003), and *Xa*21 genes (Tu et al., 1998). These were used in identifying naturally occurring resistant genes among maintainer lines, selecting homozygous alleles in F_2 populations, and confirming *Xa* genes in advance lines. DNA sequence information of these markers is shown in Table 1.

bacterial suspension before cutting 1 - 2 cm from the leaf tip. An image analysis software (ImageJ program, Rasband, n.d) was used for lesion scoring of infected leaves. Scoring system is shown in Table 2 and sample description of scanned leaves while actual lesion measurement using ImageJ program is displayed in Figure 1. The final rating was the average of six leaves/plants of maintainer line.

BLB resistance genes among the developed lines were validated by genotyping four *Xa* genes functional markers and by inoculating PXO79, which

Table 1. DNA sequence information of functional gene marker used.

Gene Marker	Forward Primer (5'→3')	Reverse Primer (5'→3')	Reference
*Xa*4	atcgatcgatcttcacgagg	tgctataaaaggcattcggg	Zfiang et al., (1998)
*xa*5	gagtcgatgtaatgtcatcagtgc	gaaggaggtatcgctttgttggac	Blair and McCouch (1997)
*Xa*7	cgatcttactggctctgcaactctgt	gcatgtctgtgtcgattcgtccgtacga	Porter et al., (2003)
*Xa*21	Atagcaactgattgcttgg	cgatcggtataacagcaaaac	Tu et al., (1998)

The following process was used in PCR analysis:

***Xa*4, *Xa*7, and *Xa*21**

Each 5.95 µL PCR reaction contained 1.5 µL (approximately 50 ng) template DNA, 1.50 µL 5X PCR buffer, 0.60 µL 25 mM MgCl2, 0.35 µL 5 mM dNTPs, 0.50 µL 10 uM each of F and R primers, and 1 µL Taq polymerase. Thermal cycles involved 94°C followed initial denaturation for 5 min, followed by 35 cycles of denaturation at 94°C for 1 min, annealing at 55°C for 1 min and extension at 72°C for 2 min, and final extension at 72°C for 5 min. Amplified fragments for *Xa*7 and *Xa*21were detected using 2% agarose while 8% non-denaturing polyacrylamide gel for the *Xa*4.

xa5

PCR reaction was carried out in 5.45 µL containing 1 µL template DNA, 1.50 µL 5X PCR buffer, 0.60 µL 25 mM MgCl2, 0.35 µL 5 mM dNTPs, 0.50 µL 10 uM each of F and R primers, and 1 µL Taq polymerase. Thermal cycling conditions followed an initial denaturation at 94°C for 5 min, followed by 34 cycles of denaturation at 94°C for 30 sec, annealing at 55°C for 30 sec and extension at 72°C for 1 min, and final extension at 72°C for 5 min. Amplified fragments for *xa5*, *X*a7 and *Xa*21were detected using 2% agarose and 8% non-denaturing polyacrylamide gel for the *Xa*4.

BLB Resistance Screening

BLB resistance gene was assessed 45 days after transplanting the 50 maintainer lines in Screen house. This was done by inoculating *Xoo* races in every six leaves/plant of maintainer lines. Clipping method was done by dipping the scissor edge into

Table 2. Scoring system used as described by Kauffman et al. (1973).

Description	Lesion Length (cm)
Highly resistant (HR)	> 1.0
Resistant (R)	1.0 - 5.0
Moderately resistant (MR)	5.1 - 10.0 cm
Moderately susceptible (MS)	10.1 - 15.0
Susceptible (S)	15.1 - 20
Highly susceptible (HS)	>20.1

Figure 1. Profile of scanned leaves showing lesion and measurement using Canon Pixma 486 scanner and ImageJ program.

represented Race 3b, in the screenhouse. PXO79 isolates inoculation provide initial information in the expression of resistance of developed lines to predominant race in Maligaya, Science City of Muñoz, Nueva Ecija.

Creation of Breeding Population and Selection

Maintainer lines that exhibited BLB resistance genes and showed resistant reaction to 13 *Xoo* races were used to generate breeding population. In addition, IRBB62 was also included for development of crosses. The generated F_1 progenies were grown in the field and evaluation started by observing the F_1 plants for excellent phenotypic acceptability (PA) expression. Foreground selection was carried out in F_2 plants and only those that exhibited homozygous alleles for *Xa*4, *xa5*, *Xa*7, and *Xa*21 markers were selected. The selected F_2 genotypes were selfed to produce F_3 seeds. Forward breeding and pure line selection were used for the selected plants from F_3 to F_6 generation. Moreover, good to excellent PA with traits for maintainer lines as described by Virmani et al., (1997) was considered in the advancement of selected plants.

Grain Quality Evaluation

The excellent grain quality of Mestizo 1 is attributed to its maintainer line IR58025B. Thus, evaluation of grain quality of developed lines is important particularly for IR58025B-derived breeding population to identify the expression of IR58025B traits. The generated grain quality information will also provide analytical reference specifically for choosing future parents and other breeding purposes. The standard method indicated in the National Cooperative Testing Manual for Rice (NCT, 1997) was used for grain quality testing and was conducted by Rice Chemistry and Food Science Division at PhilRice CES.

Maintaining Ability Assessment

To determine the maintaining ability and evaluate the presence of fertility residual effect, improved maintainer was initially testcrossed to five CMS lines with different cytosterility source. Category and pollen classification of generated F_1 plants were based on evaluation described by Virmani et al. (1997).

Research Limitation

The study searched naturally occurring BLB resistance gene among maintainer germplasm pool and used them as donor source for developing new set of maintainer line with BLB resistance. This study explored an alternative breeding approach for transferring the IRBB line resistance genes to maintainer line to produce breeding population without expressing the residual fertility effect when converted to CMS line. The presentation of a more tangible data result was restricted due to availability of resources like *Xoo* isolates and BLB differential varieties during the phenotyping of advance lines.

Results and Discussion

Reaction of Maintainer Lines Against Different Isolates

Phenotyping results showed that considerable number of the evaluated maintainer lines exhibited resistant reaction to 13 *Xoo* races. Existence of resistant maintainer line was observed in every *Xoo* race (Figure 2). Detailed reaction of 50 maintainer lines to 13 *Xoo* races and the detected *Xa* genes are shown in Table 3. Screening of resistance gene and the resolved PCR products amplified using Xa gene markers is shown in Figure 3. Maintainer line 913B have three *Xa* genes, *Xa*4+*xa*5+*Xa*7, while PhilRice-bred line PR24069B carried *Xa*4+*Xa*7 genes. Fourteen lines had *Xa4+xa5* genes: PhilRice-bred lines PR35475B, PR35474B, PR36579B, and PR35473B; and IRRI-bred line IR80151B, IR72079B, IR70369B, IR68902B, IR79156B, IR69627B, IR68897B, and IR69626B. The same *Xa* genes was found in LianB and PragathiB from China and India breeding institutions, respectively. Twenty-one maintainer lines were detected to have single gene namely Xa7, *xa*5, and *Xa*4. Thirteen lines had no confirmation for the presence of any *Xa* gene. However, based on their reaction to 13 *Xoo* races, comparison with two resistant checks (IRBB61, IRBB66), and resemblance reaction to published reaction of IRBB lines, indicated

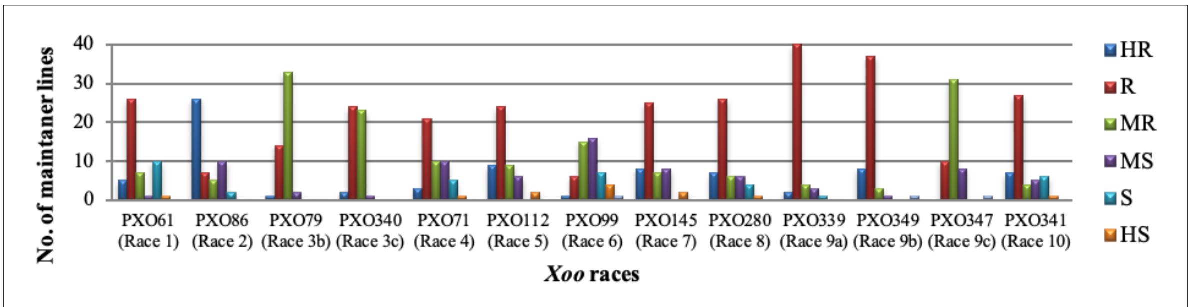

Figure 2. Reaction of different maintainer genotypes to 13 *Xoo* isolates.

that unknown resistance genes are present to these maintainer lines. This reaction could also signify the existence of gene resistance or active plant defense, in which plant resistance gene activates after pathogens intrusion (Kaloshian, 2004).

Bacterial leaf blight resistance gene has been incorporated into elite lines and modern varieties after it was identified and tagged with molecular markers in the Philippines and abroad (Hari et al., 2011; Davierwala et al., 2001; Lee et al., 2000; Kumar, 1999; Khush and Angeles, 1999; Zhang et al., 1998; Lin et al., 1996). Most of IRRI breeding materials with bacterial leaf blight resistance were utilized by national rice programs in Asia either as direct introductions or in hybridization programs. In recent years, incorporation of bacterial leaf blight resistance is one of the important objectives of rice improvement programs. The most widely exploited resistance gene before was *Xa*4 followed by *xa*5 and more (Mew et al., 1992; Khush et al., 1989). Thus, the detection of naturally occurring resistance genes among the gene pool of maintainer in this study could be a product of breeding effort for developing many resistant cultivars combatting bacterial blight disease. Maintainer lines were introduced in the PhilRice hybrid breeding program through technical collaboration between PhilRice and other R&D institutions (Xu et al., 1995; Redoña et al., 1998a; Redoña et al., 1998b). This contributed to a diversified maintainer germplasm pool where donor source for the desirable traits particularly for the resistance to diseases were sourced.

Transfer of BLB Resistance Genes and Generation Advance

During 2009 WS, four breeding combinations were produced from six select parent lines. However, only three combinations showed excellent PA expression during F_1 evaluation in 2010 DS. Early evaluation increases the chance of obtaining genotypes carrying not only the resistance gene, but also acquiring desirable traits for maintainer lines.

Parent lines of established breeding population were three maintainers having naturally occurring BLB resistance genes (PR24069B, PR24068B, IR69626B), IR58025B (maintainer line of Mestizo 1), and IRBB62 (Table 4). Out of these, IR69626B/PR24069B, IRBB62/IR58025B, and PR24068B/PR24069B crosses were generated. In 2010 WS, DNA from 984 F_2 plants of three crosses were extracted and only 453 plants exhibited homozygous alleles for *Xa* genes. Single (*Xa4*), double (*Xa4+xa5*); and (*Xa4+Xa7*), and triple (*Xa4+xa5+Xa7*) genes were detected among the F_2 plants. However, *Xa*21 gene was not found among evaluated F_2 population of IRBB62/IR58025B crosses. All the identified F_2 plants with *Xa* genes were harvested and selected plants were only established in the next generation. After F_6 generation, nine lines exhibited homogenous population including two lines from PR41325 containing *Xa4+xa5*; two lines with *Xa4+Xa7* from PR41326; two lines with *Xa4+xa5+Xa7*; and three lines with *Xa*4 from PR41327. *xa5* gene was noticed from derived lines of IRBB62/IR58025B crosses,

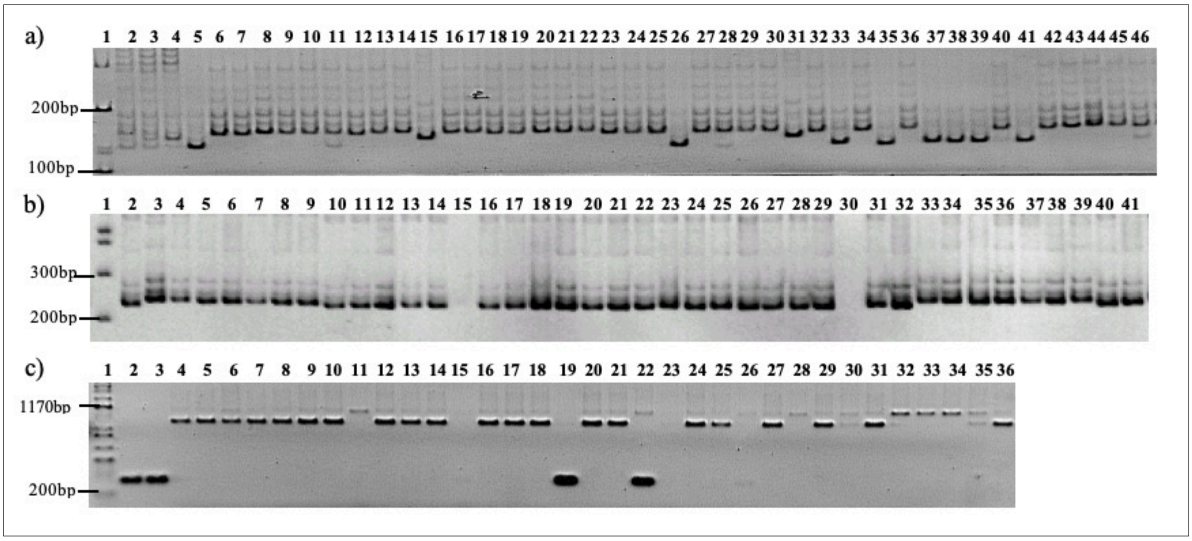

Figure 3. Polymerase chain reaction (PCR) products amplified with the specific *Xa* gene markers: (a) *Xa*4 (160bp), Lane 4-resistant check; resistant genotype: Lane 6-14;16-25; 27-30, 32, 34, 36, 40, 42-46; (b) *xa*5(240bp), Lane 3-resistant check; resistant genotypes: Lane 4-7, 33-39; (c) Xa7 (294bp), Lane 2; resistant genotype: Lane 3, 19, and 22.

Table 3. Naturally occurring Xa genes found in maintainer germplasm and their reaction against different isolates.

Maintainer line	*Xa* gene(s)	Breeding Institution	Race 1 PXO61	Race 2 PXO86	Race 3b PXO79	Race 3c PXO340	Race 4 PXO71	Race 6 PXO99	Race 5 PXO112	Race 7 PXO145	Race 8 PXO280	Race 9a PXO339	Race 10 PXO341	PXO347	PXO349
IRBB61 (R, check)	*Xa4+xa5+Xa7*	IRRI	HR	HR	HR	HR	R	MR	HR	HR	HR	HR	HR	HR	HR
IRBB66 (R, check)	*Xa4+xa5+Xa7+xa13+Xa21*	IRRI	HR	HR	HR	R	HR	R	HR	HR	R	R	HR	MR	HR
913B	*Xa4+xa5+Xa7*	India	R	MR	R	R	R	MR	HR	R	HR	R	HR	R	MR
PR24069B	*Xa4+Xa7*	PhilRice	R	HR	R	R	R	R	HR	R	R	R	MR	MR	R
PR35474B	*Xa4+xa5*	PhilRice	MR	HR	R	R	R	MR	R	R	R	R	R	MR	R
PR36579B	*Xa4+xa5*	PhilRice	R	R	MR	MR	R	MR	HR	R	R	R	HR	MS	R
PR35473B	*Xa4+xa5*	IRRI	R	HR	MR	MR	R	MR	R	R	R	R	R	MR	R
IR68897B	*Xa4+xa5*	IRRI	R	HR	MR	R	R	MR	R	R	R	R	R	MR	R
IR80151B	*Xa4+xa5*	IRRI	R	MS	MR	R	R	MS	R	R	R	R	R	MR	R
PR35475B	*Xa4+xa5*	PhilRice	R	HR	MR	MR	MR	MS	R	HR	R	R	R	MR	R
IR72079B	*Xa4+xa5*	IRRI	R	HR	MR	R	R	MS	R	R	R	R	R	MR	R
IR68902B	*Xa4+xa5*	IRRI	R	R	MR	MR	R	MS	R	R	R	R	R	MR	R
IR79156B	*Xa4+xa5*	IRRI	R	HR	MR	R	R	MS	R	MR	R	R	R	MR	R
IR69627B	*Xa4+xa5*	IRRI	R	HR	MR	MR	R	MS	R	R	R	R	R	MS	R
IR69626B	*Xa4+xa5*	IRRI	HR	MS	MR	HR	HR	MS	HR	HR	HR	HR	HR	MR	HR
IR70369B	*Xa4+xa5*	IRRI	S	MS	R	R	MR	HR	MS	MR	MR	MS	R	MR	R
LianB	*Xa4+xa5*	China	S	S	MR	MR	S	MS	MS	MR	MR	S	MR	MR	R
PragathiB	*Xa4+xa5*	India	S	HR	MR	MR	MS	S	MR	MS	MS	R	MS	MR	R
PR35735B	*Xa7*	PhilRice	S	HR	MR	MR	MS	HS	HS	HS	S	R	HS	MS	R
PR24070B	*xa5*	PhilRice	HR	R	HR	R	R	R	R	HR	HR	R	HR	R	R
PR36578B	*xa5*	PhilRice	R	HR	R	R	R	MR	HR	HR	HR	R	R	MR	R
PR35747	*xa5*	PhilRice	R	MR	R	R	R	R	HR	R	HR	HR	HR	R	HR
PR35738B	*Xa4*	PhilRice	HR	HR	R	R	HR	MR	HR	HR	HR	R	HR	R	HR
PR35736B	*Xa4*	PhilRice	S	HR	MR	MR	MS	HS	MR	MS	MS	R	S	MR	R
PR35731B	*Xa4*	PhilRice	R	HR	MR	MR	MR	MS	R	R	R	R	R	MR	R
PR24068B	*Xa4*	PhilRice	R	HR	R	R	R	MR	HR	R	R	R	R	MR	HR
PR24067B	*Xa4*	PhilRice	R	MS	MR	MR	MS	S	MR	R	R	MR	MR	R	R
IR73328B	*Xa4*	IRRI	R	HR	MR	MR	MR	MS	R	R	MR	MS	R	MS	MR
IR80156B	*Xa4*	IRRI	R	HR	MR	MR	MR	MS	R	R	R	R	R	MR	R
IR79123B	*Xa4*	IRRI	R	HR	MR	R	R	MR	R	HR	R	R	R	MR	R
PMS8	*Xa4*	India	R	MS	MR	MR	R	S	R	R	MS	MR	MR	MS	R
IR68896B	*Xa4*	IRRI	R	MS	MS	MR	MS	HS	R	MR	R	R	R	R	MS
PR35472B	*Xa4*	PhilRice	R	HR	MR	R	R	MS	R	R	R	R	R	MR	R
IR16365-5B	*Xa4*	IRRI	HR	MR	MR	R	R	R	R	R	R	R	R	MR	HR
IR69625B	*Xa4*	IRRI	S	HR	MR	MR	MS	S	MR	HS	HS	MS	S	MS	MR
IR62829B	*Xa4*	IRRI	R	MS	MR	MR	MR	-	MS	R	MR	R	R	MR	R
PR35745B	*Xa4*	PhilRice	HR	MS	R	R	HR	R	R	HR	R	R	HR	MR	R

Table 3. Continued

Maintainer line	*Xa* gene(s)	Breeding Institution	Race 1 PXO61	Race 2 PXO86	Race 3b PXO79	Race 3c PXO340	Race 4 PXO71	Race 6 PXO99	Race 5 PXO112	Race 7 PXO145	Race 8 PXO280	Race 9a PXO339	Race 10 PXO341	PXO347	PXO349
PR35744B	*Xa4*	PhilRice	R	HR	MR	R	R	MR	HR	R	R	R	R	MR	R
PR35742B	*Xa4*	PhilRice	R	HR	MR	R	R	MR	R	R	R	R	R	MR	R
PR35740B	-	PhilRice	S	HR	MR	R	S	MR	R	MS	S	R	S	MS	R
PR35739B	-	PhilRice	S	R	MR	MR	MS	MS	R	MS	MR	R	MS	MR	R
PR35737B	-	PhilRice	MR	MS	R	R	MR	S	R	MR	HR	R	R	R	R
PR35743B	-	PhilRice	S	MR	MR	MR	S	S	MR	MS	MS	R	S	MR	R
PR35741B	-	PhilRice	HS	HR	MR	MR	MS	MS	MR	MS	MS	R	S	MR	R
IR58025B	-	IRRI	MS	HR	MR	MR	S	MS	MS	MS	S	R	S	MR	R
IR70371B	-	IRRI	S	HR	MS	MR	S	S	MS	MR	S	R	MS	MR	R
IR79128B	-	IRRI	R	S	MR	MR	MS	R	R	R	R	R	MS	MS	R
IR68886B	-	IRRI	MR	MS	MR	MS	HS	HS	HS	MS	MS	MR	MS	-	-
PR35746B	-	PhilRice	MR	R	R	R	MS	MS	MS	R	R	R	R	R	R
PR37130-1	-	PhilRice	MR	R	R	R	MR	MR	MR	R	MR	R	R	MR	HR
PR37130-2	-	PhilRice	MR	R	R	R	MR	MR	MR	MR	R	MR	R	R	HR
PR37130-3	-	PhilRice	MR	MR	R	HR	MR	MR	MR	HR	R	R	R	R	HR

HR - Highly resistant, R - Resistant, MR -Moderately resistant, MS - Moderately susceptible, S - Susceptible, HS - Highly susceptible

Table 4. Select parent lines used for generating breeding population.

Maintainer lines	*Xa* gene(s)	Race 1 PXO61	Race 2 PXO86	Race 3b PXO79	Race 3c PXO340	Race 4 PXO71	Race 6 PXO99	Race 5 PXO112	Race 7 PXO145	Race 8 PXO280	Race 9a PXO339	Race 10 PXO341	PXO347	PXO349	PXO349
PR24069B	*Xa4+Xa7*	R	HR	R	R	R	R	HR	R	R	R	MR	MR	R	R
IR69626B	*Xa4+xa5*	HR	MS	MR	HR	HR	MS	HR	HR	HR	HR	HR	MR	HR	R
PR24068B	*Xa4*	R	HR	R	R	R	MR	HR	R	R	R	R	MR	HR	R
IR58025B	-	MS	HR	MR	MR	S	MS	MS	MS	S	R	S	MR	R	R
IRBB62*	*Xa4+Xa7+Xa21*	R	R	R	R	R	MR	R	R	R	MS	R	-	-	R

*Based on the published IRBB lines information (IRRI, n.d.).
HR - Highly resistant, R - Resistant, MR -Moderately resistant, MS - Moderately susceptible, S - Susceptible, HS - Highly susceptible

which probably came from IR58025B. The resistant reaction of IR58025B to six *Xoo* races and the comparable resistant reaction of IRBB5, containing *xa5* gene (IRRI, n.d.) signify that IR58025B may contain *xa5* gene. Initial evaluation of these developed lines for artificial disease infection showed that lines PR41325-2, PR41326-1, PR41326-2, PR41326-3, PR41326-4, PR41327-1, and PR41327-2 have resistant reaction while PR41325-1 have intermediate reaction (Table 5).

Table 5. Improved maintainer lines with BLB resistance genes and their reaction to isolates PXO79.

Line Designation	Combination	*Xa* Gene(s)	Race 3b PXO79
PR41325-1	IR69626B / PR24069B	*Xa4+xa5*	I
PR41325-2	IR69626B / PR24069B	*Xa4+xa5*	R
PR41326-1	IRBB62 / IR58025B	*Xa4+Xa7*	R
PR41326-2	IRBB62 / IR58025B	*Xa4+Xa7*	R
PR41326-3	IRBB62 / IR58025B	*Xa4+xa5+Xa7*	R
PR41326-4	IRBB62 / IR58025B	*Xa4+xa5+Xa7*	R
PR41327-1	PR24068B /PR24069B	*Xa4*	R
PR41327-2	PR24068B /PR24069B	*Xa4*	R
PR41327-3	PR24068B /PR24069B	*Xa4*	R

R - Resistant, I - Intermediate

Grain Quality Characteristics of Improved Maintainer Lines

The improved maintainer lines with BLB resistance showed acceptable to excellent grain quality in terms of milling potential, physical attributes, physicochemical properties, and cooking and sensory quality. Lines PR41325-2, PR41326-1, PR41327-2, and PR41327-3 showed high percentage brown rice (BR) recovery with values >80%. High percentage of milled rice (MR) with premium (Pr) classification was observed in lines PR41325-1 (71.2%), PR41325-2 (72.6%), PR41326-1, (72.1%), and PR41326-2 (71.2%) while the other lines had Grade 1 (G1) classification with values ranging from 67.2 to 69.9%. PR41325-1, PR41326-2, PR41326-3, and PR41326-4 had more than 50% head rice recovery (HR) while lines PR41326-2, PR41326-3, and PR41326-4 had premium percentage chalky grain. All the improved lines have consumer-preferred long and slender grains except for PR41326-3, which was medium and slender (Table 6). Evaluation of physicochemical properties showed interesting results. The percent protein content varied from 5.4 to 6.7, which is the usual range for protein of rice based on historical data. Amylose content (AC) was also varied in nine improved lines. PR41325-1, PR41326-2, PR41326-3, and PR41326-4 had low AC. PR41326-1 had intermediate (I) AC while PR41325-2, PR41327-1, PR41327-2, and PR41327-3 had high (H) AC. However, the high amylose lines had soft gel consistency (GC). Based on alkali spreading value (ASV), the Gelatinization Temperature (GT) of the advance lines was low (L) at 7.0 or < 70^{0}C. For cooking quality, PR41327-3 and PR41325-1 obtained the least (142.3) and highest (227.3) % height increase (a measure of volume expansion), respectively.

The result negatively correlated with their high and low AC as well as with their longest (17:37 min) and shortest cooking time (15:18 min), respectively. This result is unlikely because the highest height increase should be exhibited by the samples with high AC, which is expected in rice with hard texture. Cooked rice of the low and intermediate AC samples rice: PR41325-1, PR41326-2, PR41326-3, PR41326-4, and PR41326-1 were predicted to be tender based on their AC and GT combination (low/intermediate-low/intermediate). Meanwhile, high AC samples have hard texture as predicted by their high AC and low GT combination. Interestingly, two hard-textured lines, PR41325-2 and PR41327-2, were aromatic (Table 7). The low amylose content or tenderness and aromatic grain quality are known unique traits of IR58025B, which were observed in the four advance developed lines of IRBB62 x IR58025B crosses: tender + aromatic (PR41326-1), and tender (PR41326-2, PR41326-3, PR41326-4). Generally, eight of the improved lines have multiple traits i.e., BLB resistance+ tender + MR(Pr) + >50% HR recovery + Premium % chalky grains (PR41326-2); BLB resistance + tender + >50% HR recovery + Premium % chalky grains (PR41326-3, PR41326-4); BLB resistance + aromatic, tender + MR(Pr) + high % BR (PR41326-1); BLB resistance + aromatic, hard + MR(Pr) + high % BR (PR41325-2); BLB resistance + aromatic, hard + high % BR (PR41327-2); BLB resistance + tender + high % BR (PR41327-3); BLB resistance tender + MR(Pr) (PR41325-1) (Table 8).

Table 6. Milling potential and physical attributes of improved maintainer lines.

Line Designation	*Xa* Gene(s)	Milling Potential (%)			Physical Attributes (%)					
		BR	MR	HR	Length (mm)	Width (mm)	Shape	Trans-lucent (%)	Chalky Grains (%)	Immature Grains (%)
PR41325-1	*Xa4+xa5*	79.3 F	71.2 Pr	54.6 G1	7.0 L	1.9	3.6 S	73.04	2.5 G1	1.1 Pr
PR41325-2	*Xa4+xa5*	82.1 G	72.6 Pr	35.2 G3	7.0 L	2.1	3.4 S	43.9	4.7 G1	1.1 Pr
PR41326-1	*Xa4+Xa7*	81.2 G	72.1 Pr	48.8 G1	7.1 L	1.9	3.7 S	61.04	6.1 G2	1.0 Pr
PR41326-2	*Xa4+Xa7*	79.5 F	71.2 Pr	53.2 G1	6.6 L	1.9	3.4 S	77.76	0.5 Pr	1.1 Pr
PR41326-3	*Xa4+xa5+Xa7*	79.8 F	69.9 G1	56.9 G1	6.5 M	2.1	3.1 S	72.6	1.3 Pr	0.3 Pr
PR41326-4	*Xa4+xa5+Xa7*	78.3 F	67.2 G1	54.3 G1	6.7 L	2	3.3 S	72.84	1.2 Pr	0.7 Pr
PR41327-1	*Xa4*	79.9 F	69.9 G1	45.2 G2	7.2 L	2.1	3.5 S	51.08	6.1 G2	0.3 Pr
PR41327-2	*Xa4*	81.9 G	69.6 G1	30.2 G3	6.7 L	2.1	3.3 S	34.86	8.9 G2	0.9 Pr
PR41327-3	*Xa4*	81.6 G	69.1 G1	44.0 G2	6.9 L	2.1	3.4 S	50.78	6.0 G2	0.5 Pr

BR - Brown rice, F - Fair, G - Good, MR - Milled rice, Pr - Premium rice, HR - Head rice, G1 - Grade 1, G2 - Grade 2, G3 - Grade 3, L - Long, M - Medium, S- Slender

Table 7. Physicochemical properties and cooking and sensory quality of improved maintainer lines.

Line Designation	*Xa* gene(s)	Physicochemical Properties				Cooking and Sensory Qualities		
		% Protein	% AC	GC (mm)	GT Score	% Height Increase	Cooking Time (min:sec)	Description
PR41325-1	*Xa4+xa5*	6.71	12.48 L	-	7.0 L	227.3	15:18	Tender
PR41325-2	*Xa4+xa5*	6.18	27.49 H	100 S	7.0 L	191.3	15:48	Aromatic, hard
PR41326-1	*Xa4+Xa7*	6.51	22.42 I	-	7.0 I	192	17:20	Aromatic, tender
PR41326-2	*Xa4+Xa7*	6.05	14.22 L	-	7.0 L	175	16:52	Tender
PR41326-3	*Xa4+xa5+Xa7*	5.76	13.81 L	-	7.0 L	200	16:16	Tender
PR41326-4	*Xa4+xa5+Xa7*	5.43	14.83 L	-	7.0 L	173.9	16:50	Tender
PR41327-1	*Xa4*	5.43	27.26 H	94.0 S	7.0 L	200	16:32	Hard
PR41327-2	*Xa4*	5.93	26.64 H	66.5 S	7.0 L	168	16:33	Aromatic, hard
PR41327-3	*Xa4*	5.98	25.56 H	89.5 S	7.0 L	142.3	17:37	Tender

AC - Amylose content, L - Low, I - Intermediate, H - High, GC - Gel consistency, S - Soft, GT - Gelatinization temperature

Table 8. Multiple traits of developed maintainer lines.

Line Designation	*Xa* Gene(s)	Grain Quality Traits
PR41325-1	*Xa4+xa5*	Tender + MR(Pr)
PR41325-2	*Xa4+xa5*	Aromatic, hard + MR(Pr) + high % BR
PR41326-1	*Xa4+Xa7*	Aromatic, tender + MR(Pr) + high % BR
PR41326-2	*Xa4+Xa7*	Tender + MR(Pr) + >50% HR recovery + Premium % chalky grains
PR41326-3	*Xa4+xa5+Xa7*	Tender + >50% HR recovery + Premium % chalky grains
PR41326-4	*Xa4+xa5+Xa7*	Tender + >50% HR recovery + Premium % chalky grains
PR41327-2	*Xa4*	Aromatic, hard + high % BR
PR41327-3	*Xa4*	Tender + high % BR

BR – Brown rice; MR – Milled rice, HR – Head rice

Maintaining Ability Assessment of Improved Maintainer Lines

Initial evaluation of F_1 plants of eight improved lines using five CMS lines showed various results. Five improved lines (PR41325-1, PR41325-2, PR41326-1, PR41327-2, and PR41327-3), can maintain the sterility of all CMS lines used. The evaluated F_1 plants from these five lines exhibited unstained withered sterile (UWS) and unstained spherical sterile pollen (USS) to five cytosterility sources. The other three lines (PR41326-2, PR41326-3, and PR41326-4) can only maintain CMS lines with Chinsurah Boro II or BT type cytoplasm. The other cytoplasmic source mutagenized IR62829A, wild abortive (WA-2), and WA-3 cytoplasm produced USS and stained round light sterile (SRS) pollen when testcrossed to these three lines. In contrast, cytosterility source WA-1 exhibited stained round fertile (SRF) pollen (Table 9). This signify that the derived lines cannot be used as maintainer for the particular cytosterility source. Among the improved lines, only the lines from IRBB64/IR25025B (PR41326) was unable to maintain the five cytosterility sources used. The presence of fertility residual effect on WA-1 cytosterility source imply that these three lines may contain two dominant (*Rf*) genes with different restoration, which may be inherited from parent line IRBB62. This validates the pollen result, which showed that the stronger *Rf* genes cause the pollen fertile when crossed to WA-1 while weaker *Rf* gene produce SRS pollen when crossed to WA-2 and WA-3 cytosterility source.

Table 9. Maintaining ability evaluation of improved lines using five CMS lines.

Line Designation	*Xa* Gene(s)	CMS Cytosterilty				
		Chinsurah Boro II	Mutagenized IR62829A	WA-1	WA-2	WA-3
PR41325-1	*Xa4+xa5*	UWS/USS	UWS/USS	UWS/USS	UWS/USS	UWS/USS
PR41325-2	*Xa4+xa5*	UWS/USS	UWS/USS	UWS/USS	UWS/USS	UWS/USS
PR41326-1	*Xa4+Xa7*	UWS/USS	UWS/USS	UWS/USS	UWS/USS	UWS/USS
PR41326-2	*Xa4+Xa7*	UWS/USS	USS/SRS	SRF	USS/SRS	SRS/USS
PR41326-3	*Xa4+xa5+Xa7*	UWS/USS	USS/SRS	SRF	USS/SRS	SRS/USS
PR41326-4	*Xa4+xa5+Xa7*	UWS/USS	USS/SRS	SRF	USS/SRS	SRS/USS
PR41327-2	*Xa4*	UWS/USS	UWS/USS	UWS/USS	UWS/USS	UWS/USS
PR41327-3	*Xa4*	UWS/USS	UWS/USS	UWS/USS	UWS/USS	UWS/USS

UWS-Unstained withered sterile; USS-Unstained spherical sterile; SRS-Stained round sterile; SRS-Stained round fertile.

Conclusion

BLB is a major leaf disease, which limits yield potential of inbred and hybrid rice. BLB resistance among maintainer germplasm is the product of breeding efforts of various rice breeding programs. The established maintainer BLB reaction profile facilitates the selection of donor parent for improving BLB resistance gene and other desirable traits; thus, increasing the development of maintainer lines with multiple traits. The established maintainer line assessment for BLB resistance showed that broad resistance is present among maintainer germplasm. However, identification of other *Xa* genes was restricted due to limited resourced. Thus, a follow up investigation particularly in the identification of novel *Xa* genes among maintainer germplasm is required.

The newly developed lines in this study were produced from the development of BLB resistance gene in maintainer lines. This strategy provides substantial information particularly in overcoming the difficulty in converting into CMS lines without fertility residual effect. The advantage of this new approach is that the plants selected carry the gene of interest and the improved traits for maintainer line. In contrast with backcrossing, the genotype obtained from this method will not be improved for any character or traits except for those transferred by the donor parent (Fehr, 1987). In addition, the donor source of BLB resistance was from the background of maintainer line (having cytoplasmic fertile gene with recessive *rf* gene); thus it ensures no fertility residual effect when converted to CMS (having cytoplasmic sterile gene with recessive *rf* gene), lines with stable sterility. Aside from breeding for resistance the combined traits of both maintainer lines increase the possibility of developing multi traits specifically for grain quality.

The use of IRBB lines for introduction of other *Xa* genes to maintainer line is feasible. Application of molecular markers is needed specially for discriminating the dominant (*Rf*) gene among F_2 plants, however, this approach prevented the occurrence of fertility residual effect of developed lines when crossed to CMS with WA cytoplasm.

In developing hybrid rice, breeding for yield stability with good grain quality is still a challenge to hybrid rice breeders as right combination of parent lines is needed. The improved lines with stacked gene: PR41325-1, PR41325-2, PR41326-1, PR41326-2, PR41326-3, and PR41326-4 did not only broadened the current maintainer germplasm for BLB resistance, but also facilitated the development of CMS lines with BLB resistance and good grain quality in the future.

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YIELD RESPONSE TO THE KEY CHECKS OF PALAYCHECK® SYSTEM: AN EARLY FARM-LEVEL OBSERVATION

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Abstract

This study evaluated the contribution of each Key Check (KC) of the PalayCheck® system on yield. Specifically, a descriptive analysis was used to determine farmers' attainment of the KCs, and regression analysis to show the individual and combined effects of the KCs on yield. Majority of the farmers who practice PalayCheck® accomplished each KC, implying that PalayCheck® had enhanced the ability of farmers to adopt the recommended best farming practices. Land-preparation and nutrient-management KCs were noted as great yield enhancers. Some farmers had difficulty completing all KCs, especially the KC on nutrient management. Achieving all KCs can positively and significantly affect yield, demonstrating the interconnectedness of different production factors. Regular meetings and farmer group discussions may need to be institutionalized further to sustain a two-way information sharing among farmers, extension workers, and researchers. Extension workers and farmers may consider exerting extra effort in ensuring right amount of nutrients at the right time. Soil and plant nutrient analysis tools may be promoted further, while ensuring their availability and accessibility to rice farming communities. Credit can help financially-constrained farmers in providing sufficient nutrients to their rice plants. Technology developers should also continue designing low-cost technologies so that farmers may have extra money to spend for inputs like fertilizers.

Keywords: *Key Checks, PalayCheck®* System, *Participatory Approach, Regression Analysis.*

Introduction

Since the middle of the 1960s, the green revolution has increasingly raised rice yields with the introduction of high-yielding varieties and intensified use of agricultural chemical inputs. However, despite the availability of these technologies, yield growth almost stagnated since the 1980s. The yield gaps between techno-demo and farmers' fields were observed. These were due to several problems brought by both controllable and uncontrollable events. This situation led researchers to identify new approaches to stimulate the response of crop yields to these technologies. One of these approaches focused on the extension system.

In the past, the extension system operated under a unidirectional method of disseminating agricultural knowledge. This process treated farmers as receivers of research results, which is a one-way approach to extension. Examples of these are transfer-of-technology model and transfer-and-visit approach (Anderson and Feder, 2006). Moreover, researchers were focused on introducing new technologies to replace farmers' indigenous practices rather than investigating reasons why farmers refuse to change (FAO, 1995). Some studies reported that farmers were unresponsive to these technologies because they viewed it as unnecessary (Castillo, 1975; Bonifacio, 1994). These models failed to overcome the information asymmetry that rendered extension efforts partially effective (FAO, 1995).

An effort to incorporate farmers' views into the system was embodied through Participatory Rural Appraisal (PRA) approach. This method allowed farmers to be directly involved in developing and implementing solutions to existing farm problems (FAO, 1995; Chambers, 1994; Bonifacio, 1994). With farmers directly involved, researchers had created location-specific technologies.

This approach to extension was adopted by the Check System, an integrated crop management solution developed by Australian Agronomists of the New South Wales-Department of Primary Industries. The concept was later on applied to rice through the RiceCheck System (Lacy, 1997).

In 2004, the RiceCheck System was introduced to the Philippines. Researchers developed a local version and was called the PalayCheck® System.

Similarly, this system employs farmer-groups to facilitate crop checking and foster collaborative learning. Participants of these groups learn not only from their own experiences but also from their fellow participants. The success of this activity lies on the willingness of farmers to share experiences with one another.

Moreover, PalayCheck® involves farmers from identification of field problems to formulation of solutions. Technical persons present during farmers' meetings may give advice only upon the demand of farmers. This method combines research results and experiences of farmers as informational inputs to production. Thus, farmers are given greater participation in investigating their own production system. This can create a sense of ownership on the identified production techniques or solutions to field problems, encouraging adoption even by other farmers; thus, a participatory approach to technology transfer.

Another key feature of the PalayCheck® System is that Key Checks (KCs) are associated with good management practices. KCs are target outputs set for each crop management area that may be obtained should farmers correctly follow the PalayCheck® recommended practices. These recommendations consider the interplay or interrelatedness of factors affecting crop growth. Attaining these KCs implies that farmers had implemented the best production techniques in their farms (Lacy, 1997). Therefore, KCs are linked to higher yield; the more KCs are achieved, the higher the yield.

The PalayCheck® System remains relevant and updated. It has been used in several extension support program of the government for farmers. Corales et al. (2014) reported a moderate degree of institutionalization of PalayCheck® in select Luzon areas at the farmers' and Local Government Units' (LGU) level.

The relevance of PalayCheck® makes it a constant subject of discussions in a number of rice-related events. Specifically, questions focused on the effect of KCs on yield. There were few studies that assessed the effects of PalayCheck® on yield (Mataia et al., 2015; de Guzman, 2021). However, they did not examine the individual KC's yield effect. Existing studies utilized data that were based on farmers' recall of KC attainment at the end of each season. This paper aimed to fill the gaps in existing literatures. It investigated the yield response of each and combined KC using a more accurate data, which were gathered immediately after each production activity.

Materials and Methods

The Data

This study used the monitoring data gathered during the first year when PalayCheck® System was introduced to farmers. The areas covered were Cabadbaran in Agusan del Norte; Bayugan and Trento in Agusan del Sur; Sta. Marcela, Flora, Malekkeg, Bagutong, and Imelda in Apayao; Mlang in Cotabato; Aurora in Isabela; Llanera, Rizal, and Muñoz in Nueva Ecija; Diffun in Quirino; and Lambayong in Sultan Kudarat.

Respondents in each site were sampled. Farmers whose parcels were adjacent to each other were selected to avoid varied field conditions that may affect the findings. This sample selection also aided in farm monitoring.

Fifty-seven varieties were recorded in the original PalayCheck® database. However, varieties have varying characteristics, which can greatly affect yield. Some of these characteristics include the number of panicles and spikelets. In this study, only one variety was selected to remove the effect of varietal characteristics. PSB Rc 82 was the popular variety among the sampled farmers.

This selection further reduced the total number of observations to 240 farmers, 116 in the dry season (DS) and 124 in the wet season (WS). Table 1 shows the distribution of farmer-respondents.

Table 1. Distribution of farmer-respondents, by province and season, 2005.

Province	Number of Farmers		
	2005 DS	2005 WS	All Seasons
Agusan del Norte	0	5	5
Agusan del Sur	25	18	43
Apayao	10	10	20
Cotabato	8	0	8
Isabela	38	23	61
Nueva Ecija	19	58	77
Quirino	11	10	21
Sultan Kudarat	5	0	5
Total	**116**	**124**	**240**

DS- dry season
WS- wet season

PhilRice initially worked on seven areas of crop management in 2005 for the PalayCheck® System. Table 2 shows the corresponding KCs for each site.

Table 2. The seven Key Checks for irrigated lowland rice farms in 2005.

Management Area	KC Number	Description
Seed Quality	KC 1	Used high-quality seeds
Land Preparation	KC 2	Achieved no high or low soil spots at initial flooding after final levelling
Crop Establishment	KC 3	Achieved sufficient healthy seedlings
Nutrient Management	KC 4	Nutrients were adequate at early panicle initiation
Water Management	KC 5	Avoided excessive water and drought stress that affects the growth and yield of crop
Pest Management	KC 6	Ensured no significant yield loss due to pest
Harvest Management	KC 7	Harvested and threshed the crop at the right time.

This set of KCs was modified in 2007 to include another check on crop establishment (PhilRice, 2007) and postharvest management in 2020 (PhilRice, 2020). The assessment of other KCs was retained up to the present.

The KCs listed in Table 2 were used because these were gathered for the reference period. The dataset is more accurate because it was gathered right after each production activity and not based on farmers' recall.

Analytical Framework

As an extension product system, the PalayCheck® addresses the problem of insufficient information between researchers and farmers. Farmers possess indigenous knowledge on how to grow rice. Although these techniques were already tested by several generations, they may not be the best methods to attain the best possible yield given set of inputs. Likewise, researchers may have information on technological breakthroughs, which produced better results than traditional practices. If farmers do not have access to information on new technologies, they remain dependent on traditional practices and may not be able to take advantage of the innovations in getting higher yields. Without proper communication with farmers, researchers may also fail to grasp the actual field conditions in developing new technologies. The yield obtained using these innovations could be higher than traditional techniques; however, it may not represent the maximum potential yield with the available resources. A higher level of output can be obtained if farmers' perceptions are considered in technology development. Farmers may resist adoption because these technologies may not be suitable for their farms. PalayCheck® System then attempts to bridge this gap through a two-way information delivery scheme.

PalayCheck® uses a yield function that is represented by the equation $Y = f[L, S, X, I(\theta)]$. Where, yield is a function of labor (L), seeds (S), other inputs (X) such as pesticides, fertilizers, and water, and information (I). The factor θ represents participation of farmers that affects the flow of information within the system.

With farmers participating in the research process, enough information can be deduced, which allow a site-specific technology recommendation that can improve yield. Therefore, a positive relationship between participation and information can be realized. As participation increases, more information flows into the system, which in turn, increases output as site-specific recommendations and technologies. The relationship between participation and yield is then represented by the equation $\frac{\partial Y}{\partial \theta} > 0$.

As an integrated crop management system, PalayCheck® incorporates the interrelatedness of technologies through its KCs. The attainment of one KC is said to enhance the yield effect of the others in synergy. Researchers assert that the attainment of an additional KC can boost the effect of the other. Every additional KC achieved pushes the production away from an inefficient level of resource use to the optimum level. Therefore, practicing few KCs is insufficient to obtain the best possible yield from a given set of inputs.

The Production Function

In developing countries, farmers may opt not to apply a subset of inputs in rice production such as fertilizers and pesticides. Despite this, rice can still be produced. However, application of these inputs can enhance production and, consequently, increases yield. The underlying production process may include a feature wherein basic or essential inputs substitute with each other, and another which entails a package of inessential inputs. The appropriate application of the technology in a way shifts the production frontier upwards.

Seeds and labor are essential inputs. Seeds and labor may have limited substitutability. Poor quality seeds may be partly offset by more cultivation; hence, requiring more labor inputs. On the other hand, better seeds may allow the farmer to attain a given amount of yield with less labor inputs. Non-essential inputs such as agricultural chemicals are not required to produce an output. Farmers can still harvest rice, although yield could be higher than the non-essential inputs. Non-essential inputs act like shifters of the production function in a rice cum essential inputs space.

In micro studies involving regression estimation of a Cobb-Douglas production function, the presence of zero-valued inputs poses a problem. Some treat this problem by omitting zero-valued observations, as if they are outliers. However, this has an effect on the variance of estimators (Johnson and Rausser, 1971). Some researcher opt to replace the zeroes with small positive values, however, this method produces biased estimators (Battese, 1997).

The specification of the Cobb-Douglas production function presented below was then developed to solve this issue:

$$Y_i = AF_{1,i}^{\beta_1} F_{2,i}^{\beta_2} \dots F_{2,i}^{\beta_2} e^{a_1 X_{1,i} + a_2 X_{2,i} + \dots + a_n X_{n,i}} \mu_i$$
$$= A \prod_{i=1}^{k} F_{j,i}^{\beta_j} e^{\sum_{j=1}^{n} a_j X_{j,i}} \mu_i$$

Y_i represents rice yield; F_j the primary factors and essential inputs; X_j are non-essential intermediate inputs; β_j are the coefficients of primary and essential inputs; a_j are the coefficients of non-essential intermediate inputs; and μ_i as the error term.

Taking its logarithmic transformation, we derived at the equation below:

$$y_i = \alpha + \sum_{j=1}^{k} \beta_j f_{j,i} + \sum_{j=1}^{n} a_j X_{j,i} + \varepsilon_i$$

Where y_i, f_i, and e are the respective natural logarithms of Y, F, and μ, respectively.

The Empirical Model

This paper estimated two production functions based on equation (2). The first was used to estimate the effect of each KC on yield and the second one was for the estimation of yield effect of completing all KCs.

$$y_i = \alpha + \sum_{j=1}^{k} \beta_j f_{j,i} + \sum_{j=1}^{n} a_j X_{j,i} + \sum_{j=1}^{m} a_j Z_{j,i} + \varepsilon_i$$

Where y_i is the natural log of yield; α is the constant; f's are the natural logs of the quantities of essential inputs like seeds and labor; X's are the quantities of intermediate inputs like fertilizers and chemicals; Z's are dummy variables representing season and KCs; β, a, b are the coefficients; and ε as the error term. The dummy variable on season gets a value 1 if it is DS; 0 otherwise. KC dummy variables' value is 1 if the farmer achieved the specific KC; 0 otherwise.

The second model is specified below:

$$y_i = \alpha + \sum_{j=1}^{k} \beta_j f_{j,i} + \sum_{j=1}^{n} a_j X_{j,i} + D_i + S_i + \varepsilon_i$$

Equations (3) and (4) have the same set of explanatory variables except that instead of individual KCs, equation (4) used a dummy variable where a value of 1 means all KCs were attained; 0 otherwise. is the season variable that gets an entry of 1 if DS; 0 if WS.

Methods of Analysis

This paper employed descriptive statistics in describing KC attainment by farmers. Regression analysis was also used to determine the significance of KC attainment on yield. The production models (3) and (4) were estimated using Ordinary Least Squares (OLS) method.

Results and Discussion

Attainment of Key Checks

Table 3 shows that most farmers attained five to seven KCs. The distribution peaked at five and six in the dry season (DS) and wet season (WS), respectively. In DS, approximately 28% attained five; 24% attained seven KC. Similarly, more farmers in the wet season accomplished six (45%) and seven (26%) KCs.

The participatory approach embedded in the PalayCheck® System encouraged majority of the farmers to follow the recommendations and, consequently, attain higher number of KCs. The two-way information delivery scheme could have effectively bridged the knowledge gap between the researchers and the farmers, which resulted in recommendations that are suitable to actual field conditions. This could account for the higher percent of farmers attaining more KCs.

Results suggest that farmers had difficulty in completing the KCs. The missed KCs may have required greater financial capital or time. This additional invested capital or time could have been spent on other profitable activities.

Table 4 shows that more than 50% of farmers attained each KC in both seasons. This means that majority can follow the best production practices correctly. This may be partly attributed to the participatory nature of the PalayCheck® extension system. As farmers' perceptions were considered in generating recommendations, rice growers were more encouraged to adopt the KCs.

In DS, approximately 91% of farmers properly managed their water supply (KC 5) while 94% harvested crops at the right time (KC 7). Only 47%

of the DS farmers practiced KC 1 (seed quality). Crop establishment (KC 3) and nutrient management (KC 4) were the second least attained; only 60% of the farmers in the DS attained the KCs. These least prioritized KCs require relatively higher cash investment.

Farmers' attainment of KCs was almost the same in 2005 WS. KC 7 remained to be easily practiced (98%); followed by KC 2 (land preparation), attained by 94%. The least attained were KC 1 and KC 4.

Overall, the most attained KCs were on harvest management and land preparation. These KCs are relatively easier to follow because these are not as complex as the other crop management recommendations. These KCs do not also require much time and cash. Farmers had difficulty adopting high-quality seeds and nutrient management, which may be attributed partly to insufficient capital to purchase these inputs. Based on the Rice-based Farm Household Survey (RBFHS) results of PhilRice, farmers identified lack of capital as one of their common problems in 2011 - 2012. Adopting high-quality seeds and applying sufficient fertilizers to rice plants may be a costly decision for farmers.

In another perspective, missing KC 4 also imply that farmers may have applied fertilizers without conducting soil analysis. In 2016, only 1% and 3% of sample farmers of RBFHS used Minus-one Element Technique (MOET) and Rice Crop Manager (RCM), respectively (Flores, 2018). Availability and accessibility of soil analysis tools may also be an issue.

Yield Response to Key Checks

A regression analysis was conducted using OLS method on equation (3) (Table 5). The estimated model resulted in a significant F-value, meaning that the model fits the data. The resulting adjusted R-squared means that the chosen variables were able to explain 40% of the variability in yield.

While holding other factors constant, KC 2 (land preparation) and KC 4 (nutrient management) assessed at 90% and 99% level of confidence, respectively, generated positive and significant estimators. Based on computed semi-elasticity, KC 2 can increase yield by 15%; 21% for KC 4.

KC 2 involves preparing an area with no high and low soil spots at initial flooding after final harrowing. This means that the entire area should be properly leveled, a necessary condition for good crop growth and management. To obtain this, farmers should puddle the soil very well to incorporate rice

Table 3. Distribution of farmers based on the number of KCs attained, 2005.

Number of Key Checks Attained	2005 DS		2005 WS		All Seasons	
	Frequency	Percent	Frequency	Percent	Frequency	Percent
1	1	1	0	0	1	0.4
2	3	3	1	1	4	2
3	15	13	0	0	15	6
4	13	11	9	7	22	9
5	32	28	26	21	58	24
6	24	21	56	45	80	33
7	28	24	32	26	60	25
Total	**116**	**100**	**124**	**100**	**240**	**100**

Table 4. Distribution of farmers per KC attained, 2005.

Key Checks	Distribution of Farmers					
	2005 DS		2005 DS		All Seasons	
	Frequency	Percent	Frequency	Percent	Frequency	Percent
KC 1	55	47	79	64	134	56
KC 2	103	89	117	94	220	92
KC 3	70	60	113	91	183	76
KC 4	70	60	80	65	150	63
KC 5	106	91	110	89	216	90
KC 6	91	78	107	86	198	83
KC 7	109	94	122	98	231	96

Table 5. Regression results of estimating the effects of individual Key Checks on yield, 2005.

Explanatory Variables	Coefficient	Robust Standard Error		Elasticity/ Semi-elasticity
Season	0.2994	0.1122	***	0.3490
Seeds (logged)	0.0380	0.0470		0.0380
Labor (logged)	0.1574	0.0552	***	0.1574
Nitrogen	0.0026	0.0006	***	0.0026
Phosphorous	0.0010	0.0007		0.0010
Potassium	-0.0013	0.0012		-0.0013
Pesticides	0.0191	0.0105	*	0.0191
KC 1	0.0333	0.0374		0.0339
KC 2	0.1398	0.0799	*	0.1500
KC 3	-0.0099	0.0557		-0.0099
KC 4	0.1907	0.0443	***	0.2101
KC 5	0.0697	0.0611		0.0722
KC 6	-0.0412	0.0600		-0.0404
KC 7	0.2272	0.1561		0.2550
Constant	6.6821	0.3940	***	6.6821
Number of observations		240		
F(computed)		15.03		
Prob>F		0.00		
R-squared		0.4015		

*, **, *** means coefficients are significantly different from zero at 90%, 95%, and 99% level of confidence, respectively.

straw, stubbles, and weeds. Good ditches, dikes, and drainage are also necessary to obtain this key check. These practices can minimize the occurrence of weeds and snails, promote efficient water management and nutrient uptake, and helps crops to grow and mature uniformly. Good land preparation is a pre-requisite to good crop growth and management (PhilRice, 2020).

The regression also identified KC 4 as an influential factor in improving yields. Its attainment depends on how farmers manage the nutrient needs of the plants. Supply of nutrients is most crucial at early panicle initiation (EPI) because panicles develop at this stage. This KC may be attained by providing adequate supply of fertilizers at the right time as nutrients are necessary to boost plant growth and health. Unfortunately, it was mentioned in an earlier section that KC 4 was one of the least attained KCs.

The model failed to determine yield's response to KC 1 (seed quality), KC 3 (crop establishment), KC 5 (water management), and KC 6 (pest management). This implies that these KCs, if taken individually, may not have a large impact compared to the two key checks identified in Table 4. Thus, the estimation had difficulty detecting their influence on yield. Yield may be less sensitive to these KCs if taken one at a time.

Experts recognize that rice growing is a complex process that makes production factors interdependent and interrelated with each other (PhilRice, 2020). With this, another estimation was done to determine if yield will respond to these KCs if collectively gathered. Results in Table 6 shows that completing all key checks had positive and significant coefficient. This implies that attaining one KC can amplify the effect of the others; thus, if taken together, these KCs can positively influence yield. Therefore, these KCs are all important indicators of good rice production.

Table 6. Regression results of estimating yield effects of all Key Checks, 2005.

Explanatory Variables	Coefficient	Robust Standard Error	
Season[b]	0.2907	0.1259	**
Seeds[c]	0.0314	0.0476	
Labor[c]	0.1678	0.0584	***
Nitrogen	0.0031	0.0005	***
Phosphorous	0.0013	0.0007	*
Potassium	-0.0010	0.0014	
Pesticides	0.0275	0.0106	***
All Key Checks	0.0807	0.0469	*
Constant	7.0897	0.3815	***
Number of observations		240	
F(computed)		15.97	
Prob>F		0.00	
R-squared		0.3243	

*, **, *** means coefficients are significantly different from zero at 90%, 95%, and 99% level of confidence, respectively.

Conclusion

The attainment of Key Checks may be reflective of PalayCheck's® strategy of extending technologies to farmers. Farmers can be directly involved in measuring their performance and analyzing their own production process. Through this technique, farmers were encouraged to embed their own knowledge in the system. As such, the technique promotes greater understanding of the complexities of rice production.

For this strategy to be effective, extension workers should encourage farmers to attend and actively participate in meetings and group discussions. Two-way communication among farmers, extension workers, and researchers is enhanced. These meetings and discussions can be institutionalized, especially in places where there are strong and active farmer-leaders and dedicated extension workers. There is a high probability that these meetings may not be sustained as the opportunity cost of farmers' participation in such activity could be high.

Farmers should give full attention to land preparation and nutrient management key checks. Attainment of KC 2 was high but low for KC 4. Therefore, extension workers and farmers need efforts in ensuring that the right amount of nutrients are applied at the right time.

One important consideration on this may be an intensified promotion of soil and plant nutrient diagnostic tools such as MOET, Leaf Color Chart (LCC)/LCC App, and RCM. However, these tools have to be available and accessible to farmers. Farmers' access to low-cost credit may also be explored to help augment limited budget. Rice technology developers need to design low-cost technologies that may have comparable yield effect with those of the relatively expensive ones. These will help farmers save and have budget for other inputs like fertilizers.

Regression analysis showed that KCs, if collectively taken, can synergize effects on yield. However, many farmers had difficulty completing the KCs. Farmers and extension workers may need to revisit the frequently missed KCs and identify strategies on addressing the challenges. Information sharing within and between farmer groups has to be strengthened because it serves as a vehicle for improved management practices.

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PHILRICE AS AN INNOVATIONS BROKER: THE CASE OF THE PROJECT 'WHAT IS A CLIMATE CHANGE-ADAPTIVE SCHOOL?'

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Abstract

Innovations brokering enables extraction of new or old knowledge from the wider environment. In agriculture organizations, performing this function means having the ability to benefit from the collective wisdom of the players in the innovation system to either address an issue or simply to advance the sector forward. This paper discussed how a public research institute can perform the roles of an innovations broker. In doing this, a case study of the 'What is a climate change-adaptive school?' project of the Philippine Rice Research Institute (PhilRice) is presented. Focus groups and interviews served as the main methods used in data collection. Through the project, PhilRice accomplished the three roles usually performed by an innovations broker. These roles are demand articulation, network composition, and innovation process management. Overall, innovation brokers may help provide avenues for self-reflection on how the actors can do their work and mediate conflicts in the innovation system. This paper concludes that PhilRice became an innovations broker serendipitously. It is not a function that the Institute consciously performs. Questions relating to the level of institutional transformation that is needed for a public research institute to perform innovations brokering roles are enhanced.

Keywords: *Climate Change, Innovations Brokering, Innovation System, Public Agricultural Research Institute.*

Introduction

The importance of innovations cannot be overemphasized. Innovative entities are able to respond to the challenges of the times. In agriculture organizations, being innovative means being able to respond to the demands of major issues like climate change.

Hence, it is important to ask how can organizations become more innovative? Or, how can innovations be propelled by agriculture organizations? Among the many ways to answer these questions is through innovations brokering.

Innovations brokerage has gained following in the agricultural extension literature (Rivera and Sulaiman, 2009). Broadly, it refers to how entities facilitate interaction among different actors to enhance innovation capacity (Howells, 2006). This interaction enables the extraction of new or old knowledge from the wider environment (Horne, 2008). Innovation, it should be emphasized, is not just the domain of research institutions. It is a product of interactions among a heterogenous set of actors (Chowdhury et al., 2014; Sumberg, 2005).

Heterogenous actors in the innovation system helps diminish the surprise when the system does not work as expected (Intarakumnerd and Chaminade, 2011; Woolthuis et al., 2005). When this happens, innovations brokers are needed the most. There are many reports as to how innovations brokerage has benefited the agriculture sector in different countries like The Netherlands. An example is the case of the Agricultural Knowledge Centre North Holland, an organization jointly funded by the government and the private sector. As an intermediary, it had helped in the search for 'flower bulb disease detector' to reduce cost and automate detection. It performed the work by searching for available knowledge from all available sources, not merely from public research institutes (Klerkx et al., 2009).

In the literature, innovations brokerage is not very common in agriculture-focused public research institutes. There are cases of research institutes acting as innovations brokers such as the case of the International Potato Centre (IPC). The IPC helped link the farmers to market through the Papa Andina project (Devaux et al., 2009).

In this paper, therefore, we problematize how brokering becomes possible in a public agriculture research institute. Specifically, researchers' experience, albeit serendipitously, in brokering under the auspices of a PhilRice project on conceptualizing climate change-adaptive schools was discussed.

Klerkx et al. (2009) note that innovations brokerage in public institutions has been the domain of agricultural extension systems with very limited and unimpressive results. Likewise, public institutions have a myriad of limitations in their operations especially that they are fraught with bureaucracies and what not. The reader is alerted that this paper focuses solely on the application of the innovations brokering concept to public agriculture research institutes.

Value-Added of Information Brokering and Challenges in Setting Up Formal Information Brokering Functions

Innovations brokering has several value additions in an organization. Among them are providing avenues for self-reflection on how the actors can do their work and mediating conflicts. In providing avenues for self-reflection, an example would be to facilitate discussions on how businesses and extension workers could improve their ways of communicating. Another would be to provide avenues to discuss how they can improve their work. As regards to mediating conflicts, innovations brokers may devise ways to ensure that parties who may otherwise refuse to collaborate should sit down to iron out differences to push the innovation system forward. The key is to unpack the barriers to effective communication. Klerkx et al. (2009) note that the overall impact of having innovations brokering functions in an organization is an improved innovation system.

There are, however, challenges in formally setting up innovations brokering functions in organization (Klerkx and Gildemacher, 2012). The first of these challenges is learning new skills among staff members in the organization who may be tasked to perform innovation brokerage roles. For example, a staff member may have to learn new skills and concepts such as those from sociology or conflict management strategies and move away from the traditional technology transfer skills. Another challenge is to be detached from projects and ideas. Having an in-house innovation brokering function may prove challenging among staff members who may have biases or strong attachment towards certain ideas or ways of thinking. Being distant is amongst the requirements for being an "honest broker". It would also be challenging to take credit for results because it can prove annoying to other parties in the innovation system who may lose ownership of both the product and the process. Depending on the level of maturity of the innovation system in particular contexts, the innovation broker may have to deal with different layers and types of issues such as clientelism, social exclusion, nepotism, and corruption. In an organization, the level of flexibility required for the successful realization of innovations brokerage roles may prove challenging to implement with rigid rules and standards.

Theoretical Framing

Innovations brokers are people and organizations involved in boundary work as they are the go-betweens in an innovation system (Howells, 2006 and Hellin, 2012). It can be defined that an innovation system "is a network of organizations and individuals that are focused on bringing new products, new processes, and new forms of organization into social and economic use."

Klerkx et al. (2009) note that innovation brokers perform the following roles:

Demand articulation: Articulating innovation needs and visions and corresponding demands in terms of technology, knowledge, funding, and policy achieved through problem diagnosis and foresight exercises.

Network composition: Facilitation of linkages among relevant actors, i.e., scanning, scoping, filtering, and matchmaking of possible cooperation partners (Howells, 2006).

Innovation process management: Enhancing alignment in the often-heterogeneous networks, constituted by actors from different institutional backgrounds and reference frames related to norms, values, incentive and reward systems.

The main role of an innovations broker is to ensure interaction among the actors in an innovation system (Tesfaye et al., 2010). Klerkx et al. (2009) note that managing conflict is central to the roles that innovations brokers must play. In relation to the three major functions above, (Batterink et al., 2010) advance the framework on network orchestration (Figure 1), which draws from the earlier work of Dhanaraj and Parkhe (2006). Network orchestration refers to "the set of deliberate actions undertaken by a network orchestrator as it seeks to create value with and extract value from the network" (Batterink et al., 2010). The network orchestrator in this definition refers to the innovations broker. This framework has several propositions under each function to demonstrate how successful network orchestration can be realized.

The framework above emerged from case studies in small and medium enterprises in France, Germany, and The Netherlands. This framework was adopted in analyzing the findings of this current research. It also pays to mention that the three-pronged functions of innovations brokers are among the most cited frameworks in the innovations brokering literature. Hence, there is wisdom in adopting the above framework in this current study.

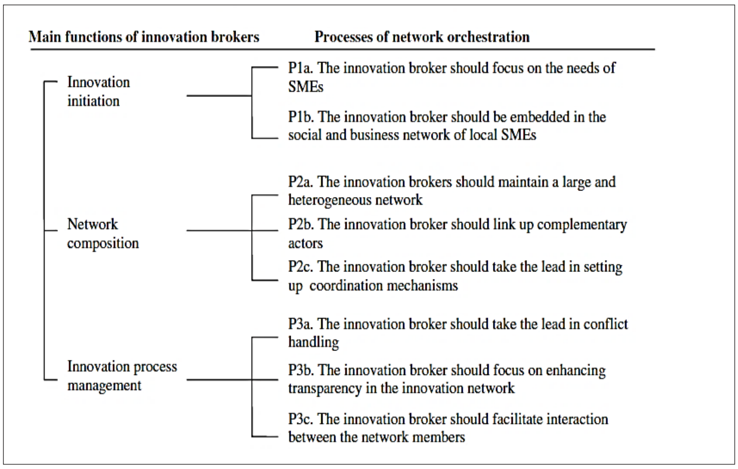

Figure 1. Framework for successful network orchestration (Batterink et al., 2010).

This paper is a welcome addition to the dearth of studies on innovations brokering in the context of public agriculture research institutes (Chowdhury et al., 2014). As earlier alluded, information brokering among public research institutes is not very common. Scholars note that most public agriculture research institutes have decided to focus on the traditional technology transfer functions as opposed to taking on the more challenging functions on innovations brokerage. By pursuing this research route, the main aim was to identify possible ways to improve the interaction of the actors in the innovation system in which public agriculture research institutes belong. The specific research questions were: (1) What are the innovations brokerage functions that a public agriculture research institution performs? (2) What are the limitations of these functions?

Materials and Methods

In responding to the research questions put forward in this paper, the researchers reflected on their experiences in implementing a project aimed at characterizing a climate change-adaptive school. In doing this, we had a debriefing among ourselves, which focused on how we served as catalyst in the process of implementing the project. In addition to the group debriefing, we also reviewed the process documentation book (Manalo et al., 2019) that we wrote and paid attention to the instances of information brokering that we did. We also reviewed the transcripts of focus groups and interviews with participating teachers and school officials that were conducted under the project. Our field observation notes also served as data sources for this paper. The project was implemented in 12 participating high schools spread across the country. Comprehensive information on the methodology of the whole project is available in Manalo et al. (2019).

Findings

In this section, we share how the project was implemented. The objective is to pay attention to the details as to how a public research institute may facilitate innovation brokering. The brokering functions seen in the project as presented in this section will then be unpacked in the Discussion section. A more comprehensive presentation of the project implementation of each of the participating schools is described in Manalo et al. (2019).

About the Project

The Philippines ranks high on climate change vulnerability. Its more than 2 million rice farmers rely on rice production as their main source of livelihood. The motivation in doing the project was to have some clear entry points for schools in the climate change adaptation discourse with emphasis on the agriculture sector. In this project, the aim was to mobilize schools as demonstration hubs of climate change-adaptive technologies and strategies in general. Mobilizing schools for this purpose is a strategic move considering that they are located even in the remotest communities in the Philippines. For anyone not familiar with the context in the Philippines, the country is the world's second largest archipelago next to Indonesia. Amongst the agricultural extension issues in the country is serving the farmers who are in the remote communities.

Mobilizing schools is also opportune given that certain secondary schools specialize in Agricultural Crops Production under the K-to-12 curriculum. In 2015, the Philippines also passed the Rural Farm Schools Act, which works well for this project in terms of collaboration with the Department of Education.

Under the Project, 12 public high schools from different parts of the country were invited to participate. These schools were among the best implementers of the previous Infomediary Campaign project of PhilRice (Manalo et al., 2019). It was a project that engaged young people in agriculture.

Implementation (During the Training)

Teachers and their school administrators (i.e., principals in other schools) and in some instances the Technical-Vocational Education heads were invited to attend a one-week training on Climate Change and Rice Production. The decision to involve school officials was based on the information that were gathered in the previous Infomediary Campaign project. One of the concerns in implementing the Campaign was that some school administrators were not fully supportive of the teachers. Among the reasons for not supporting was that the school administrators did not fully understand what the teacher was doing and that the whole project simply did not communicate to them. Hence, in the project on conceptualizing a climate change-adaptive school, the key school officials were involved and all activities expected of the participating teachers. This way, the officials know the amount of work that should be delivered under the project.

In the training, the teachers were introduced to the different climate change-adaptive technologies of PhilRice. An example is the "capillarigation", which is a water management technology for vegetables (one of the foci of the training was crop diversification). The participants also went on a study tour around the farm tourism sites near the PhilRice Central Experiment Station in Nueva Ecija. In these sites, the participants saw several innovations on water management and farm setups. In the same training, the school personnel were also informed about available funding for their agriculture-related projects by tapping the local Department of Agriculture (DA) office in their area. They were also taught about the possibility of attending training programs under its Agricultural Training Institute. The Institute offers a number of training programs such as an organic agriculture and other rice-based enterprises.

Post-training Activities

After the training, the teachers were each given PhP 50,000 ($ 1,000) as seed fund to implement various components of their climate change-adaptive school. A Facebook Group was set up for the teachers to disseminate the activities in their respective schools. The project team moderated the Facebook Group. Weekly regular posts were announced during project implementation. The team also facilitated some requests for endorsement to the local DA Offices made by participating teachers. There were instances when the team helped in doing concept proposals to access funding. The team did random visits to the project sites. During the visits, focus group discussions were conducted with the participating teachers as well as the school officials. Questions usually revolved around how the project was being carried out especially if there were some issues during implementation. Should there be concerns, the team helped in solving and/or realizing them. An instance was the request for availability of certified seeds in our site in Sarangani in Central Mindanao. Certified seeds are preferred because of their 10% yield advantage over the regular seeds that are being exchanged by farmers. The team purchased and shipped the seeds from Nueva Ecija to Sarangani.

The participating schools separately implemented the project, spicing it up with plenty of innovations. It should be highlighted that they integrated many technologies or strategies that they did not learn from the training. These technologies are captured in the book, "What is a climate change-adaptive school?" (Manalo et al., 2019). In applying their learning, participating schools modified the technologies or practices introduced during the training. Among these technologies is the carbonizer, a simple machine. The participating teacher and his students recycled trash such as empty paint cans and used fasteners, which greatly reduced the cost of producing the machine. Our participating school in La Union (Northern Philippines) managed to collaborate with a private company for their vegetable garden. They also partnered with the DA local office in setting up a seed production area in their school.

Results and Discussion

The three functions of an innovations broker, stipulated by Klerkx et al. (2009) as performed in the PhilRice case were noted. The first is demand articulation. By creating the need to think about how to conceptualize a climate change-adaptive school, PhilRice was articulating a demand. It raised the need to clarify entry points for schools in the climate change adaptation discourse with a focus on agriculture. PhilRice was able to get the schools in figuring out how to go about this necessity. In performing the demand function, the team bears similarity to the roles played by the International Potato Centre's researchers in Peru and Bolivia who acted as 'trigger factors'. They were the ones who dropped the idea that there might be a market for colourful native potatoes among farmers in their research sites (Devaux et al., 2009).

The second function was network composition. The project was quite strong in this. The team linked the schools to different innovations, and by extension the innovators; funding agencies (the schools were oriented that they could actually tap the resources of the local DA); and training hubs (the schools were informed that they could liaise with the local offices of the Agricultural Training Institute and farm tourism sites). These had enabled the schools to collaborate with other organizations in their areas. These linking activities performed by the Campaign team typified the proposition on linking complementary actors as well as leading in coming up with coordination mechanisms. During the process, many strategies were implemented, which the participating schools did not learn from the project training. They were able to pick up the key principles and aims of the project, and implemented it from there. Their collaborations were not confined to government institutions but also with private organizations. In performing this function, the PhilRice team might have shown the 'soft skills' needed to be a successful innovations broker. An example of soft skills is "to make use of contextual information and develop affective domains of learning that influence leadership and problem-solving skills" (Chowdhury et al., 2014).

The third function was innovation process management. This was realized in many ways. The first was through conflict prevention by inviting school administrators and other key officials to participate in the training. In doing this, the team established some level of mutual trust (Leeuwis and van den Ban, 2004) with its participating schools by showing that it is highly committed to make the project successful. At some point, this also relates to the project done by the International Livestock Research Institute where they addressed issues on power imbalances in a pastoral community in Kenya (Kristjanson et al., 2009).

The second was by setting up the closed Facebook (FB) Group for the participating schools to share their practices and at the same time emulate from each other. This act typifies fostering transparency in the innovation network, which refers to the "timely, accurate, open, and adequate communication among the people in the network… to foster commitment" (Batterink et al., 2010). Setting up of the FB Group established some working procedures (Leeuwis and van den Ban, 2004). In a way, this is similar with the stakeholder platforms by the IPC researchers in their work in Peru and Bolivia with the aim of ensuring continuity of interaction among stakeholders (Devaux et al., 2009). The FB Group also triggered new forms of innovations as the participants saw how their peers in other schools were implementing the project. The third was the provision of PhP 50,000 ($1,000) seed fund for each of the participating schools. The fund enabled them to pursue some innovations that were beyond the reach of the budget in their respective schools. Fourth, the overall coordination and facilitation roles played by PhilRice researchers reinforces the point made by Devaux et al. (2009) on the importance of competent external facilitation in doing innovations brokering.

In hindsight, it would be good to reflect on the factors that enabled the team to perform those functions. The first that comes to mind was the awareness of the situation in the participating schools. As mentioned in the Methodology section, the participating schools in the project were the best performing schools in the Infomediary Campaign project that was previously implemented. Hence, there was already prior knowledge of the issues in those schools as well as the development context in their respective areas. The knowledge derived from the situation have prompted the team to do those interventions (e.g., inviting the school principals to the training, not just the participating teachers). It pays to recognize that the team members of the campaign were mostly development communicators and agricultural extension workers. Hence, the importance of removing the barriers to effective communication (Klerkx and Gildemacher, 2012) as well as linking actors that may help the project to be successful was quite organic to the team. Lastly, it pays to recognize that PhilRice staff were not new to coordination, which is a daily routine work at the Development Communication Division at PhilRice. These three capabilities: doing a careful and thorough analysis of the situation, expertise in communication, and coordination skills contributed to performing innovations brokering functions by the team. These capabilities are among the list of capabilities listed in the network orchestration framework by Klerkx and Gildemacher (2012). Other important capabilities and/or enabling mechanisms cited by the same authors include "improving understanding of how to implement innovation brokering effectively as a tool for development" and "improving human capacity to play the role of innovation broker". (Klerkx and Gildemacher, 2012).

What is the significance of these findings? First, as implementers, the team did not know then that they were already doing innovations brokering. The project was set up in a way to conceptualize a climate change-adaptive school and how it can be realized. It was exploratory. Hence, the innovations brokerage functions listed above were performed subconsciously.

This project shows that innovations brokering is possible within the domain of a public agriculture research institution. The first way whereby this can be realized is on a project basis, which is similar with the case of the International Potato Center (Devaux et al., 2019) which brokering happened through a project. This is an important point to make as with the case of PhilRice — its key mandate is to serve as the lead organization in rice R&D in the Philippines. While it maintains a cohort of organizations comprising the national Rice R&D network, innovations brokering is not even implied within its key functions. As shown in this project, brokering may easily be realized or take place organically depending on how a project is executed. This realization corroborates the point made in the literature that some entities with brokering functions may just be performing them on the side of their traditional functions (Howells, 2006). A performed function may fit as a "collateral benefit".

In the literature, a point surfaces that among the issues in innovations brokering is the possible conflict in the identity of the broker. To explain, a broker pushing for one advocacy may find it difficult to be a broker of another entity that sponsors something that opposes the advocacy. This issue did not transpire in this project. PhilRice became a flexible broker among entities. In hindsight, what made this possible was the setup of the project - it was exploring possible strategies and/or technologies that can be showcased. Hence, technologies were offered as options. It also helped that PhilRice is a research institute, which promotes location-specific recommendations.

Another issue derived from this study was its limitations in the innovations brokering setup as a public research institution. The answer to this may be an open secret to anyone who has dealt with government and its bureaucracy. Brokering was made possible in this project because of its setup. Even so, there were instances during project implementation when more collaborations could have been brokered but did not materialize owing to the voluminous administrative requirements. While there were several attempts to forge collaborations, they simply could not be realized given the project's timeframe.

Conclusion

Drawing on the experiences of this project, the extent in which innovations brokering can be performed in a public research institution depends heavily on how it is central to the pursuit of a certain project. At PhilRice, brokering is subconsciously being practiced, perhaps instinctively and to some extent serendipitously, but not necessarily a mandate of the Institute. It might be interesting to know about the mode of institutional transformation it would take should a public research institution like PhilRice take innovations brokering as among its core functions. What laws could enable public research institutes to seamlessly perform innovations brokering?

Recommendation

There were some limitations in the analysis of this current research. First, it was limited to a single case study. Doing multi-case synthesis would have provided more insightful results. Second, the study was limited to a framework drawn from the case studies in Europe. On one hand, this is likely to be the case considering that there were dearth of studies conducted on innovations brokering involving public agriculture research institutes in developing countries. Hence, working on a framework that factors in the nuances of the Asian context or the developing county contexts in general, would also have yielded different results.

In relation to the more practical aspects of innovations brokering, it would do well to conduct workshops and interviews with experts and practitioners to brainstorm on the breadth and depth of innovations brokering that may transpire in public agriculture research institutes. The output of which is a database of insights as to how to fully operationalize this concept. There has to be careful identification and scrutiny of both the actors and the development context in which the brokering of innovations may take place.

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LONG DARK PERIOD TREATMENT ON RICE: IMPLICATION ON RAPID GENERATION ADVANCE IN VARIETY DEVELOPMENT

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Abstract

Efficiency in rice breeding is accomplished in various methods. One of these is induced flowering through artificial means, which adjust early flowering in the rapid generation advancement of lines in variety development. Current breeding efforts are well planned and efficiently conducted to reduce breeding cycle; thereby, saving time, land use, labor, and other related resources. Rapid Generation Advance (RGA) facility is a means to resolve problems on long cycles, expensive, and tedious field selection of rice lines. This study evaluated flowering induction of different maturing rice genotypes using long dark period and proposed for the improvement of RGA facility at Philippine Rice Research Institute (PhilRice). Results show that long dark period treatment (15 h) and short day period (9 h) hasten flowering and maturity of very early, early, medium, and late maturing rice varieties compared with those planted in the normal daylength field conditions. Maturity for PSB Rc 18 (medium maturing) was earlier by 14 days, 16 days for NSIC Rc 222 (early maturing), 27 days for PSB Rc 44 (late maturing), and 23 days for PSB Rc 10 (very early maturing) under the long dark period RGA treatment. Currently, PhilRice RGA facility, which can accommodate thousands of breeding lines has no provision for long dark period treatment; thus, it is recommended to install a black cover to achieve four filial generation in a year.

Keywords: *Dark Period, Flowering, Rapid Generation Advance, Rice, Variety Development.*

Introduction

Flowering induction is one of the most important physiological responses of rice to daylength. This can be explored by the breeders either for crossing activities or faster generation advance of the breeding lines (Manigbas and Lambio, 2015). Rice is a short-day plant but its flowering period can be delayed or prevented using long-day treatment (Liang and Liu, 1983; Shimamoto and Yokoi, 2005; Izawa, 2007). Flowering is induced in a controlled condition where daylength can be manipulated using the Rapid Generation Advance (RGA) technique. Manigbas and Lambio (2015) described the efficient use of RGA to reduce the breeding cycle in rice. The process was done by long dark period (15 h) or short day (9 h) period at the PhilRice old RGA facility. The RGA facility produced four filial generations in a year as compared with conventional field planting, which can only yield two filial generations. The RGA technique produces homozygous lines within a short period and enables the breeders to advance thousands of plants and number of populations in minimal space and time; thus, saving time, resources, and labor costs.

The RGA technique is not a new concept in advancing rice populations in a short time. Vergara et al. (1982) and Mackill et al. (1996) used this method in rice breeding using closely spaced plants and artificially short-day periods to minimize growth duration. Collard et al. (2017) revisited the evidence in favor of using RGA as a routine breeding method at the International Rice Research Institute (IRRI) to re-establish RGA on a large scale as the main breeding method for irrigated rice breeding. The method is typically similar to the breeding procedure of single seed descent (SSD) proposed by Goulden in 1939 and modified by Brim in 1960 (Manigbas et al., 2015). SSD is a method that rapidly fixes genes in a population and attains a genetically stable generation in a short period of time (Goulden, 1939; Brim, 1966). As the name indicates, it uses only one seed per population cross combination that is planted every generation (i.e., F_2 to F_6 generation) until full homozygosity is achieved.

Temperature and photoperiod are environmental conditions that affect flowering in rice. Temperature affects photoperiod-sensitive and non-photoperiod sensitive plants. High temperature accelerates while low temperature delays heading (Ahn and Vergara, 1969; Hosoi and Takahashi, 1973; Gomosta and Vergara, 1983). In most cases, progress was most rapid at 24 - 26° C, in which the optimum temperature was much cooler than expected from previously published values of times to panicle emergence in a less extensive range of photothermal regimes (Summerfield et al., 1992). They concluded that development rate is a linear function of temperature and photoperiod with no interaction, which is common to many species including rice.

A small room in PhilRice Central Experiment Station (CES) was converted into an RGA facility in 2006 to advance rice lines for high temperature tolerance breeding. F_2 plants were subjected to long dark period (15 h) and short day period (9 h) until F_6 generation (Manigbas and Lambio, 2015). Rice plant can easily respond to short day or long dark period. Under normal field conditions, average daylength is usually 11 h and flowering could not be enhanced. Thus, short day treatment of less than 10 - 8 h period can generate more favorable results. The duration of treatment was proposed based on the average critical daylength to induce flowering of 9 h short and 15 long dark period and on the logistics of field workers reporting for work at 7 am - 4 pm. The long dark period treatment was stopped when 50% of the plants in each population already flowered. Rice plants were harvested when a single seed is already matured, then planted immediately; thereby, reducing the time of the generation cycle. Other seeds are kept in the cold room for safekeeping. In the F_6 generation, all plants were harvested and planted in the field for selection and evaluation. The RGA facility can accommodate 7,920 plants per planting, which requires and area of 320 m^2 in the field but only 11.9 m^2 in the darkroom. This is a huge area difference between field and RGA facility; thus, saving time, labor, space, and resources.

In 2020, PhilRice CES built a large RGA facility similar to International Rice Research Institute (IRRI) but without the provision of a long dark period treatment. This is ten times or bigger than the 2006 converted dark room RGA facility at PhilRice. The new RGA can run generation advancement of hundreds of thousands of plants and populations in a single planting with an equivalent of a greater number of hectares when planted in the field. However, the facility could be further improved to allow long dark period exposure of the plants by installing a cover so that more generations can be advanced in a year.

To maximize the utilization of the new RGA facility of PhilRice, which has initially no provision for long dark period treatment, this study evaluated the flowering induction of different maturing rice genotypes using long dark period and proposed for the improvement of the new RGA facility.

Materials and Methods

The study was conducted at PhilRice CES, Nueva Ecija in 2021 dry season. Four released varieties (Table 1) with different maturity were planted in push carts (Figure 1) for RGA or long dark period and in the field for normal daylength period treatments. The experiment was laid out in Completely Randomized Design (RCBD) in the field with three replications but with no replication in RGA due to small space and limited carts. However, each cart has similar amounts of seeds with the field setup to satisfy the optimum number of plants in both treatments. Direct wet-seeding was applied to both treatments.

The varieties were directly seeded in February 6, 2021 both in RGA push carts and field condition.

Table 1. PSB and NSIC approved varieties used in the experiment and their major characteristics.

Variety	Maturity (days after sowing, DAS)	Classification	Ecosystem	Ave. Yield (t ha $^{-1}$)
PSB Rc 10	106	Very early	Irrigated lowland	4.8
PSB Rc 18	123	Intermediate	Irrigated lowland	5.1
PSB Rc 44	144	Late	Cool elevated	4.1
NSIC Rc 222	114	Early	Irrigated lowland	5.7

Figure 1. Transportable push carts for RGA facility with a 1.41 m x 0.74 m x 0.23 m dimension.

Two hundred grams of pre-germinated seeds (~8,000 plants) of each variety were sown in the field and push cart. Push carts' floor were lined up with plastic acetate to prevent water seepage. On top of the plastic, a 2-in soil layer was overlaid and cups filled with soil were embedded. There were 265 (size #5) cups placed in each push cart. Six pre-germinated seeds were sown and covered with a thin layer of dry pulverized soil in each cup. Germinated seedlings were watered when it reached 1-in tall.

In the field, the experiment was laid out in RCBD with three replications. A 200 g pre-germinated seeds (~8,000 plants) were direct-seeded in a 1 m x 1 m plot. Each plot was divided into 19 furrows with 1 in distance from each other. Furrows were 1-in wide and half inch deep. Approximately 10 g of seeds were sown for each furrow which was covered by a thin layer of dry pulverized soil. Plots were flushed with water after the seedlings reached 1- in tall.

Water was maintained during the growth period and fertilizers were applied when needed to maintain greenness of the plants in RGA push carts and in the field.

Darkroom Treatment

Each push cart can accommodate 200 g of pre-germinated seeds. Thirty days after sowing, push carts were placed inside the darkroom for long dark period treatment (Figure 2). The treatment started at 16:00 h and ended at 7:00 h to provide 15 h dark period and 9 h light period. This was done until the plants had 50% flowered and further maintained outside of the darkroom until maturity.

Field Treatment

Pre-germinated seeds were directly seeded in the field and maintained without dark period treatment. Plants were exposed under normal daylength period and were observed until physiological maturity.

Data Gathering

After field and RGA establishment, the following data were gathered: (1) days to 10%, 50%, and 100% flowering, (2) plant height, (3) occurrence of pests and diseases, and (4) maturity. The automatic weather station (AWS) in the field data was used to determine the daylength.

Results and Discussion

Rice plants were observed and important data were gathered in the field and RGA facility at PhilRice. The RGA facility is an old small room converted into a darkroom where long dark and short-day period can be manipulated to induce flowering of rice plants. Rice is a short-day plant and its development towards flowering is rapid in shorter days (i.e., long dark periods) than in longer days (i.e., short dark periods) (Summerfield et al., 1992).

The four varieties used in this study with different maturity days (Table 1) showed different responses in terms of 10, 50, and 100% days to flowering under RGA and field conditions (Figure 3). All of these varieties, regardless of published maturity data (PhilRice Binhing Palay app on google), flowered earlier by 7 to 17 days under long dark period treatment (15 h dark and 9 h light) than the natural field conditions, which

Figure 2. A small room converted into RGA facility at PhilRice. Push carts are manually placed inside the room at 16:00 h and then brought outside at 7:00 h to complete the 15 h dark period and 9 h daylength.

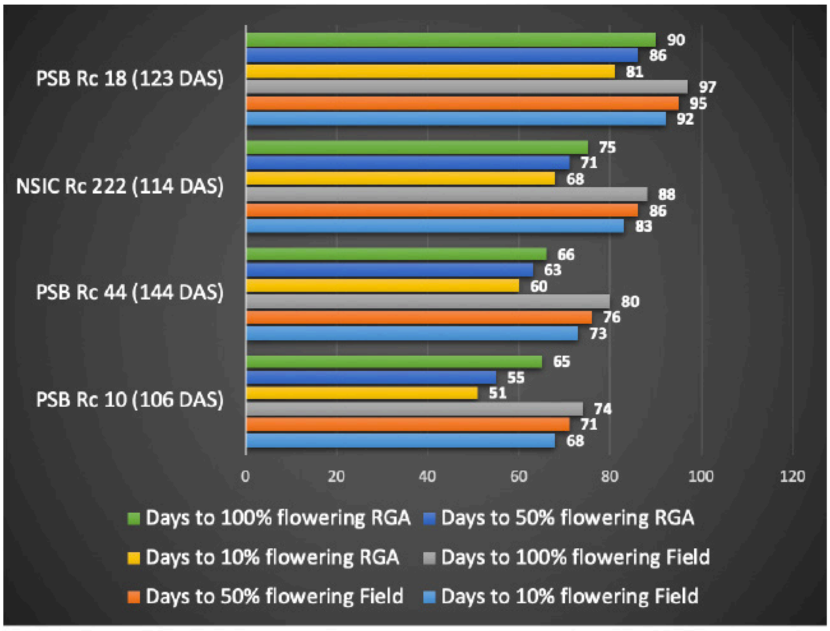

Figure 3. Different days to flowering (i.e., 10, 50, and 100%) of the four varieties tested under RGA facility and normal field condition.

had an average of 11 h daylength. All of the varieties were more responsive to long dark period at 10 and 50% days to flowering because they flowered 9 to 17 days ahead than in the normal field conditions. At 100% days to flowering, their difference ranges from 7 to 14 days. The RGA long dark period treatment enhanced flowering by two-week difference than when planted under normal field daylength condition.

The four varieties varied in terms of maturity when treated under long dark period and in field natural conditions. Maturity for PSB Rc 18 (medium maturing) was earlier by 14 days, 16 days for NSIC Rc 222 (early maturing), 27 days for PSB Rc 44 (late maturing), and 23 days for PSB Rc 10 (very early maturing) under the long dark period RGA treatment (Figure 4). Their days to harvest under field conditions were similar to the published maturity in the PhilRice Binhing Palay App except for PSB Rc 44 (late maturing, 144 days), which was 108 days. PSB Rc 44, known as Gohang, was released for national recommendation in 1995 for cool elevated condition. The background parent of this variety is a *japonica* from RPKN2 suited for cooler environment. This characteristic may had enabled this variety to change its flowering and maturity when grown in warmer conditions. Similar to the findings of Summerfield et al., (1992), there are considerable differences among genotypes in relative sensitivity rate of progress towards panicle emergence to temperature and photoperiod. The *japonica* cultivars tended to be more sensitive to temperature and less sensitive to photoperiod than *indica* cultivars. The warm non-elevated growing conditions at PhilRice CES may have caused the early flowering and maturity of this variety. In any case of the varieties tested, the long dark period treatment in RGA has consistently shorten the maturity duration compared with the normal field conditions.

Varieties had similar plant height at harvest for both long day period treatment in RGA and normal field condition (Figure 5). Plants under darkroom treatment were slightly taller due to the absence of light during late afternoon and early morning. There was no effect on the method of seeding and plant population because they were direct seeded with similar seeding rate (Figure 6a, 6b).

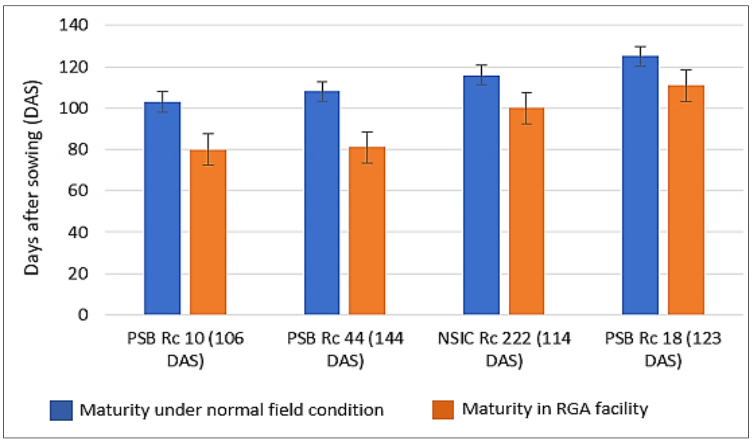

Figure 4. Actual maturity period (i.e., days after sowing) of the four varieties tested under normal field condition and in RGA facility.

Implication on Variety Development

One of the objectives of variety development and release is to increase efficiency in selection and shorten the generation advance or breeding cycle of the materials. The development of rice varieties normally takes 10 years and another two years for seed production and use of farmers. In the advent of new technologies and facilities, variety development can now be shortened by 2 - 3 years. The new RGA facility of PhilRice, which can accommodate thousands of lines in one facility like the glasshouse (Figure 7) is an alternative way to hasten the generation advance of breeding lines.

The current PhilRice facility lacks coverage for dark period treatment of various breeding materials that will undergo RGA. This study shows the importance of building an RGA facility larger than its current infrastructure and with a provision of an additional requirement for dark periods. Results showed that long dark period treatment in RGA can enhance flowering of rice plants with various maturity earlier than the normal field daylength conditions. Generation of breeding materials with long dark treatment in RGA was already produced in two weeks. By using long dark period, all plants will mature at the same time, resulting in a more efficient operations in the glasshouse especially

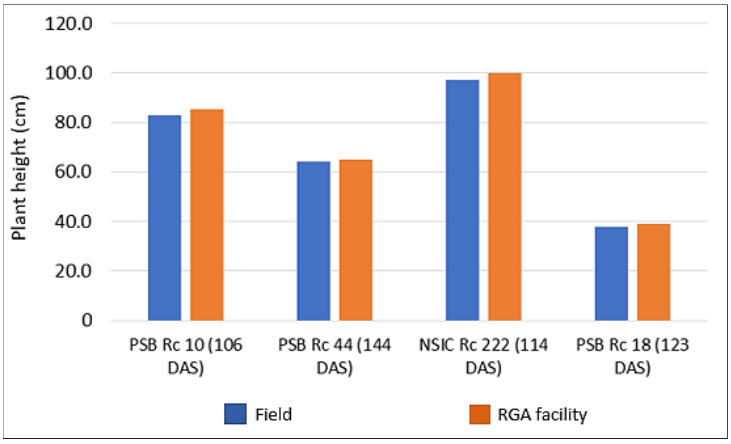

Figure 5. Plant height at maturity of the four varieties tested under normal field condition and in RGA facility.

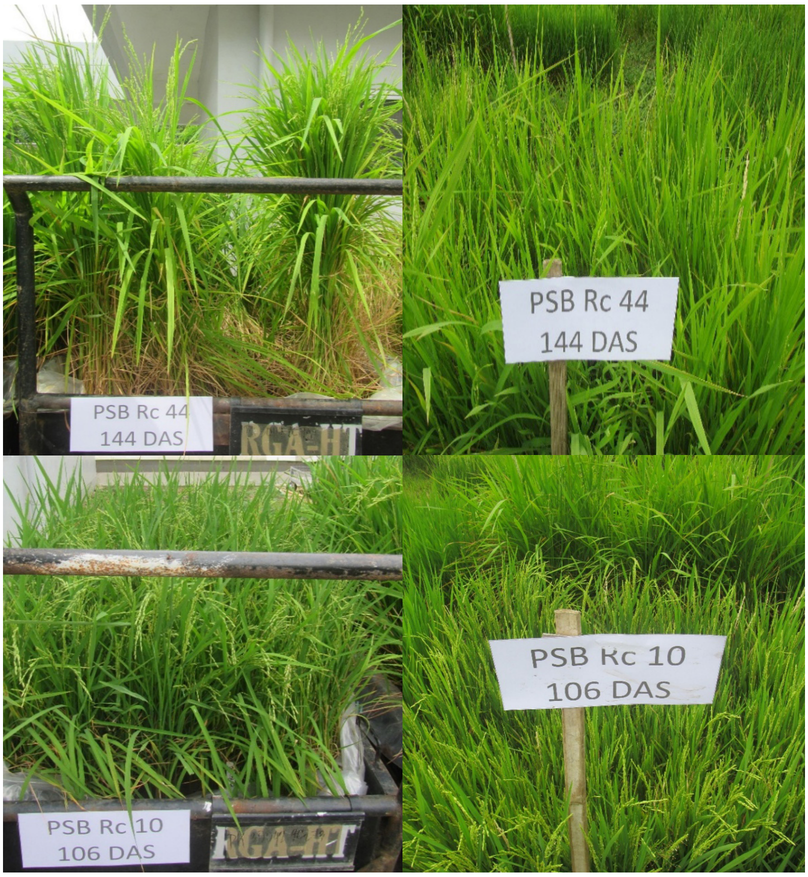

Figure 6a. Flowering of the rice varieties planted in the RGA push carts and in the field 74 days after sowing.

during planting and harvesting. In this scheme, four generations could be achieved in a year compared with only two generations produced without long dark period treatment. Manigbas and Lambio (2015) have shown that four generations a year is possible under long dark period treatment.

In rice breeding, it takes F_6 generations to produce homozygous uniform lines. Under normal field conditions, this takes three years needing a larger area for cultivation and more field workers. On the other hand, producing F_6 uniform lines through the RGA facility with long dark period treatment only takes one and a half year; saving time, space, and logistics. Best lines can also be selected on the second year and yield trials thereafter, completing only until 4 - 5 years. Without the RGA facility and provisions of long dark period, it takes 8 - 9 years.

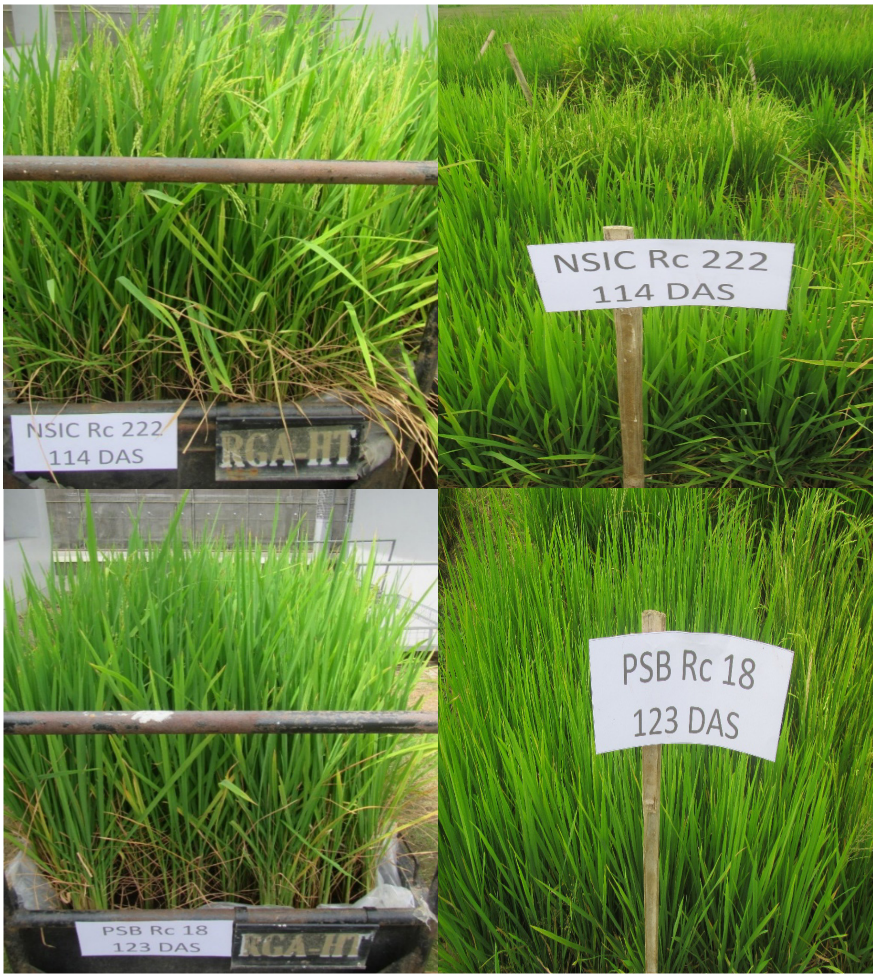

Figure 6b. Flowering of the rice varieties planted in the RGA push carts and in the field 74 days after sowing.

Figure 7. The new PhilRice RGA facility.

Conclusion

The four varieties with known maturity varied in their flowering and maturity when subjected to long dark period treatment in RGA and normal field conditions. The long dark period treatment in RGA had 14 - 23 days earlier advantage in maturity than in untreated normal daylength under field conditions. With this method, 3 - 4 filial generation can be produced in a year using long dark period treatment in the new RGA facility. This saves time, labor, and resources in breeding line development and incorporation of desirable traits for new varieties.

Recommendation

This study shows that flowering in rice can be enhanced faster through long dark treatment in RGA than under normal field conditions. It is recommended that the new RGA facility with no provision of dark period treatment at PhilRice should be provided with black cloth or material that could cover the facility at desired number of hours for dark periods. This could be enhanced by either manual or automatic operations inside the RGA facility. This way, the generation advance of thousands of breeding lines will be more efficient as it saves time and labor and speeds up breeding cycle in a year from the usual two generations to the study-derived four generations.

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AUTOMATED SIZE AND SHAPE MEASUREMENT FOR BROWN AND MILLED RICE USING DIGITAL IMAGE PROCESSING

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Abstract

Rice kernel size measurement is an important determinant of rice yield and consumer acceptability. Although manual measurements are accurate and repeatable, they are laborious, eye-straining, and with low throughput rate. This study aimed to develop a validated method for rice kernel size measurement consisting of digital image acquisition and image processing algorithm. Purposively, varying sizes of 1,000 kernels from 34 brown and milled rice samples were measured using an analog caliper to achieve a uniform distribution for length and width. Images were captured using a flatbed scanner (800 DPI [dots per inch]) and a singulation template. Python OpenCV program was used to fit minimum enclosing rectangle (MER) or ellipse to define the digital measurement of length and width then linear regression models were fitted. Based on MER, the model was consistent and had high correlation values (Rc = 0.973 - 0.998), acceptable standard error of prediction (SEP) of 0.080-0.093 mm, and consistent correlation coefficient for prediction (Rp = 0.961 - 0.998). Validation using 56 kernels showed that SE (0.08 mm) and accuracy based on coefficient of determination (r^2 = 0.95 - 0.99) were consistent with the model development results. The manual and automated measurements took 44 - 48 sec/kernel and 12 sec/kernel, respectively. The developed system can replace the manual measurement with high speed and accuracy, which could fast track the physical evaluation of numerous rice entries.

Keywords: *Kernel Size, Machine Vision, Python OpenCV, Regression Model, Standard Error.*

Introduction

Rice is an important cereal staple food that provides 21% of the world food requirement and 76% of the calorific intake of the people in Southeast Asia. The total production must be increased by at least 40% (Fitzgerald et al., 2009) to meet the world demand for rice by 2030. Increasing rice yields had been the focus of many researches through breeding and crop production management. Preferably, new rice varieties must have high yield potential, consumer accepted shape, improved nutritional profile, good resistance to pest and diseases and tolerance to both abiotic and biotic stresses (Huang et al., 2013). There are three main components that dictate grain yield of rice; the number of panicles per plant, the number of grains spikelets per panicle, and the average weight of each grain. The grain weight, often expressed as 1,000 - grain weight, is affected by the grain's length, width and thickness, or more descriptively, the grain size. The shape of the kernel is often described by the ratio of length-to-width, which strongly affects the preferences of consumer groups. Most Southeast Asian people prefer the long and slender grains while the Korean and Japanese prefer the short and round types.

As the yield and quality of rice grains are strongly associated to the grain size and shape, these parameters are integral part of a grain physicochemical quality determination. The manual procedures involve measurement of individual randomly selected whole milled rice kernel length and width of two sets containing 10 kernels each. The computation for the average length and the ratio of average length to average width defines the shape classification. This has been incorporated into a routine procedure of the National Cooperative Testing (NCT) of promising selections prior to their recommendation for release as new rice varieties (NCT, 1997). The NCT classified milled rice either short (Sh), medium (M), long (L) or extra long (EL) based on length (≤ 5.4 mm, 5.5 - 6.5 mm, 6.6 - 7.4 mm and ≥ 7.5 mm, respectively); and slender (S), intermediate (I), and bold (B) based on length to width ratio (≥ 3.0, 2.1 - 3.0, and ≤ 2.0, respectively). To accomplish the measurement of length and width, an analog caliper is often used to attain a good precision. However, this requires dexterity in positioning the kernels on the caliper. With its negative impact on the eyesight of the technician, frequent rests have to be enforced especially when a large number of samples are queued for size determination.

Filipino consumers generally prefer long and slender rice kernels. Therefore, the standards for rice variety call for milled rice kernel lengths of 6.6 - 7.4 mm with length-to-width ratio above 3.0. As the bran is still retained in the brown rice, gradual demand for this type of rice is created primarily for health reasons and as a step towards rice security. While the grain size standards were defined only for milled rice forms, the grain size characteristics should be more stable in the brown rice form than the milled rice because no further physical transformation during the whitening operation occurs in the grains. Thus, measuring sizes on the brown rice is more reflective of the effects of breeding and crop management on the yield outcome of rice.

Various studies were conducted to automate the measurement procedures based on image capture of the grains and digital image processing. Flatbed scanners and digital consumer cameras had been effective enough to capture image of randomly presented grains on a glass platten. To make the size measurement, early algorithms rely on fitting ellipses in the isolated kernel image with the lengths of major axis to represent the grain length and on the minor axis for the width. Although this approach is quite simple and robust, significant errors could be committed; thus, the need for much better algorithms. One novel way is to extract some features of the kernels (ellipse axis lengths, bounding box size, area, perimeter, and several length measurements) and use artificial neural networks as the regressing model to better compute for length and width.

At the Philippine Rice Research Institute (PhilRice), a system called Milled Grain Classifier was developed that can process 6.2 g of sample in less than five minutes rather than the conventional approach of 30 g in as long as 96 min (Sarol, 2016). The system, however, requires a tedious placement of grains in straight rows and columns, which was quite limiting especially when hundreds of samples were to be processed. The needed alternative system entails the operator to simply dump a sample in, the images are quickly scanned, and kernels are automatically measured. There are some instruments being promoted to make measurements such as the SeedCount (SC600R, Next Instruments, New South Wales, Australia as cited by Armstrong et al., 2005) or the Grain Scanner RSQI 10A (Satake Corporation, 2016). However, these tools could be expensive because they are imported and unavailable to the local suppliers. Developing local version of the abovementioned instruments using locally available devices is necessary to make the system ready and accessible.

This research aimed to develop a grain size and shape measurement system for randomly oriented grain kernels of brown and milled rice samples based on a commercial flatbed scanner and open source software OpenCV in Python. Specifically, it aimed to develop a digital image acquisition and a digital image processing algorithm for grain size and shape determination.

Materials and Methods

Preparation and Size Measurement of Samples

To obtain rice kernels of widely varying sizes, 300 g rough rice from 34 rice samples were weighed and de-husked using a Satake laboratory rubber roll huller to obtain brown rice samples, and consequently milled through a McGill No. 3 whitener to produce the milled rice samples. Whole brown rice and milled rice of more than 1,000 kernels each were purposefully selected to achieve a uniform distribution of kernel length and width. Each kernel was placed in separate brown coin envelopes and sequentially labeled to track their identity throughout the experiment. Using an analog dial caliper, the length and width of each kernel were measured in millimeter (mm) with a precision of one-hundredth of an mm by two adequately trained technicians.

Imaging of Kernel Samples

A flatbed scanner (ScanJet Model G3110, Hewlett Packard, Palo Alto, California, USA) was used to scan color images of the rice kernels. A black rubber mat template having 20 cells (5 rows x 4 columns) was used to ensure singulation of the kernels with one kernel placed in each cell. Another black rubber mat with no holes was placed on top to serve as the background of the template to greatly facilitate segmentation of the kernels during image processing. All the image enhancements of the scanning software were turned off (e.g., contrast and hue adjustments, sharpening and thresholding). An 800 DPI scanning resolution was selected for all the images that were saved in uncompressed bitmap file format.

Digital Measurement of Kernel Sizes

A Python version 2.7.15 program was written using the OpenCV revision 4557 and Numpy version 1.15.1 scientific libraries. The general flow of the program is shown in Figure 1. Each image file was loaded and converted into grayscale using the cvtColor function and then converted into a binary image using the threshold function at gray level value of 100 for milled rice and 80 for brown rice. Each cell containing a kernel was then cropped out from the binary image, and contours of blobs (binary large objects) were detected using the findContours function. The largest blob area (using cv2. contourArea() function) was determined, and then minimum rotated enclosing rectangle (MER, using function cv2.minAreaRect()) and an ellipse (using cv2.fitEllipse() function) were fitted onto the largest blob. The dimensions of the rectangle and the lengths of the axes (major and minor) were determined and linearly converted to mm by multiplying them with 25.4 mm/in and divided by the 800 DPI (dots per inch) scan resolution. The tabulated data were then saved to an output text file for later statistical processing.

Regression Analysis

For each grain type, the manually measured kernel length values were regressed against the rectangle length and ellipse major axis length using the statistical functions of the spreadsheet program. Similarly, the manually measured kernel width values were regressed against the rectangle width and ellipse minor axis measurements. Because of the robustness of the regression statistical method, some manual measurement errors were observed whenever there were large differences between the measured and regressed or predicted values. The technicians were directed to investigate the error cases, and often repeat the measurements of the dimension in question.

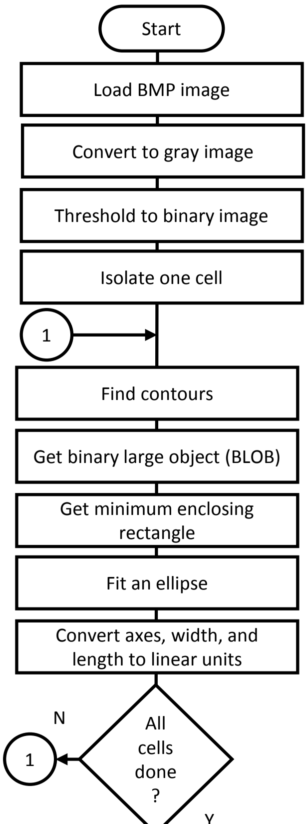

Figure 1. Flowchart of image processing for kernel size measurement.

The manually measured kernel lengths were sorted in ascending order and were labeled 1, 2, and 3 repeatedly to properly assess the model performance. Kernels labeled 1 and 2 were assigned to the calibration set while labeled 3 were assigned to the prediction set. A regression model was fitted using the calibration set and used for model-estimated measurements. In this step, the standard error of calibration (SEC) and linear correlation coefficient for calibration (Rc) were computed. Using the same calibration model, the digitally measured lengths of the kernels in the prediction set were adjusted and regressed against the manual measurements. The standard error of prediction (SEP) and linear correlation coefficient for prediction (Rp) were computed from the prediction set. The same procedure was used in assessing the model performance for estimating the width of the kernels.

Size Distribution of Kernels

Three samples (i.e., short, medium, and long) for brown rice and its corresponding milled rice were selected. For each entry, about $N > 700$ whole kernels were obtained and placed on the scanner while keeping them fully separated from each other. The kernels were then scanned at the same 800 DPI setting and saved in bitmap format. Another program was written to estimate the length and width of the kernels using the model developed in the previous step.

Validation of Models

To evaluate the soundness of the algorithm and the regression models for MER, a set of 56 kernels each of milled and brown rice were validated. These were manually and digitally measured from rice samples that were not part of the original model development set. A new flatbed scanner was used to capture images of the manually singulated kernels. Using the same Python program, the length and width of the kernels were computed from the scanned images. Procedures for regression analysis were also applied to compare the manual measurements and the digital measurements. The time it took to manually measure the kernels, length of program execution, and setup duration were also included.

Results and Discussion

Model Development

Fitting an ellipse or a rotated rectangle are two model approaches to digitally measure the size of rice grain kernels from images. The efficacy of these approaches depends on accurately defining the outline of a grain kernel, which can be sensitively affected by (1) the selected resolution when the image was scanned, (2) shape of the grain kernel, and (3) threshold level setting in the image processing program. The scanner hardware assumes that the target objects are placed fully flat on the scanning glass. The edges tended to be blurry or out of focus when the kernels were scanned because the cross-section of the kernel is naturally not rectangular (i.e., elliptical). The threshold level must be selected so that most part of the edges are captured without significantly introducing artifacts in other parts of the images. Figure 2 shows an image of a kernel of milled rice, its binary (threshold) image and the fitted MER and ellipse to estimate the length and width of the kernel. From the outline contour of the image, an enclosing rectangle was algorithmically rotated to find a minimum areal coverage to enclose the whole kernel. The fitted ellipse had its centroid coincident with the centroid of the binary image of the kernel, and its outline passed through the most parts of the contour. In most cases, the MER efficiently marked the outermost stretches of the length with its perpendicular width. However, the ellipse tended to over-fit or under-fit the kernel contour. A plausible advantage of using an ellipse as the geometric model over the rotated rectangle is its lesser susceptibility to the presence of protrusions on the kernels. These protrusions happen especially with brown rice kernels, in which a part of the silver lining testa may have been torn out and clearly appear in the scanned images. Figure 3 illustrates this scenario where the MER tried to ensure that the protrusion is covered. The ellipse gracefully fitted well with the contour of the kernel image.

Testing of Models

Table 1 shows the statistical profile of manually measured length and width of the brown and milled rice kernels for the calibration and prediction or validation sets. To achieve robust comparison of digital and manual measurements, a good span of kernel sizes must be included in the analysis. By selecting the kernels, a uniform distribution of sample can be confidently achieved so that all possible sizes are equally represented. Using a randomized kernel selection approach, a normal distribution of measurements is achieved, which tended to cluster around the mean and under-represent the outer fringes of the range of sizes. This makes the model development less efficacious. The calibration set had almost similar statistical profile as the prediction or validation set to satisfy the requirement that samples should be drawn from similar distribution. Additionally, a large number of kernels would ensure valid model development.

Figures 4 - 7 show the scatter plots for measuring length and width of brown and milled rice kernels using MER and ellipse fitted geometric models of both calibration and prediction data sets. In all cases, kernel length was better measured using the MER than the ellipse. High correlation values (Rc = 0.973 - 0.998) were obtained for calibration models. The standard error of prediction (SEP) was less than 0.1 mm (0.080 - 0.093 mm), which was acceptable with the grain length classification falling in the 0.1 mm resolution. Meanwhile, for the measurement of kernel width, MER and ellipse had almost similar model performance. The apparent wide spread of data points for width about the regression line seemed to emphasize the difficulty of measuring the dimension both for the manual and digital measurement. The spherical cross section of the grains disenabled stable positioning of the kernels when using the analog dial device. Similarly, when kernels were placed on top of

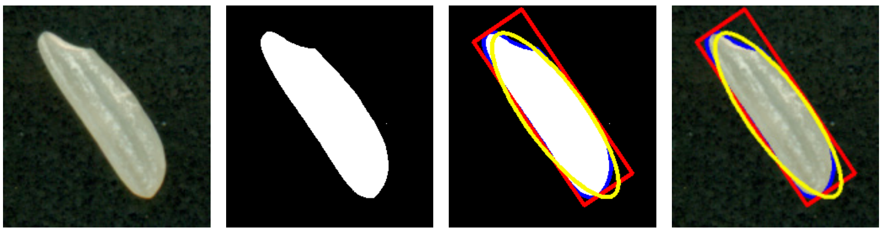

Figure 2. Image processing steps for a kernel of milled rice. (a) original image, (b) binary image, (c) contour (blue), minimum enclosing rectangle (red) and ellipse (yellow) fitted to the kernel of binary image, and (d) contour, minimum enclosing rectangle and ellipse fitted to the kernel of original image.

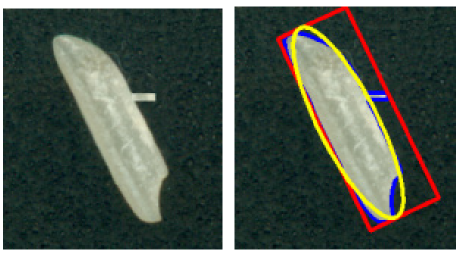

Figure 3. Image indicating if a kernel had a protruding part where the minimum enclosing rectangle tended to extend well to the end of protrusion while the ellipse stayed within the bounds of the kernel.

Table 1. Statistical profile of grain kernel length and width of samples for calibration and prediction sets.

	Brown Rice		Milled Rice	
	Length, mm	Width, mm	Length, mm	Width, mm
	Calibration			
Mean	6.63	2.39	6.33	2.27
SD	1.24	0.40	1.10	0.33
CV, %	18.6	16.8	17.4	14.4
Minimum	4.56	1.78	4.52	1.62
Maximum	9.40	3.32	9.32	3.02
N	740	740	731	731
	Prediction			
Mean	6.64	2.39	6.33	2.28
SD	1.24	0.40	1.10	0.33
CV, %	18.7	16.9	17.4	14.5
Minimum	4.66	1.82	4.53	1.63
Maximum	9.45	3.34	9.08	3.14
N	370	370	365	365

SD - Standard deviation; CV - Coefficient of variation, N - Number of kernels

the scanner glass, some may have inevitably shifted a bit, resulting in the axis for width not having perfectly leveled (parallel) with the glass panel, as explained earlier. However, during the model validation step when the apparent digital measurements for both length and width were adjusted using the calibration models, the adjusted values fitted well with the manual measurements (Rp = 0.961 - 0.998).

A large number of kernels had to be accurately measured and a frequency distribution had to be drawn from these measurements so that the grain size profile of a sample can be described. More than 700 kernels were randomly obtained from three entries of brown rice and from their corresponding milled rice for a total of six sample sets. Manual measurement of lengths and widths are tedious and slow. With the system for digital measurements developed, the kernels were randomly placed on the flatbed scanner while keeping each kernel separated from one another. Figure 8 shows an example of the scanned kernels and the fitting of MER and ellipse to digitally estimate the sizes. Each kernel was labeled by a number so that their lengths and widths can be traced from the output tabulation. The frequency distribution profiles of the three selected entries are shown in Figure 9. The rice sample with longer kernel length (ST-27) seemed to have a wider variability than the shorter entry (SP-21) because of higher apparent variability of grain size gene expression for long grains. There could be no observable pattern for width variability in the selected entries. This clearly illustrates that the tool provides deeper insights for breeders in targeting grain size traits and their phenotypic expressions rather than looking alone at the central value estimates of grain size as usually practiced.

The study successfully measured the length and width as determinants of kernel sizes. The determination of shape as a descriptor of the kernels' geometry can now be easily derived from the length and width measurements. The dimensionless length-to-width (L/W) ratio can be computed from the mean values of length and width for a sample entry and classify the sample into any of the three shape classes. For the three selected samples, the size profiles of kernels are shown in Table 2. As the shape classification was established for milled rice, similar approach can be used for brown rice.

Validation of Models

The models were validated using a new set of kernels of wide range of sizes. Table 3 shows the statistical profile of the randomly selected 56 kernels from seven rice samples. The validation of computer vision models using the MER showed consistent and highly acceptable performance statistics as can be seen in Figures 10 - 13.

The time needed for the measurement of the validation kernels were recorded and the summary is presented in Table 4. The dimensions of a kernel, on the average, took 44 - 48 sec, which accounted for setup and rest times. On the other hand, the automated measurement using the Python program took only 240 sec on the average for 20 kernels or an individual time of 12 sec per kernel including setup time. Thus, with the computer vision system, a very high throughput rate can be achieved. This process does not lead anymore to drudgery nor create undue strain to the operator.

Table 2. Statistical profile of kernel sizes of three selected rice samples.

	Brown Rice			Milled Rice		
	CT-09	SP-21	ST-27	CT-09	SP-21	ST-27
			Length, mm			
Maximum	7.6	6.1	9.3	9.7	5.9	9.3
Minimum	5.8	4.7	4.4	5.2	4.4	6.3
Mean		6.6	5.3	7.8	6.2	4.9
SD	0.2	0.2	0.8	0.3	0.2	0.5
CV%	3.6	3.3	10.5	5.1	3.5	6.9
			Width, mm			
Maximum	2.6	3.4	3	2.5	3.6	2.8
Minimum	1.8	2.4	1.8	1.6	2.3	1.7
Mean	2.1	2.8	2.3	2	2.8	2.2
SD	0.1	0.1	0.2	0.1	0.1	0.1
CV%	5.4	4.7	7.5	5.7	5.2	6.8
N	1200	1297	950	999	988	780
L/W Ratio	3.1	1.9	3.4	3.1	1.8	3.5
Shape	Slen-der	Bold	Slen-der	Slen-der	Bold	Slen-der

SD: Standard Deviation, CV%: Coefficient of Variation
N: No. of Samples, L/W: Length-to-width ratio

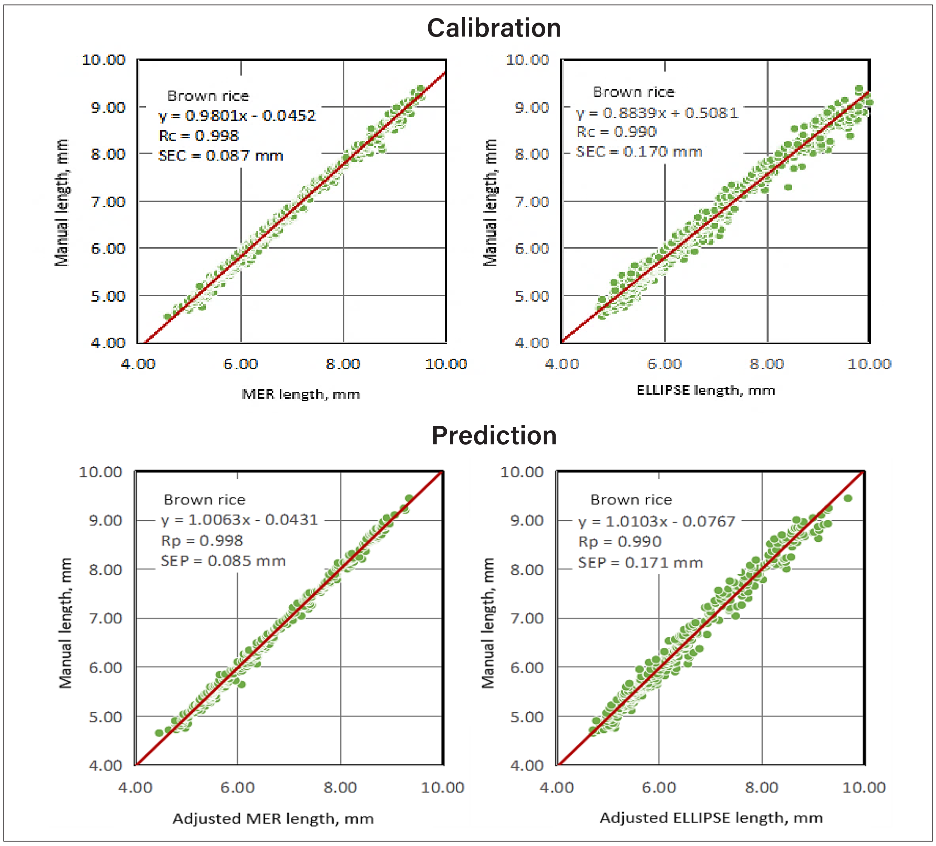

Figure 4. Scatter plots of calibration and prediction sets for length of brown rice kernels using fitted MER and ellipse.

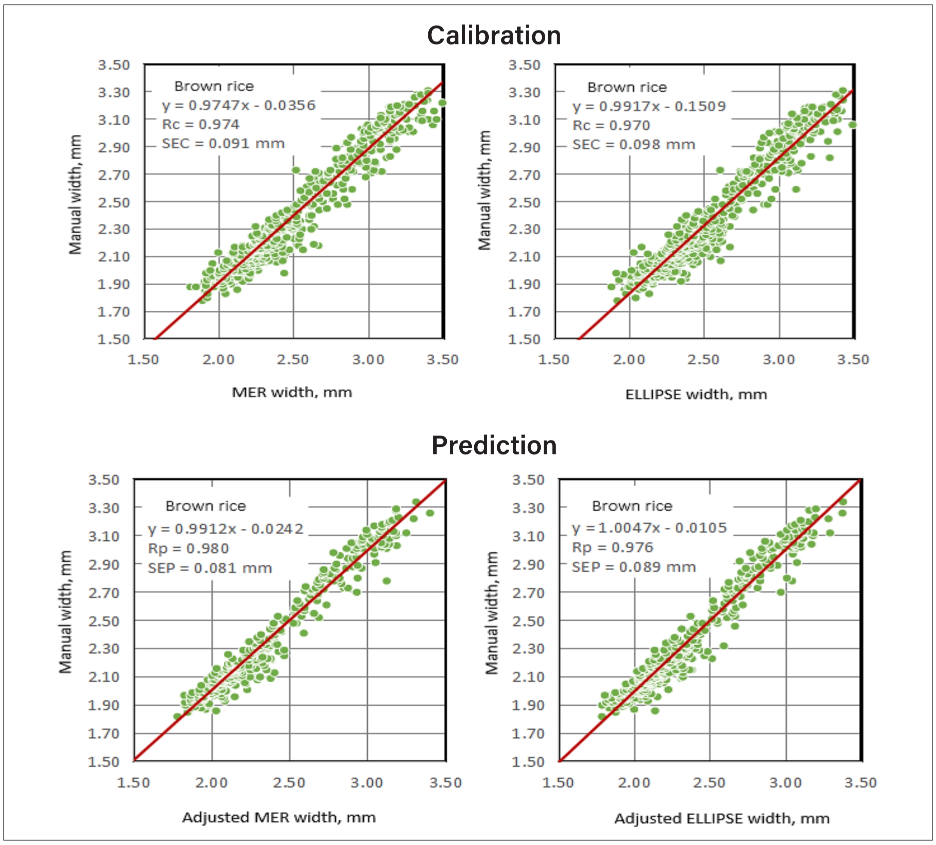

Figure 5. Scatter plots of calibration and prediction sets for width of brown rice kernels using fitted MER and ellipse.

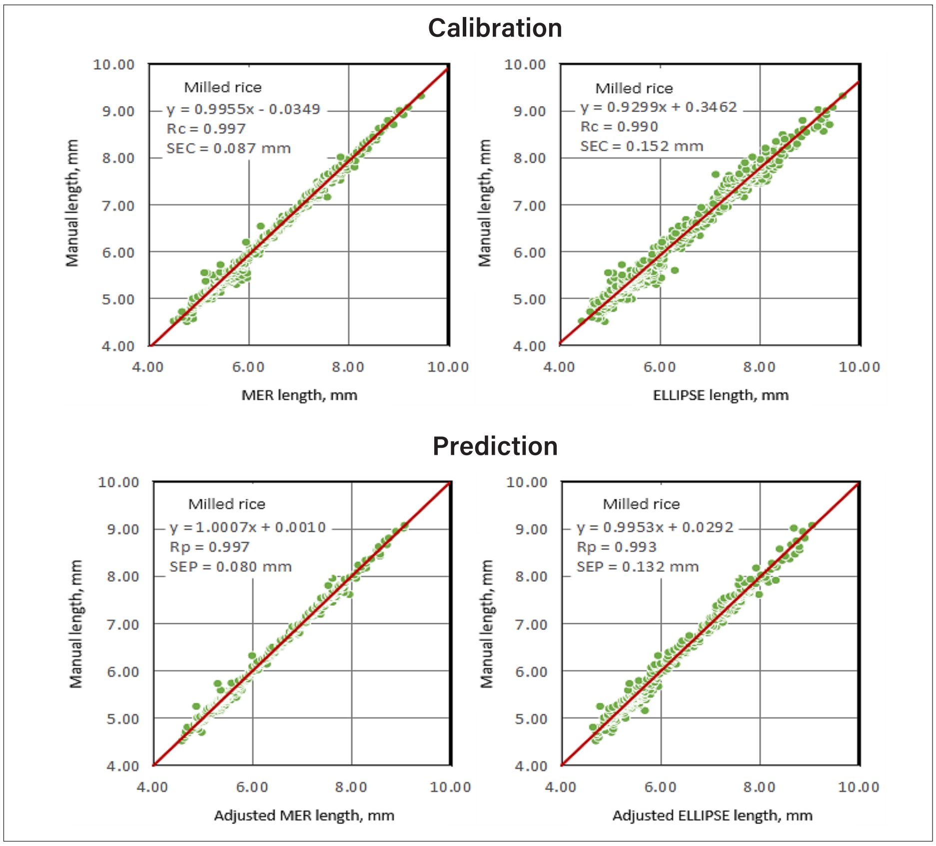

Figure 6. Scatter plots of calibration and prediction sets for length of milled rice kernels using fitted MER and ellipse.

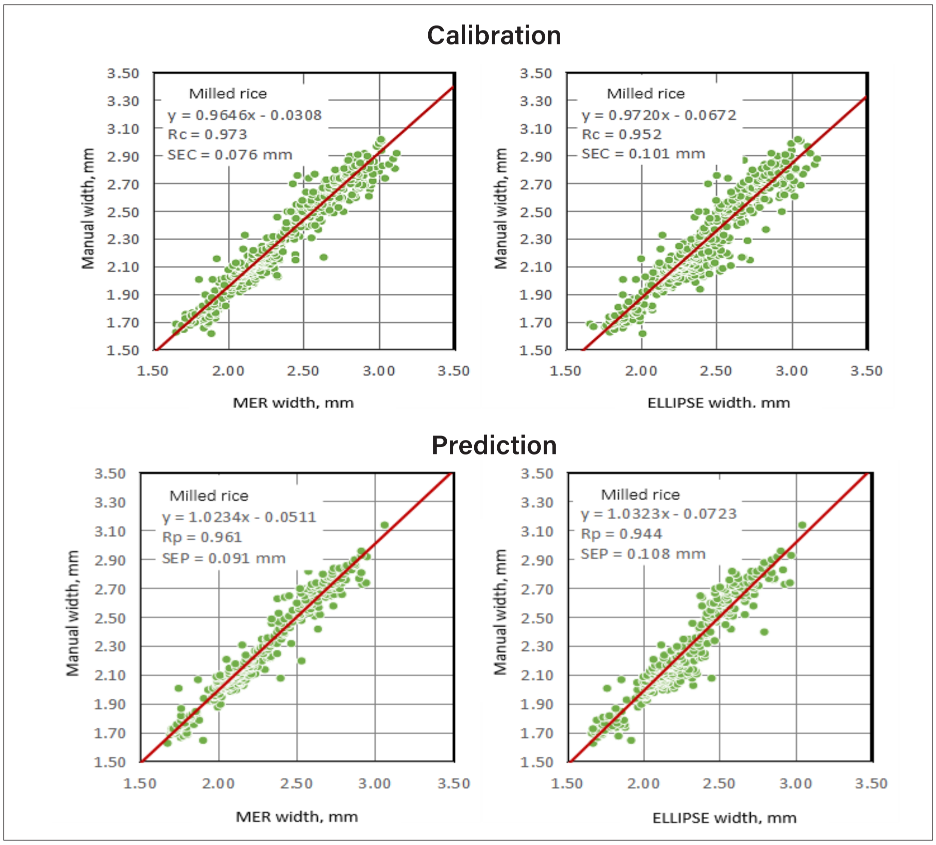

Figure 7. Scatter plots of calibration and prediction sets for width of milled rice kernels using fitted MER and ellipse.

Figure 8. The length and width of randomly oriented kernels that can be automatically determined by the program, in which each kernel was labeled with a number.

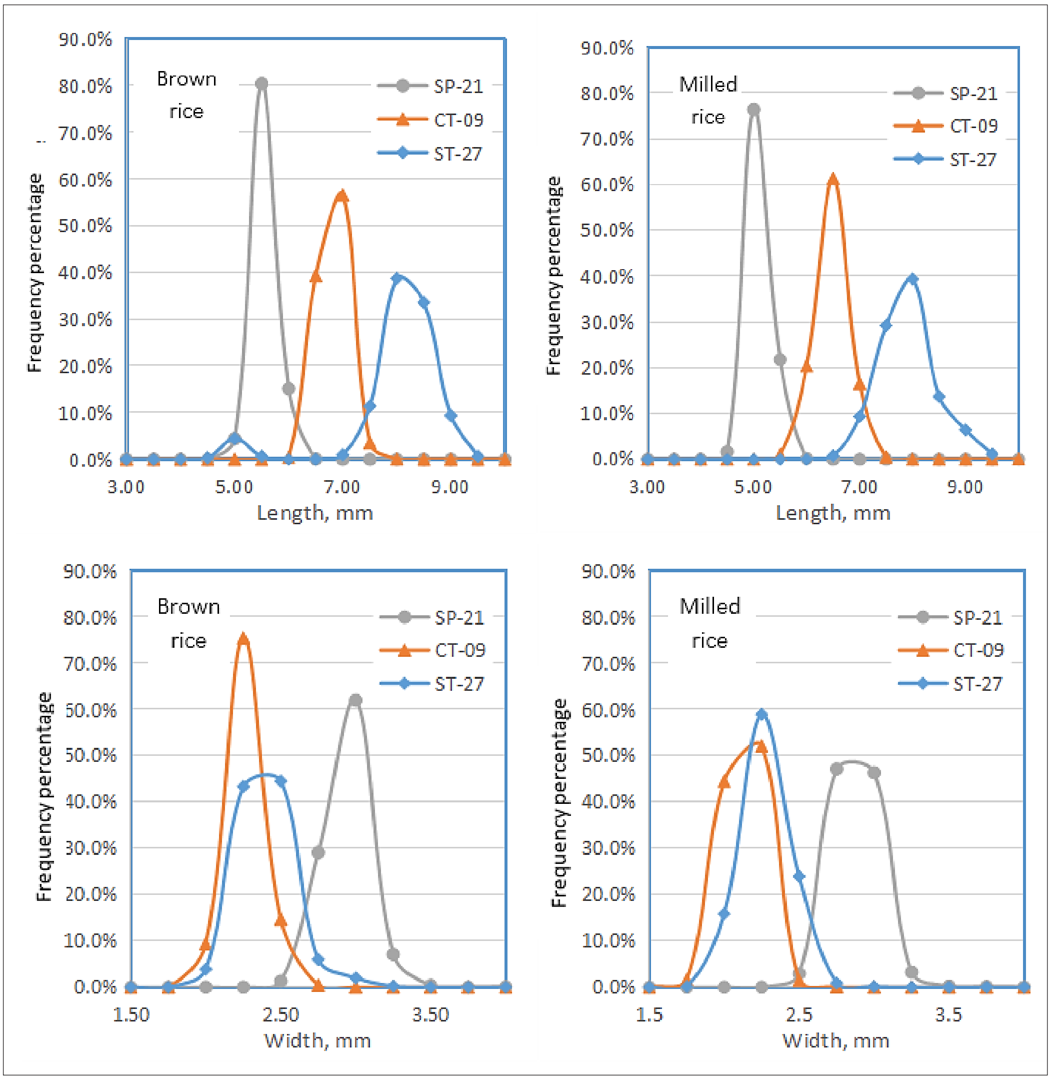

Figure 9. Length and width profiles of brown and milled rice forms of the selected samples.

Table 3. Kernel dimensions in mm of validation set kernels.

	Milled Rice		Brown Rice	
	Length	Width	Length	Width
Mean	6.08	2.35	6.18	2.43
SD	0.64	0.30	0.67	0.34
Maximum	7.30	2.93	7.42	3.16
Minimum	4.72	1.90	4.78	1.97
N	56	56	56	56

Table 4. Time in seconds to manually measure dimensions of a single kernel.

	Milled Rice	Brown Rice
Mean	48.1	44.3
SD	11.6	8.7
Maximum	103.7	73.9
Minimum	33.4	31
N	56	56

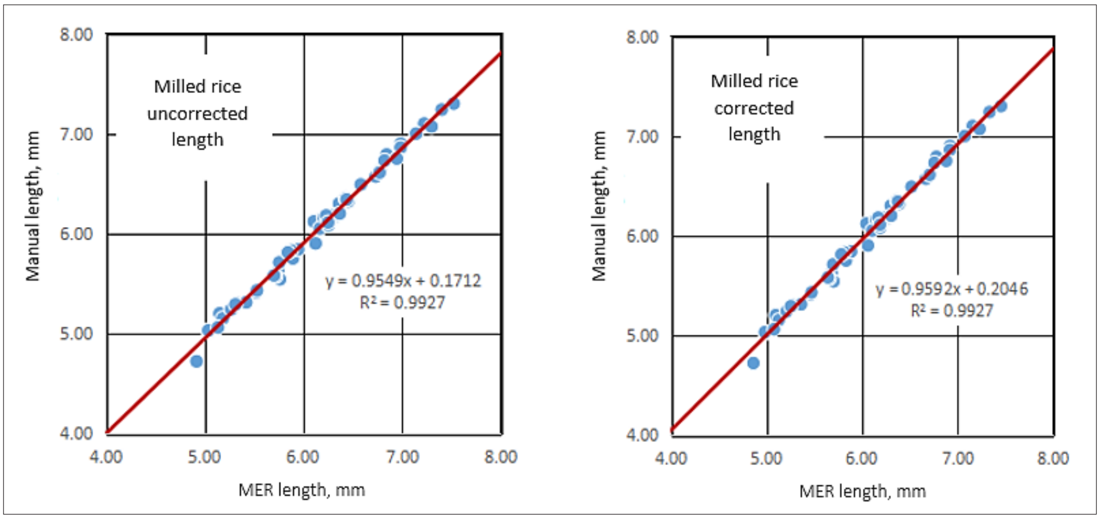

Figure 10. Digitally measured uncorrected and corrected lengths using regression model of milled rice kernels.

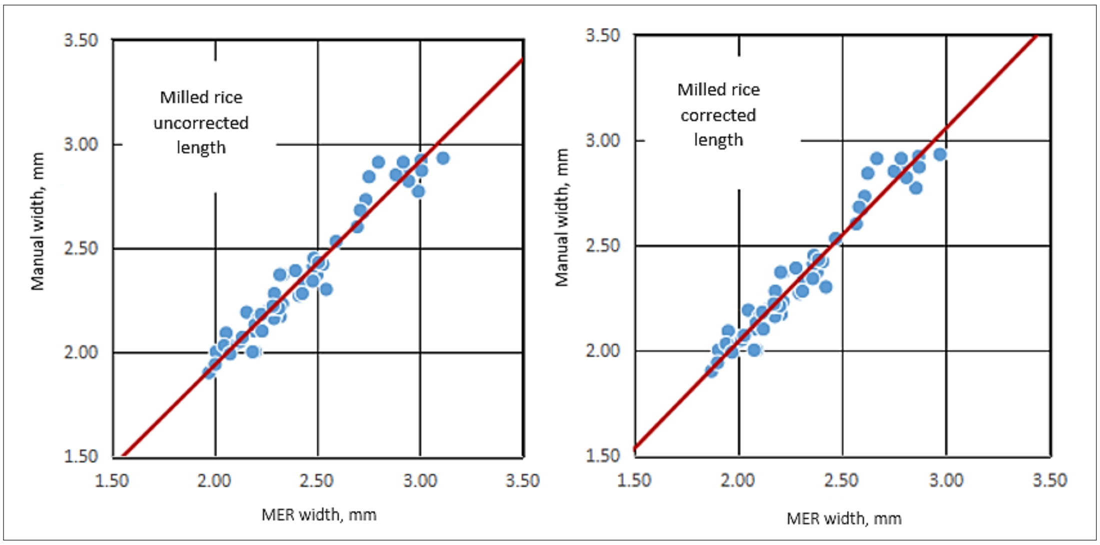

Figure 11. Digitally measured uncorrected and corrected widths using regression model of milled rice kernels.

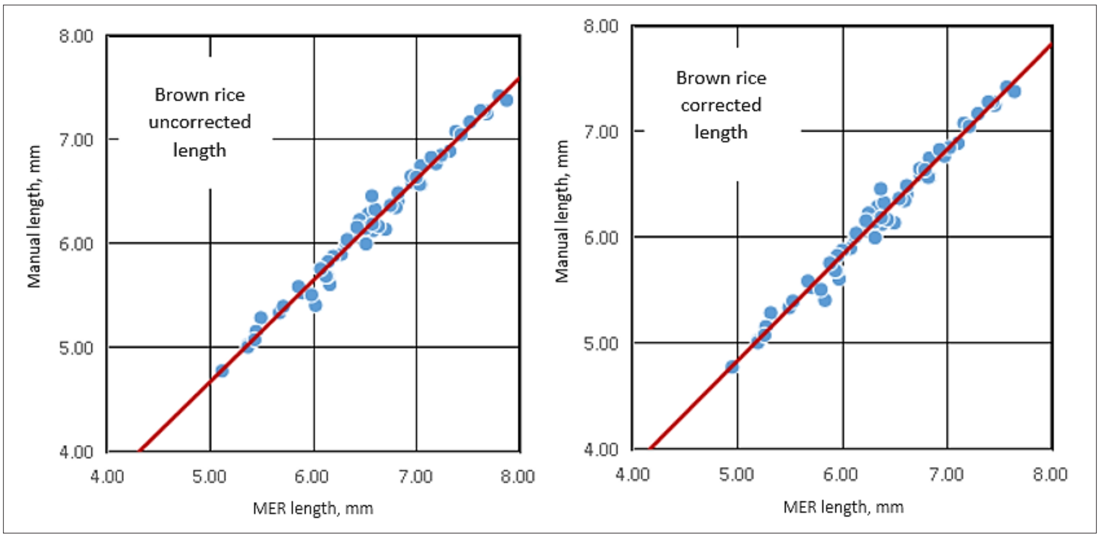

Figure 12. Digitally measured uncorrected and corrected lengths using regression model of brown rice kernels-validation set.

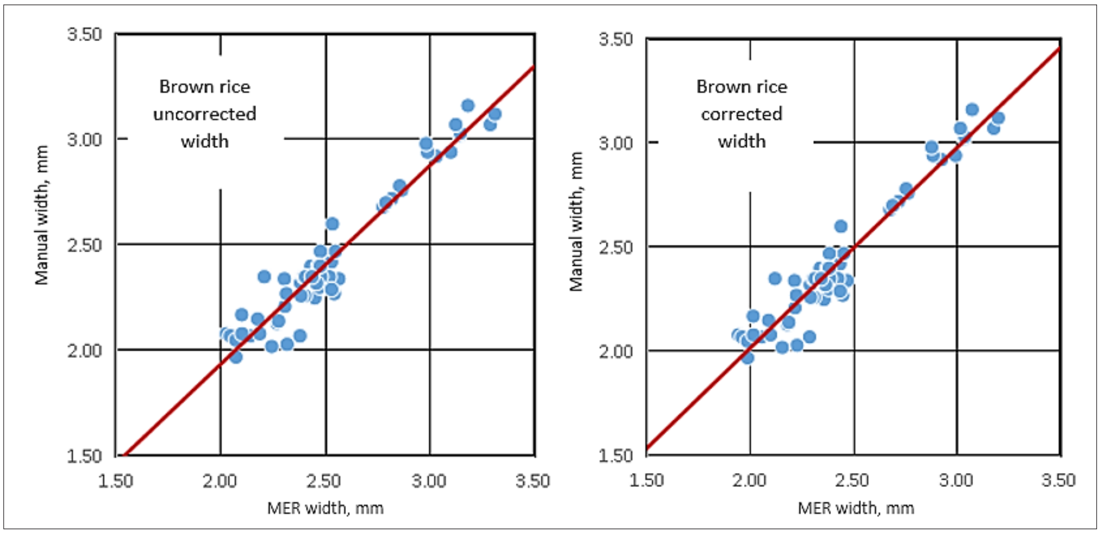

Figure. 13. Digitally measured uncorrected and corrected widths using regression model of brown rice kernels-validation set.

Conclusion

A grain size measurement system's hardware and software components were established based on commercial flatbed scanner and open source technology, the OpenCV in Python programming language. The systematic approach for calibration and performance evaluation achieved higher accuracy in estimating the grain sizes based on the geometric model of minimum enclosing rectangle than using an elliptical model. The high coefficient of correlation and near-unity slope of regression models are sufficient for accurate grain length estimation even using the un-adjusted measurement, which can be used to gain confidence in measuring. However, there was a wider variability in the measurement of kernel width owing to their spherical cross-section geometry, which could introduce positional errors during manual measurement and lesser definition of kernel extents during image processing.

The developed system could speedily measure grain sizes from a large number of kernels to better define the size distribution profile and gain more insight in the expression of genes during the breeding process. It can also replace the manual measurement of rice length and width with high speed and accuracy, which can significantly fast track the physical evaluation of numerous rice samples in the laboratory.

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FIELD PERFORMANCE OF IMPROVED SOMACLONES FROM *IN VITRO* CULTURE RICE VARIETY PSB Rc 68 UNDER COMPLETE SUBMERGENCE

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Abstract

The potential of *in vitro* culture (IVC) to induce somaclonal variation for crop improvement has been proven since it was introduced in crop breeding. Challenges of climate change include developing rice varieties with market value and high chances of adoption. These varieties must be highly adaptive to the changing environment and have good grain quality. IVC using young inflorescence of the submergence tolerant rice variety, PSB Rc 68, was done in 2008 wet season to improve its grain quality, specifically its amylose content. The activity generated a diverse IVC_2 population from which good phenotype plants were selected, and were consequently evaluated for grain quality. The IVC lines were assessed for grain quality traits from which 44 lines with improved grain quality were identified. These were screened for submergence tolerance at seedling stage from which 20 (45%) tolerant IVC lines with survival (75 - 91%) rate comparable with the tolerant check, FR13A (91%), and superior to the wild type, PSB Rc 68 (68%) were selected. Nine lines produced seeds and were evaluated for tolerance to submergence stress at vegetative stage and field performance under non-stress and stress conditions in 2016 dry season. Field evaluation showed the comparable performance of the IVC lines to the tolerant check and wild type in terms of survival and yield under non-stress and submergence condition. These lines are ready for nomination to the National Cooperative Test for evaluation and possible varietal release for submergence-prone rice ecosystem.

Keywords: *Grain Quality, In Vitro Culture, Somaclonal Variation, Submergence, Survival, Stress.*

Introduction

Drastic change was observed in climate patterns, which causes tremendous rainfall and frequent strong typhoons, resulting in extreme flooding in low-lying rice areas that affects crop yields. The development of rice varieties that can survive under complete submergence is one of mitigating technologies. However, tolerance should be accompanied with acceptable phenotype, grain yield, and good grain quality for these factors dictate the release, market value, and adoption of rice (Laborte et al., 2015). Currently, three rice varieties were released for flood-prone rice ecosystem: PSB Rc 68 (*Sacobia),* which was released in 1997; IR64-Sub1 (*Submarino* 1), released in 2009; and NSIC 2020 Rc 590 (*Submarino* 2*)* released in 2020. However, PSB Rc 68 has poor grain quality while IR64-Sub1 is susceptible to disease.

The efficiency of somaclonal variation, induced through tissue culture, in improving crops has been proven since it was introduced in breeding for crops. Somaclonal variation is common in plants regenerated from callus tissues. The genetic alterations commonly induced in somaclones are: changes in the number of chromosomes, alterations in chromosomal structure causing translocations, deletions, insertions, or duplications, and DNA sequence-base mutations (Sharma and Khanna, 2019). These alterations in the crop's genetic structure cause changes in the phenotype. Plants regenerated from cultured explants and isolated protoplasts can show considerable differences from the donor plants that is considered as a potential source of improvements in plants (Larkin and Scowcroft, 1981). This study utilized tissue culture technique using young rice inflorescence to improve PSB Rc 68, specifically its grain quality. The objective was to generate improved breeding lines with retained tolerance to submergence.

Materials and Method

PSB Rc 68, locally known as *Sacobia*, is known for its tolerance to complete submergence. The variety was developed by the International Rice Research Institute (IRRI) and was approved for released by the National Seed Industry Council (NSIC) in 1997 for rainfed lowland rice ecosystem. Despite of its desirable traits (Figure 1), PSB Rc 68 has high amylose content (28.7%) and high-intermediate alkali spreading value (3.8) associated to hard cooked rice texture (Juliano et al., 2009), making it less appealing to farmers and consumers.

Line Generation Using Inflorescence Culture Technique

Tillers with immature inflorescences were collected and sectioned 12 cm (Fig. 2a) from the base. These basal sections contain 2 cm long young inflorescences (Fig. 2b) disinfested with 50% (v/v) sodium hypochlorite (98% active ingredient) for 15 min with agitation at 200 rpm using Thermo-Scientific MaxQ200 Orbital Shaker, and were rinsed twice with sterilized distilled water. Twenty individual spikelets (Figure 2c) were dissected under a pre-sterilized laminar hoodand were inoculated in callus induction (CIM) containing 30 ml Murashige and Skoog (MS) culture media (Murashige and Skoog, 1962). Cultures were incubated in dark condition at 25^{0}C for four weeks until callus was formed. Callused spikelets were inoculated in MS regeneration medium (RM) with 1 mgL^{-1} Kinetin and 0.5 mgL^{-1} NAA. Cultures were incubated in light condition at 8-h photoperiod at 27^{o}C until plants were regenerated. Fully developed plants were removed *in vitro* and hardened for adaptation for 3 - 5 days before transplanted in the screen house. Regenerated plants were grown to maturity to generate the IVC_2 population. There were 350 IVC_2 plants generated and evaluated for phenotypic acceptability for line selection (IVC_3) from which 82 plants were selected. The selections were evaluated in 2009 dry season (DS) and wet season (WS) for uniformity and stability.

Evaluation for Grain Quality Traits

The selected IVC_3 lines were evaluated for grain quality, milling recovery, physical attributes, and physico-chemical traits to identify which among the selected lines were improved for grain quality traits, especially for percent amylose content (AC). The evaluation was conducted in 2009 WS based on the evaluation protocol published in the NCT Manual (PhilRice, 1997).

Screening for Submergence Tolerance at Seedling Stage

The lines with improved grain quality traits were selected and were screened for submergence tolerance at seedling stage in 2011 WS under cemented tank condition. The screening was based on the established protocol of Philippine Rice Research Institute (PhilRice). Score for tolerance was based on the Standard Evaluation System (SES) for Rice (IRRI, 1996).

Traits	Values	Classification
Agronomic		
Maturity	116 DAS	medium maturing
Plant height	116 cm	intermediate height
Average yield	3.4 t ha^{-1}	
Maximum yield	4.4 t ha^{-1}	
Grain quality		
Percent brown rice	75.20%	Fair
Percent milled rice	63.50%	Grade 2
Percent head rice	29.40%	Very poor (aa)
Grain length	7.2 mm	Long
Grain shape	3.2 mm	Slender
Chalkiness	8.30%	Grade 2
Percent amylose content	28.70%	High
ASV	3.8	High-intermediate
Percent crude protein	5.50%	
Other traits		
Submergence stress		Tolerant
Drought stress		Tolerant
Pest/Disease (blast)		Intermediate

Figure 1. Agronomic, grain quality, and other desirable traits of PSB Rc 68; field crop stand (a) and plant type (b) of PSB Rc 68 (*Sacobia*).

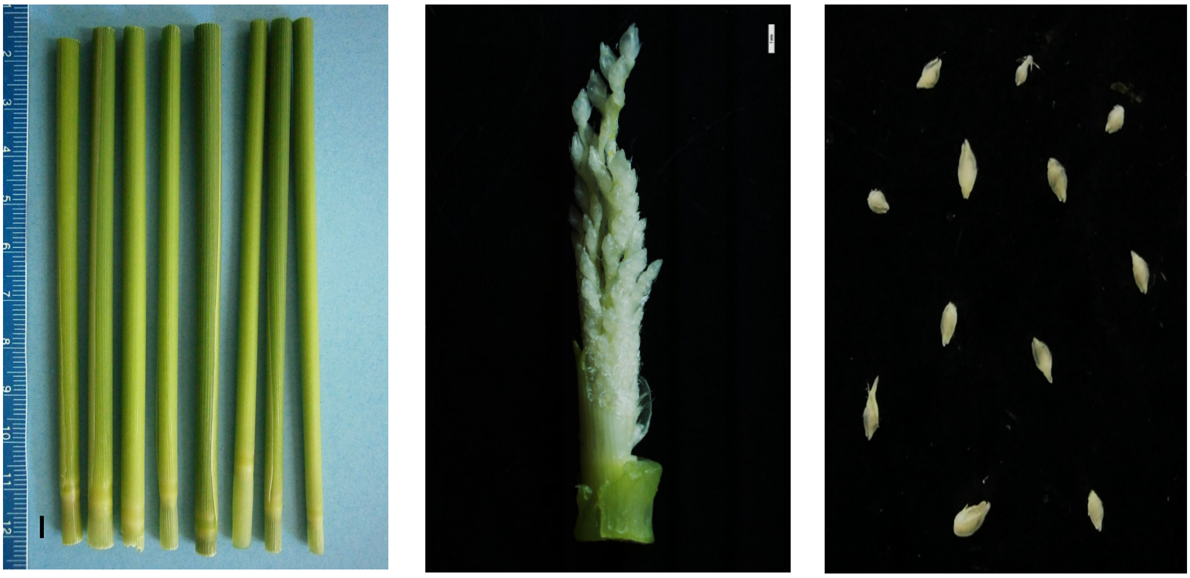


Figure 2. Sectioned tillers, (a) 12 cm length; (b) immature inflorescence, 2 cm length; (c) dissected spikelets in callus induction medium (CIM).

Evaluation for Submergence Stress Tolerance at Vegetative Stage, and Field Performance Under Non-Stress and Complete Submergence Stress

The 50 g seeds of the test entries were sown in a raised seed beds with furrows and were covered with moist sawdust with used sacks. The seedlings were transplanted 21 days after sowing. For the submergence stress (STR) condition, the experimental plot size was 1.44 m^2, comprised of 6 rows and 6 hills, spaced at 20 cm in between hills and rows, and were replicated twice. Submergence was imposed 22 days after transplanting for six days. De-submergence was done when the susceptible check, IR42, was already soft, deteriorated, and dead. Percent survival (%) was determined at 21 days after de-submergence. For the non-stress (NSTR) condition, the entries were established in a 16 m^2 plot with 20 rows and 20 hills, spaced at 20 cm in between rows and hills, and were replicated twice. Water parameters including depth, temperature, pH, electric conductivity (EC), total dissolve solids (TDS), and dissolve oxygen (DO) were measured to determine the quality of the water during complete submergence. The entries were evaluated for agronomic traits, days to heading, plant height, tiller number per hill and grain yield under non-stress (NSTR), and submergence stress (STR) conditions. Under NSTR, data collection was based on the procedure published in the Field Operations Manual (PhilRice, 2007), while under STR, agronomic data were collected from 12 sample plants. Grain yield was collected from all surviving plants harvested and computed from the total plot size.

Results and Discussion

In Vitro Culture Response and Line Generation

In 2008 WS, 300 excised spikelets from immature inflorescences of PSB Rc 68 were cultured from which 55 (18%) spikelets were induced to callus formation. These calli regenerated 177 plantlets, from which 82 (46%) plants survived to maturity and generated 350 IVC_2 plants. The IVC_2 plants were established in field condition in which 82 (23%) plants were selected based on phenotypic acceptability. Genetic and epigenetic alterations are induced by plant tissue culture, which may result in desirable and heritable changes in their phenotypes (Dong et al., 2013). Uniformity evaluation in 2009 DS and WS showed that the selected lines were uniform in terms of agronomic traits (Table 1). Seeds were bulked and analyzed for grain quality.

Table 1. Variability statistics within the line for agronomic traits evaluated in 2009 DS and WS for uniformity evaluation.

Agronomic Trait	2009 DS			2009 WS		
	Standard Deviation	Coefficient of Variance	Pr>F	Standard Deviation	Coefficient of Variance	Pr>F
Days to heading*	0-1.4	0-1.79	0.2431^{ns}	0-1.9	0-2.48	0.2513^{ns}
Days to 80% maturity	0-1.4	0-1.30	0.6543^{ns}	0-1.9	0-1.79	0.6672^{ns}
Culm length	0-1.5	0-2.03	0.5432^{ns}	0-1.6	0-2.03	0.5422^{ns}
Plant height	0.1.5	0-2.24	0.7621^{ns}	0-1.6	0-1.57	0.7589^{ns}

*Emergence of first head

Evaluation for Grain Quality Traits

Physico-chemical Properties: Amylose Content, Gelatinization Temperature, Crude Protein

AC was reduced in 67 (82%) IVC lines by 7 - 42% (Figure 3a), shifting the classification from high to low. Alkali spreading value (intermediate) were retained in 100% of the IVC lines generated, while increase in %crude protein was obtained (Figure 3b). AC is a major factor determining the texture of cooked rice and GT directly dictates cooking time (Juliano, 2007). Both properties contribute to the release of a rice variety because these affect cooked rice texture (Laborte et al., 2015). Tissue culture is one of the efficient strategies that generates lines with improved biochemical properties (El-Banna and Khatab, 2012). Forty-four (54%) IVC lines were selected based on improved grain quality.

Milling Recovery

The percent brown rice (%BR) of the 82 IVC lines ranged from 57 to 89%, from which 78 (95%) lines had improved %BR from poor to fair and good (Figure 4a). Furthermore, improvement in the percent milled rice (%MR) was observed in the majority (89%) of the IVC lines, shifting the classification from Grade 2 to Grade 1 and Premium (Figure 4b). These improvements in %BR and %MR resulted in the improvement of percent head rice (%HR) recovery of the 65 (79%) IVC lines, shifting their classification from very poor (aa) to Grades 1 to 3 and Premium (Figure 4c). Induce mutation techniques such as mutagenesis and *in vitro* culture improves milling and head rice recovery at an average of 5% and 4.9%, respectively (Pillai et al., 2021). Total milled and head rice recovery are very important physical quality measures in the approval and release of a rice variety for cultivation and commercialization (Xangsayasane et al., 2018). Correlation analysis (Table 2) of the milling recovery parameters showed moderate correlations. Positive correlation existing between %BR and %MR (r=0.4997) and %BR and %HR (r=0.2962) were significant, while insignificant weak negative correlation exists between %MR and %HR (r=-0.0061). Milling recovery is the measure of how much bran is removed from the brown rice kernel (Chen and Bergman, 2005). It affects milling and influences consumer acceptance. Milling recovery also influences the color and the cooking attributes of rice. Milled brown rice is inefficient in absorbing water and does not cook as quickly as milled rice (Tong et al., 2019).

Physical Attributes: Grain Size, Shape, and Chalky Grains

Milled rice of the IVC lines and of the wildtype, PSB Rc 68, were measured for grain length, width, and shape. Reduction in grain length by 5.4% (0.4 mm) to 8.4% (0.6 mm) was observed in the majority (49%) of the IVC lines. However, this reduction was not significant to change the long grain length classification of the lines. Significant reduction in grain length was observed in 30 (37%) IVC lines, shifting their classification from long to medium. The alterations in grain length did not affect the grain shape classification of the IVC lines. Somaclonal variation is a genetic variation in crops occurring during *in vitro* culture (Karp, 1994; Ahloowalia, 1986), leading to mutations expected to occur in one gene resulting in expected phenotype of the crops (Bozorgipour and Snape, 1997). In the case of the IVC lines, reduction in their grain size maybe considered a negative effect. However, the negative effect of tissue culture in grain size is minimal as the classification was retained. Negative mutation results in a protein that does not function normally or may not function at all. This non-functioning protein results in different phenotypes of the crop (Oladosu et al., 2015). These harmful mutations maybe visible in the vegetative, reproductive, or primordial structures of the plants

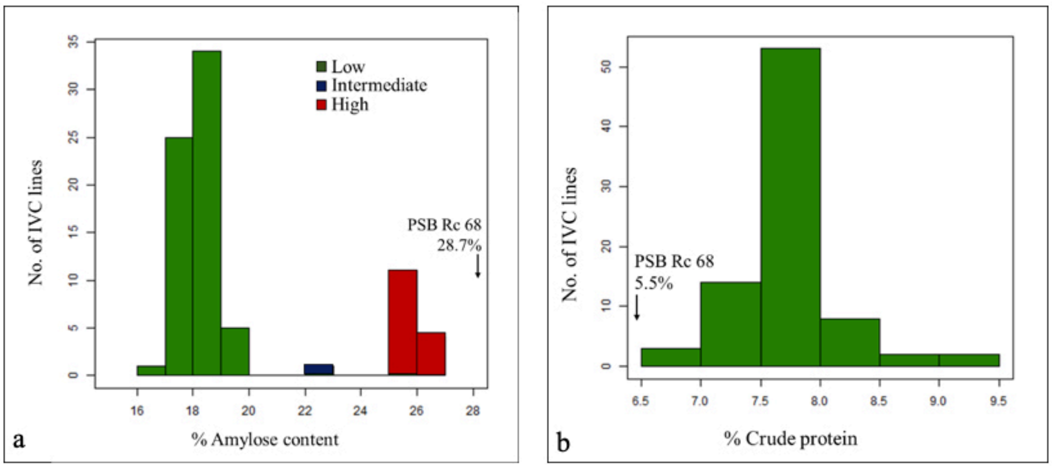
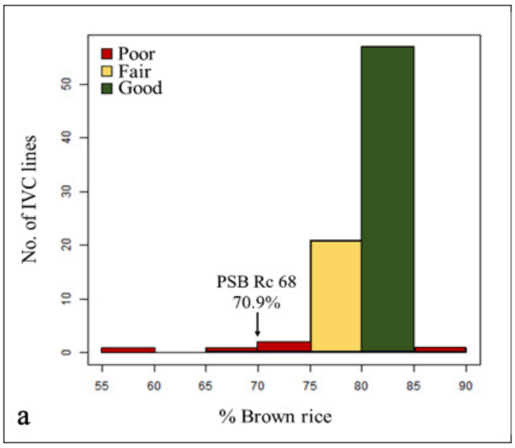
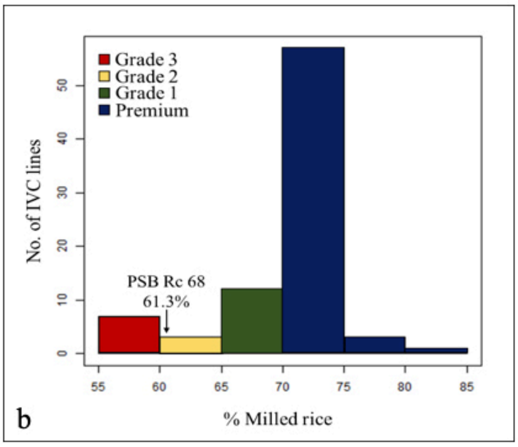


Figure 3. Amylose content (a) and crude protein (b) of the IVC lines compared with the wildtype.





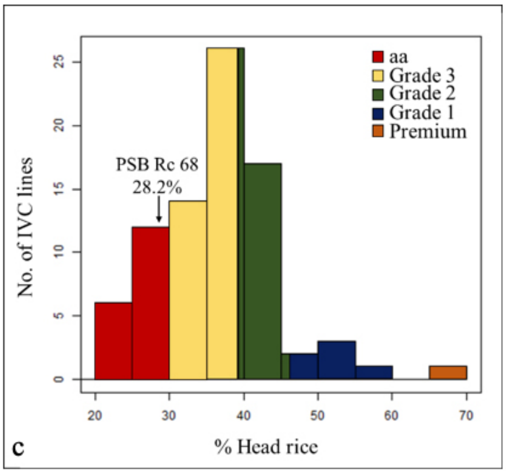


Figure 4. Brown rice (a), milled rice (b), and head rice (c) of the IVC lines classified based on the published categories in the NCT Manual, PhilRice, 1997.

such as seeds, which affect their functions (Parry et al., 2009). The grain length of somaclones derived from four *indica* rice in the study of Kang-Le et al. in 1989 showed significant reduction of 0.04 mm to 0.24 mm.

Chalky grains (%) was improved in 26 (32%) IVC lines, shifting the classification from Grade 2 to Grade 1. Retained Grade 2 class of chalky grains was obtained in 56 (68%) IVC lines. Chalkiness is a major concern in rice breeding because it is one of the key factors in determining quality and price. Chalkiness disappears during cooking; therefore, has no effect on cooked rice taste, aroma, and tenderness (Ohsawa et al., 2007). However, it affects the appearance of milled grains (Sreenivasulu et al., 2015; Mishra et al., 2019). The development of numerous air spaces between loosely packed starch granules and the resulting change in light reflection cause grains to become chalky (Tashiro and Wardlaw, 1991). Inducing variability via somaclonal variation in six genotypes of rice, in the study by Torrealba in 2006, identified three somaclones from CT10310-15-3-2P-4-3 with better grain quality, white belly reduction, and decreased endosperm chalkiness.

Screening for Submergence stress at seedling stage

Survival of the test entries and check varieties after 21 days from de-submergence ranged from 25 to 92%. Survival of the 43 (98%) IVC lines (42 - 91%) were comparable with FR13A (92.2%), while all lines were comparable with PSB Rc 68 (68%). From these lines, 20 (45.5%) were moderately tolerant, 20 (45.5%) susceptible and 4 (9%) highly susceptible (Figure 5). Somaclonal variation offers several advantages, one of which is its efficiency in eliminating one or a few defects in variety, while retaining its desirable characteristics (Acquaah, 2007). In the case of PSB Rc 68, somaclonal variation eliminated its poor grain quality while retaining its tolerance to submergence. The screening identified 20 lines possessing good phenotype (Figure 6) with improved grain quality and tolerance to submergence at seedling stage. However, only 9 (45%) lines were able to seed set. Submergence stress affects seed setting due to the decrease of photosynthetic intensity and photosynthetic areas resulting in reduction of photosynthetic activities and insufficient assimilate production (Setter et al., 1989). These selected IVC

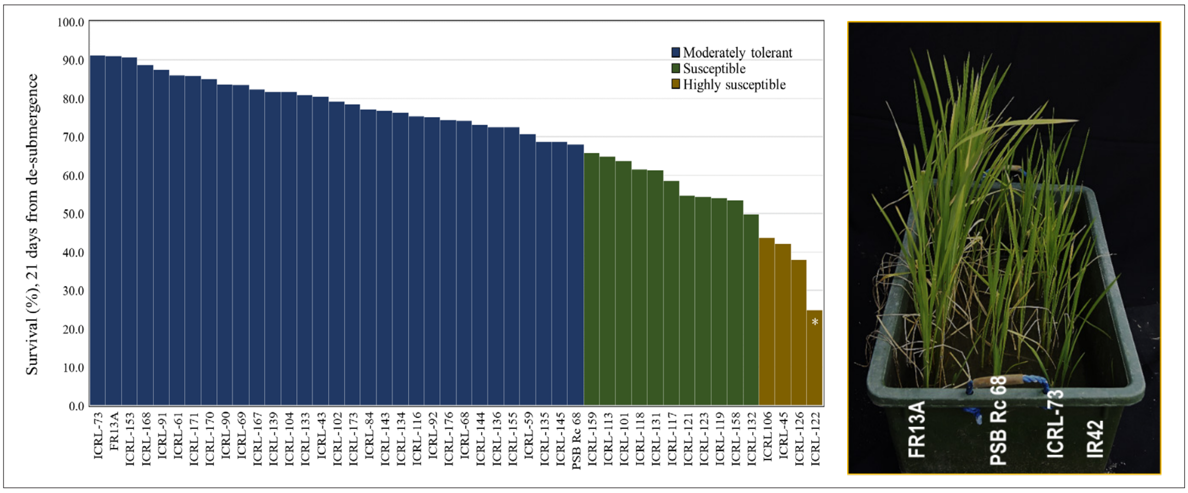


Figure 5. Survival of the IVC lines under submergence in comparison with the tolerant check, FR13A, and the wildtype, PSB Rc 68.
**Significantly inferior to FR13A by Tukey's at Alpha=0.05*

lines (Table 3) were evaluated for field performance under non-stress and submergence conditions in 2016 DS.

Field Performance Trial under Submergence and Non-Stress Condition

Water quality during submergence (Figure 7a) was monitored until the stress was terminated 6 days from imposition. The depth of the water during the submergence period ranged 90 - 99 m deep. The pH ranged 7.9 - 8.7, indicating a slightly alkaline level that is attributed to the presence of sulfate and carbonate-containing compounds mixed with the water (Trick et al., 2008). The pH of the water exerts additional stress to the submerged rice plants; thereby, affecting survival and growth (Liang et al., 2017). The temperature and EC of the water during the duration of submergence stress ranged 28 - 34°C and 159 - 263 μScm^{-1}, respectively. Water temperature influences rice growth and survival under submergence (Rafii et al., 2020). The cool temperature reading of the water provided a cool environment; however, low temperature is highly correlated to alcohol fermentation causing rapid plant decay (Noguchi, 2007). The TDS and DO ranged 45 - 76 ppm and 6 - 12 mgL^{-1}, respectively. High TDS decreases the dissolvability of oxygen under water, resulting in lesser available oxygen. This condition provides a rapid-decaying environment to plants under submergence (Butler and Ford, 2018).

Survival under submergence

Percent survival (%) of the 9 lines at vegetative stage was assessed 21 days after de-submergence and were compared with the tolerant checks and their wildtype, PSB Rc 68 (Table 4). Significant variation (Pr>F=0.0483) in survival was observed among the test genotypes. Comparable survival with FR13A (100%) was observed in 2 (22%) IVC lines, SUB38, and SUB39 with survival of 96% and 99%, respectively, while the remaining 7 (78%) IVC lines were comparable with PSB Rc 68 (89%).

Grain Yield Under Non-Stress and Stress Condition

Grain yield under non-stress (NSTR) condition ranged 3.2 - 7.8 t ha^{-1}, while under stress (STR) yield varied from 2.1 to 3.9 t ha^{-1} (Table 3). Significant yield reduction due to stress was incurred by IR64

Table 3. Survived tolerant IVC lines with improved agronomic and grain quality traits.

No.	Field Code	IVC Code	Entry Name
1	SUB34	ICRL-2008WS-61	PR41398-ICRL2008WS-PSB Rc68 11-4-1
2	SUB35	ICRL-2008WS-91	PR41398-ICRL2008WS-PSB Rc68 20-1-2
3	SUB36	ICRL-2008WS-134	PR41398-ICRL2008WS-PSB Rc68 27-2-3
4	SUB37	ICRL-2008WS-143	PR41398-ICRL2008WS-PSB Rc68 29-2-1
5	SUB38	ICRL-2008WS-170	PR41398-ICRL2008WS-PSB Rc68 34-1-1
6	SUB39	ICRL-2008WS-171	PR41398-ICRL2008WS-PSB Rc68 34-2-1
7	SUB40	ICRL-2008WS-69	PR41398-ICRL2008WS-PSB Rc68 12-1-2
8	SUB3	ICRL-2008WS-84	PR41398-ICRL2008WS-PSB Rc68 18-2-1
9	SUB4	ICRL-2008WS-43	PR41398-ICRL2008WS-PSB Rc68 30-1-2

Note: Field code of the IVC lines will be used in the succeeding discussions.

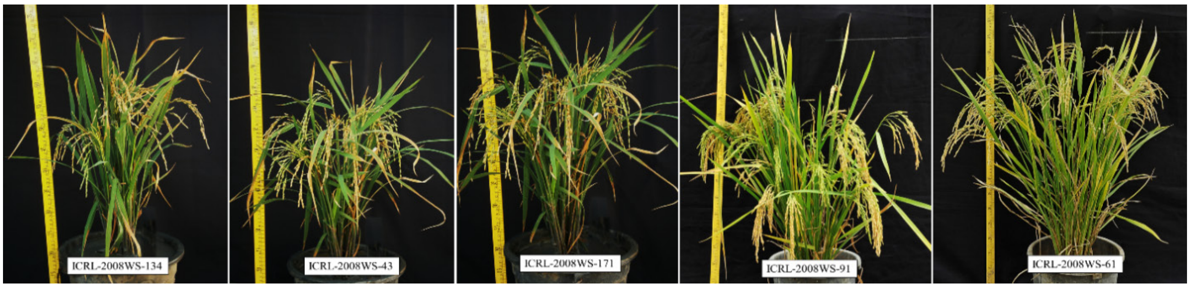


Figure 6. Phenotype of some of the selected IVC lines with tolerance to submergence at seedling stage and improved grain quality traits.

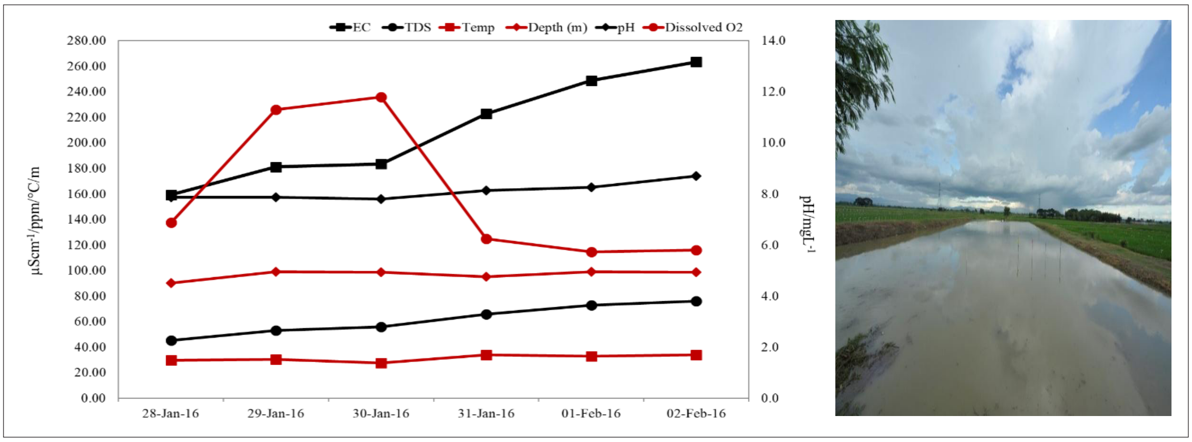


Figure 7. Quality of the water (a) during complete submergence (a); water quality, 3 days from submergence stress imposition (b).

Table 4. Survival at 21 days after de-submergence, grain yield under NSTR and STR, and yield reduction as effected by submergence for six days of the IVC lines and checks, 2016 DS.

No.	Field Code	Survival (%)	Comparative Survival (%)/FR13A	Grain Yield (t ha^{-1})		Yield Reduction	
				NSTR	STR	t ha^{-1}	%
1	FR13A	100^{a}					
2	IR42	47^{b*}	47.2				
3	IR64	63^{ab}	62.5	6.170	2.714	3.456	56.0^{**}
4	IR64-Sub1	72^{ab}	72.2	4.856	2.078	2.778	45.0
5	PSB Rc68	89^{ab}	88.9	7.773	3.865	3.907	63.3^{**}
6	SUB34	64^{ab}	63.9	5.187	2.714	2.473	40.1
7	SUB35	69^{ab}	68.8	3.287	2.517	0.770	12.5
8	SUB36	90^{ab}	90.0	5.618	3.067	2.551	41.3
9	SUB37	90^{ab}	90.3	6.481	3.953	2.528	41.0
10	SUB38	96^{a}	95.8	3.632	3.385	0.247	4.0
11	SUB39	99^{a}	98.6	4.813	3.444	1.370	22.2
12	SB40	78^{ab}	77.5	4.466	2.989	1.477	23.9
13	SUB3	89^{ab}	88.9	4.737	3.001	1.736	28.1
14	SUB4	82^{ab}	81.9	3.193	2.969	0.224	3.6
Minimum		47	47	3.193	2.078	0.224	3.6
Maximum		100	99	7.773	3.953	3.907	63.3
Range		53	51	4.580	1.875	3.683	59.7
Mean		80	79	5.018	3.058	1.960	31.8
Standard Deviation		15.7	15.2	1.353	0.541	1.192	19.3
Coefficient of Variation		19.5	19.3	27.0	17.7	60.8	60.8
Pr>F		0.0483		0.4435	0.8274		

Note: Means with the same letters are not significantly different by Tukey's, Alpha=0.05
*Significantly different compared with the tolerant checkFR13Aand wildtype PSB Rc68
**Significant reduction in grain yields due to complete submergence stress

(56%) and PSB Rc 68 (63.3%). Reduction in yield among the IVC lines were insignificant, even though there were yield reductions of 4 (0.247 t ha^{-1}) to 45% (2.778 t ha^{-1}). Under both NSTR and STR, yield of the IVC lines were comparable with the checks PSB Rc 68, IR64, and IR64-Sub1. Under both conditions, no significant genotypic differences were observed for grain yield. This indicates that the performance of IVC lines is comparable with the checks under non-stress and submerged growing conditions.

Under submergence, strong correlation (Table 4) of survival and grain yield was significant (r=0.7275). Regression analysis (Figure 8) of the two parameters showed that genotypes with higher survival have higher grain yield than those with low survival or are intolerant. Plant survival is an important determinant of grain yield (Singh et al., 2009) under submergence.

Table 4. Pearson's correlation of survival and grain yield under complete submergence.

Parameter		Survival	Grain Yield
Survival	r	1	0.7275
	p-value		0.0073
	n	14	12

Data of several studies (Singh et al., 2009; 2013; Iftekharuddaula et al., 2011; Dar et al., 2013) show that survival after complete submergence, as well as the ability of the plant to recover and resume growth faster after the water subsided, are functionally related and are important determinants of grain yield in flood-prone areas. Immediate resumption of growth after submergence supports generation of new photosynthesizing shoots (Panda et al., 2008) that are significant contributor to grain yield. Tolerant plants recovered faster in terms of biomass, leaf area, and tillers (Zhang et al., 2015). Meanwhile, slow recovery of intolerant plants is attributed to post-flooding processes. Injuries are not immediately manifested right after de-submergence but develop progressively during post-submergence as results of the damage due to reactive oxygen species (Hunter et al., 1983; Monk et al., 1989; Gutteridge and Halliwell, 1990; Crawford, 1992; Ella et al., 2003).

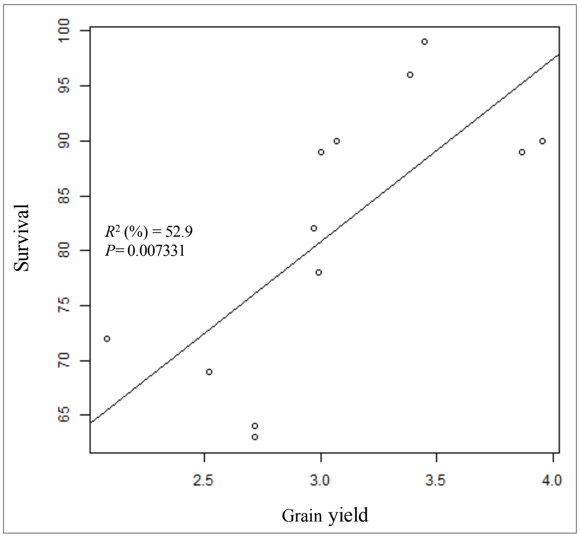


Figure 8. Regression analysis of survival and yield under submergence.

Comparative agronomic traits under NSTR and STR condition

The major agronomic traits significantly affected by submergence were heading, maturity, plant height, and grain yield (Mackill et al., 2010). Combined analysis for STR and NSTR showed significant genotype main effect for plant height (Pr>F=0.001), while there was no significant interaction for days to heading, maturity, number of tiller, and grain yield (Table 5). Significant condition main effect was significant in all traits except in the number of productive tiller (Pr>F=0.0633). Variance component analysis showed that the genotype attributes 9% of the total variation for yield, days to heading, 0.31%; days to maturity, -2% (Table 5), tiller, -12%, and plant height, 9% (Table 6). Meanwhile, growing condition attributes 46% of the variation for grain yield, 95% for days to heading, 92% for days to maturity, 7% for tiller per hill, and 34% for plant height. The genotype x condition interaction attributes 30% and 24% of the total variation observed in productive tiller and plant height, respectively. Heading, maturity, and height varied significantly with the growing condition with broad-sense heritability (H_2) values of 0.01, 0.01, 0.4 and 0.3, respectively.

Days to Heading and Maturity

Under NSTR, days to heading of the IVC lines ranged 82 - 84 days after seeding (DAS), and under STR, 91 - 95 DAS. Heading days of the genotypes under NSTR and STR were comparable with the wildtype, PSB Rc68, and checks, IR64, and IR64-Sub1. The heading stage is recognized as the most sensitive rice trait to complete submergence (Ujiie et al., 1956; Matsushima, 1968; Reddy et al., 1985). Delay in days to heading was observed among the test lines under submergence stress (Figure 10a). Estimate analysis showed significant delay by 8 - 12 days. The delay in flowering is due to the need of the rice plants additional time to recover and resume normal vegetative growth and to repair the damages brought about the submergence stress (Nugraha et al., 2012; Purwoko et al., 2019). Days to maturity of the IVC lines under NSTR and STR ranged 109 - 115 DAS and 120 - 124 DAS, respectively. No genotypic differences were observed on this trait, indicating a comparable maturity day between the IVC lines, the wildtype, and check varieties. However, submergence stress caused a significant delay in maturity by 8 - 15 days in all test entries except in SUB39 (Fig. 10b).

Table 5. ANOVA and variance components for grain yield, heading, and maturity, 2019 DS.

Source of Variation	DF	Grain Yield		Days to Heading		Days to Maturity	
		MS	Pr>F	MS	Pr>F	MS	Pr>F
Genotype (G)	11	2.86	0.26^{ns}	2.84	0.36^{ns}	2.11	0.98^{ns}
Condition (C)	1	44.70	0.0002*	1271.02	<0.0001*	1312.5	<0.0001*
Genotype* condition (GC)	11	1.45	0.49^{ns}	2.16	0.67^{ns}	6.16	0.43^{ns}
Error (E)	21	2.13		2.81		5.52	
R-Square		0.69		0.94		0.86	
Coefficient of variance		36.2		1.79		2.23	
Variance Component		**Grain Yield**		**Days to Heading**		**Days to Maturity**	
		Value	**%**	**Value**	**%**	**Value**	**%**
V_G		0.35	8.95	0.17	0.31	-1.01	-1.71
V_C		1.80	45.70	52.87	95.22	54.43	91.85
V_{GC}		-0.34	-8.68	-0.33	-0.59	0.32	0.54
V_E		2.13	54.03	2.81	5.07	5.52	9.32
Total		3.94	100	55.52	100	59.26	100
h^2		0.2		0.01		0.01	

V_G-variation due to genotype, V_C-variation due to condition, V_{GC}-variation due to genotype*condition, V_E-variation due to error; h^2-heritability; ns-not significant/*significant effect at alpha=0.05
DF- degrees of freedom, MS – mean of squares

Table 6. ANOVA and variance components for productive tiller and plant height, 2019 DS.

Source of Variation	DF	Productive Tiller		Plant Height	
		MS	Pr>F	MS	Pr>F
Genotype (G)	11	6.79	0.91^{ns}	65.21	0.0001*
Condition (C)	1	24.08	0.06^{ns}	507.0	0.0003*
Genotype* condition (GC)	11	10.36	0.16^{ns}	45.0	0.083^{ns}
Error (E)	21	5.75		18.5	
R-Square		0.34		0.60	
Coefficient of variance		17.86		5.42	
Variance Component		**Productive Tiller**		**Plant Height**	
		Value	**%**	**Value**	**%**
V_G		-0.89	-11.54	5.05	9.01
V_C		0.57	7.40	19.25	34.34
V_{GC}		2.30	29.78	13.25	23.64
V_E		5.750	74.36	18.50	33.01
Total		7.73	100	56.05	100
h^2		0.4		0.3	

V_G-variation due to genotype, V_C-variation due to condition, V_{GC}-variation due to genotype*condition, V_E-variation due to error; h^2-heritability; ns-not significant/*significant effect at alpha=0.05
DF- degrees of freedom, MS – mean of squares

Ripening of rice grains under maturity is delayed to provide enough time for assimilates remobilization from straw to the grain (Yang et al., 2010; Zhang et al., 2010).

Plant Height and Productive Tiller Per Hill at Maturity

Plant height under NSTR ranged 88 - 97 cm, comparable with the check varieties IR64 and IR64-Sub1 and significantly shorter than the wildtype, PSB Rc 68 (116 cm). Under STR, plant height was 79 - 87 cm, which is comparable with IR64 and IR64-Sub1, but significantly shorter than PSB Rc 68 (105 cm). Plant height was significantly shorter in 7 (78%) IVC lines under STR than in NSTR condition (Figure 10c), which may be attributed to the quiescence mechanism during the submergence period (Nakazano et al., 2012). Tolerant plants have reduced plant height because after de-submergence, the plants require more energy to recovery rather than to grow, resulting in compensation on biomass and plant height (Nugraha et al., 2012). Reduction in plant height was not significant in IR64, IR64-Sub1, SUB3, SUB37, and SUB40. Genotypic difference was not observed for productive tiller per hill under both NSTR and STR conditions. Significant increase and reduction in productive tiller by 36% and 32% were observed in PSB Rc 68 and SUB38, respectively.

Figure 9. Test lines 21 DAT (a); complete submergence, 4 days after imposition (b) de-submerged test lines, 2 days from stress termination (c); survived lines, 21 days after de-submergence (d), 2016 DS.

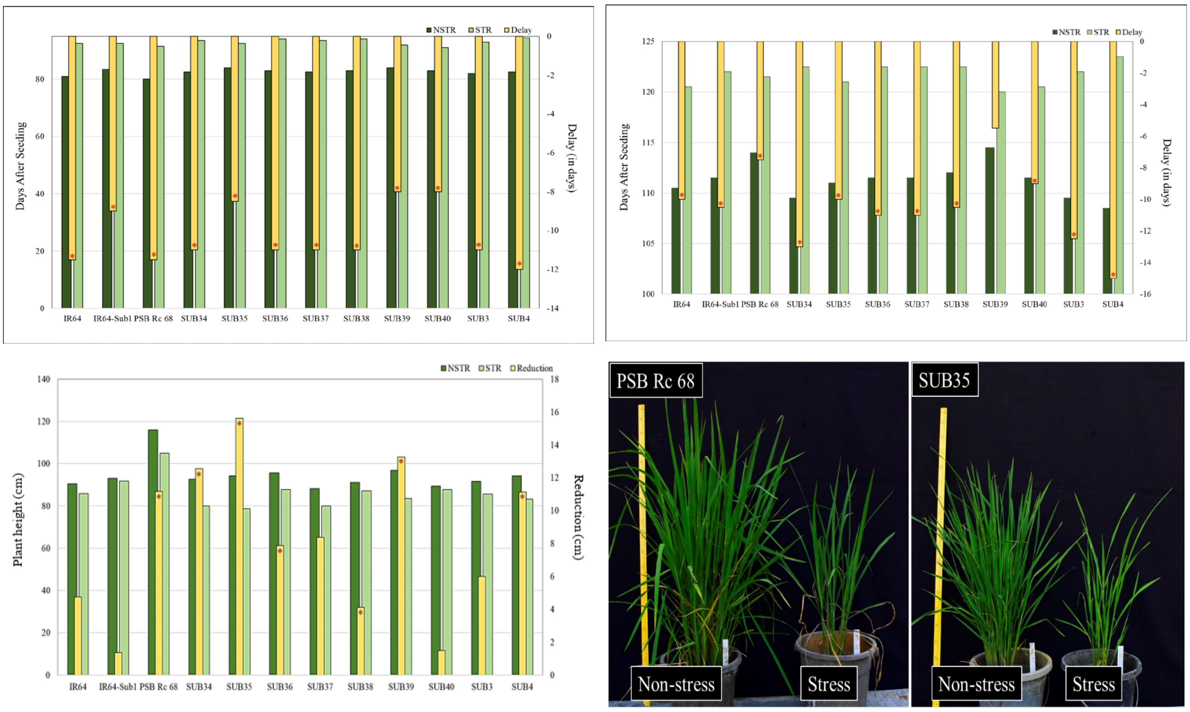


Figure 10. Days to heading (a), days to maturity (b), and plant height (c) as effected by complete submergence stress. Plant height (d) of PSB Rc 68 and SUB35 at 75 DAS. Plants under submerged and stress conditions manifesting stunting growth, 2016 DS.

Conclusion

In vitro culture (IVC) of PSB Rc 68 resulted in the generation of nine promising lines with acceptable phenotype and improved grain quality, specifically amylose content, contributing to the tenderness of its cooked rice.

These promising IVC lines had retained tolerance to submergence stress both at seedling and vegetative stages, with survival comparable with the tolerant check FR13A and the wildtype PSB Rc 68. Field evaluation trial of the IVC lines under non-stress and submergence stress condition, exhibited their comparable performance to the wildtype, PSB Rc 68, IR64, and IR64-Sub1 under both growing conditions. Better survival under complete submergence, acceptable yield potential and yield, and improved grain quality make these lines more advantageous than its wildtype (PSB Rc 68, FR13A) and released variety IR64-Sub1 (*Submarino* 1). The results proved the efficiency of tissue culture in improving undesirable traits of rice while retaining desirable traits. Based on field performance, these IVC lines are ready for nomination to the National Cooperative Test (NCT), for evaluation under submergence condition.

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GENDER MAINSTREAMING IN PHILIPPINE RICE RESEARCH AND DEVELOPMENT: PROGRESS, CHALLENGES, AND OPPORTUNITIES

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Abstract

The increasing ascendancy of 'gender mainstreaming' as the central approach to improving gender equity has largely determined strategies to integrate a gender focus in the Philippine Rice Research for Development (RiceR4D). This paper explored the impetus for and processes of change as implemented by Philippine Rice Research Institute (PhilRice) in the four major entry points in gender mainstreaming framework (GMEF), namely: policies, capacity development, enabling mechanisms, and banner projects. It assessed the GMEF parameters applied in specific strategies and its influence in rice farming communities. This involves extensive documentary reviews (242 documents), surveys, and key informant interviews. By using the theory of change as a preliminary framework, this paper documented the process undertaken by PhilRice relative to gender mainstreaming efforts for rice R4D. By employing social epistemology framework, the paper proffered that gender mainstreaming strategy in rice R4D, though complex, is highly possible and desirable. Thematic analysis showed progress in the four major entry points. There was a notable increase in the level of integration in policies (3.35 points) and active capability enhancement activities (5.4 points) that paved the way for increase in the gender and development (GAD) verified application in the organization. However, the results also raised other issues that the rice R4D efforts need to address for it to be fully mainstreamed. These issues are related to program structures, sustainability, policy formulation, the commitment of other actors involved, and the whole aspect of attitudinal change both at individual and collective level.

Keywords: *Attitudinal Change, Gender Mainstreaming, Gender and Development, Social Epistemology.*

Introduction

The Philippines ranked 8th in closing the gender gap with overall score of 0.799 based on the 2018 Global Gender Gap Report (WEF, 2018). This means that male and female citizens of the nation have an equal educational attainment (1.0), nearly equal health and survival (0.979), and economic participation and opportunity (0.801). However, there is a slightly broad gender gap in terms of political empowerment (0.416).

Economic participation and opportunity as the top empowerment mechanism provide both male and female with basic and practical needs. In agriculture, hunting, and forestry sector, only 25% of employed are women (PSA, 2018a). The specific contribution of the woman laborer is often undervalued and, in some cases, this remains 'invisible'. In terms of wage differential, there was a PhP 28.92 difference in the daily nominal wage rate of male (PhP 284.72) and female (PhP 255.80) agricultural workers (PSA, 2018b). Specifically, for *palay* farm workers, there was a PhP 16.75 difference in the daily nominal wage rate of male (PhP 307.40) and female (PhP 290.65) workers. Though there is still a gender differential, through time, the participation and wage gaps have been narrowing.

This narrowed gap can be attributed to the relative success of gender mainstreaming. However, there is a dearth of data that may provide tangible and tenable information pointing to the role of government agencies in ensuring that 'no Filipino will be left behind' – the primary goal of GAD. More importantly, they do not supply the necessary data regarding gender mainstreaming – the promotion and fulfillment of women's human rights and elimination of gender discrimination in the systems, structures, policies, programs, processes, and procedures of government agencies (PCW, 2016a). It must be noted that gender mainstreaming is one of the most undocumented areas on GAD. This dearth of data provides opportunity in conducting a deeper and further analysis for better and clearer understanding of gender mainstreaming as a strategy in achieving inclusive development.

In the Philippine public sector, gender mainstreaming as a development tool can be traced back to Section 37 of the Republic Act No. 9710 or the Magna Carta of Women (PCW, 2008). It mandates all government agencies to assess the implications for women and men of any planned action including legislation, policies, or programs in all areas and at all levels. This is in consonance with what the Beijing Platform for Action (BPFA) asserted in recognizing the role of gender mainstreaming in decreasing the impact of persistent and increasing burden of poverty, inequality in economic structures, and policies in all forms of productive activities. It also includes access to resources, inequality between men and women in the sharing of power and decision-making at all levels, insufficient mechanisms at all levels to promote the advancement of women (PCW, 2016b)

Being the primary institute for rice R4D, PhilRice started its gender mainstreaming in 2017. Given this time frame, a simple self-assessment was deemed necessary to foster gender-responsive agenda. There is a need to locate or plot the approximate state of gender mainstreaming in terms of progress, challenges, and opportunities in rice R&D's policies, capacity developments, institutional enabling mechanisms, and projects in the institute's R4D efforts and intervention.

Hence, this study evaluated the progress of gender mainstreaming in the rice R4D; identified the challenges in implementing gender mainstreaming in the rice R4D; and determined the opportunities that gender mainstreaming in rice R4D can offer.

Theoretical Framework

The study used a combination of Theory of Change (ToC) [Jameel, 2014] and Social Epistemology Framework (Goldman, 2004). ToC is useful in locating the progress of gender mainstreaming implementation in the rice R4D and the challenges posted by its implementation. On the other hand, Social Epistemology Framework (SEF) [Goldman, 2004] was used in determining the opportunities that gender mainstreaming in rice R&D offers.

ToC is an ongoing process of reflection to explore change and how it happens – and it meaning in a particular context, sector, and/or group of people (Jameel, 2014). It is also a structured way of thinking concerning change and the impact organizations would like to achieve. For this theory, an integrated approach is used for project design, implementation, monitoring and evaluation, and communication. It emphasizes the (a) complexity of development challenges; (b) learning within and between programming cycles; (c) developing and managing partnerships and partnership strategies; and (d) articulating shared vision and strategy for how change can happen (UNDG, 2014).

SEF, on the other hand, looks at the patterns of interaction among epistemic agents that influence the beliefs of each individual (Goldman, 2004). SEF reads in between networks or patterns of communication, influence the quality of beliefs thereby generated. Therefore, many contemporary philosophers argued that institutions, organizations, and associations could be a proper subject of intentional and epistemic states (Gilbert, 1989; Nelson, 1993; Tuomela, 1995; Searle, 1995). In this setup, organizations like PhilRice becomes a subject. Goldman (2004) argues that what the experts know often pales by comparison with the knowledge dispersed in society at large. By harvesting this dispersed knowledge, a social epistemic engine can foster better epistemic consequences.

SEF incorporates the unorganized and dispersed knowledge existing in the society. Thus, it can be said that GAD is incorporated in the policies and projects of PhilRice if GAD operates as a 'signal' or 'warning' to the Institute. Opportunities, as a result of openness to GAD, continue to appear unexpectedly. Intrinsically, gender mainstreaming may be deemed successful.

The PhilRice Gender Mainstreaming Framework (PGMF) [Figure 1] was developed by combining both theories and integrating them to form one working framework for analysis. The PGMF uses the ToC as a basic framework for the progress and challenges; and utilizes the SEF to identify opportunities relevant to the organization.

With the ToC, it is assumed that PhilRice mainstreamed GAD through the establishment of the GAD Focal Point System (GFPS) — a committee that leads GAD-related efforts of the Institute. Moreover, PhilRice allocated at least 0.20% of its annual budget for the direct operation of the GFPS. These resources are expected to influence PhilRice policies and projects. These are all predicted to continuously increase the awareness and knowledge of internal and external clients of PhilRice about GAD and sustain gender mainstreaming efforts through established enabling mechanisms. In return, these likely contribute in the gender mainstreaming process and development of GAD-sensitive or responsive internal and external clients as well as systems, processes, procedures, and structures. All of these are further anticipated to yield inclusive rice R4D; expanded economic opportunities in the rice industry; human resource development; and reduced poverty and vulnerability among rice farmers and their families.

SEF, on the other hand, assumes primarily that there are three possibilities when we use an organization as a subject of epistemic/knowledge flow and change. Conclusion-driven social epistemology suggests that the acceptability of a certain concept depends on the 'majority' of the people's verdict and

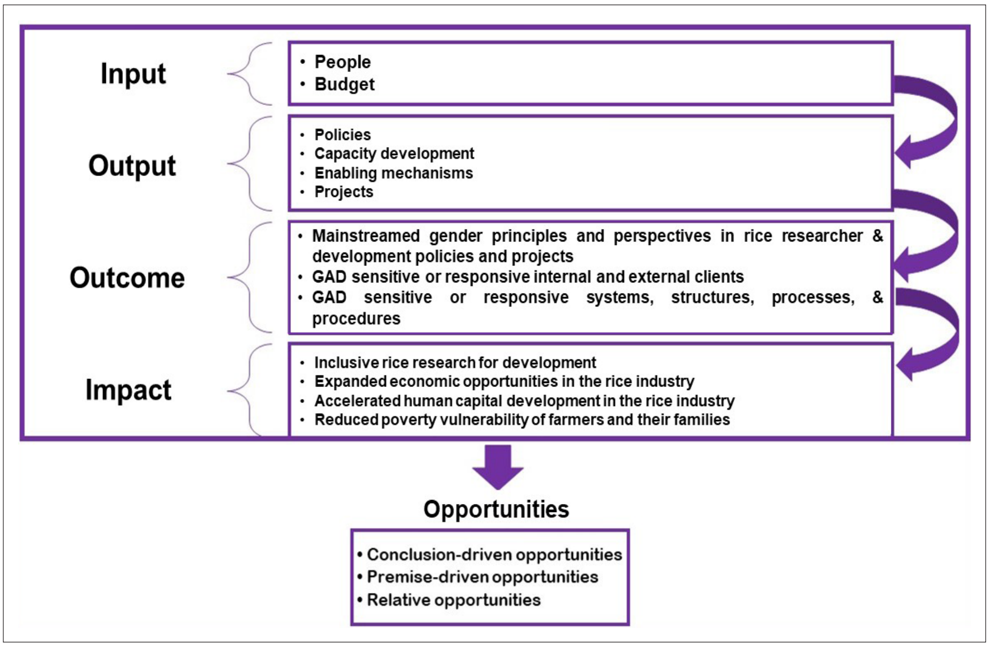

Figure 1. The PhilRice Gender Mainstreaming Framework.

that opportunities come from the propositions of the 'minority' (Goldman, 2004). Alternatively, premise-driven suggests that the acceptability of a certain concept depends on the 'weight of the truth' behind the people's verdicts and the process to achieve it. Opportunities, then, come from the most basic step to achieve the goal.

The last possibility is the epistemic relativism, which suggests that the acceptability or validity of a certain concept depends on the specific context, society, culture, or individual (Goldman et al., 2011). Thus, opportunities come from both the practical and strategic needs of the organization as reflected on the Gender Mainstreaming Evaluation Framework (GMEF) scores.

Materials and Methods

Research Design

The research is basically a study of an institution that employs a combination of qualitative and quantitative research designs. While generally descriptive and illustrative, the data were analyzed using simple quantitative methods. The data were gathered to evaluate the progress of gender mainstreaming in the rice R4D, identify the challenges in implementing gender mainstreaming in the rice R4D; and determine the opportunities that gender mainstreaming in rice R4D can offer.

Qualitative methods were used to establish hypothetical links between variables. These hypothetical links, then, were solidified into in-depth premise/s that could be tested quantitatively.

The main source of data was the documentary review of gender-related electronic and printed materials produced or released by PhilRice since January 1, 2016 - July 30, 2019. Documentary review as a social science research method was used because it is as good and sometimes even more cost effective (Mogalakwe, 2006). This method is also efficient for it is less time-consuming, unobtrusive, non-reactive, stable, exact, and has broad coverage (Bowen, 2009).

There was an innovation in the employed documentary review because it followed a structured checklist – the GMEF. The later mention framework was in response to the call of BPFA to use gender mainstreaming as a strategy in incorporating GAD in all aspects of operations, services, and programs or projects of all country signatories (PCW, 2016a). As a self-assessment tool, it has four entry points namely: policy; people; enabling mechanism (EM); and programs, projects, and activities (PAPs). Policy pertains to the "official statements and pronouncements of support for gender mainstreaming issued by the organization" while people refers to the "relevant stakeholders who assume the task of gender mainstreaming", grouped as sponsor, change agent, target, and advocate. EM focuses on the systems and mechanisms installed in the organization and the funds allocated for GAD activities such as GFPS and Knowledge Management System. PAPs pertain to flagship programs, activities, and projects that serve as a strategic entry point to mainstream GAD in an organization.

Each entry point is divided into five levels, in which each question has an increasing level of difficulty (PCW, 2016a). Level 1 is the Foundation

Formation with an equivalent point of 0 - 7.99. Level 2 is the Installation of Strategic Mechanisms, which has a corresponding point of 8 - 14.99. Level 3 is the GAD Application with a resultant point of 15 - 19.99. Level 4 is the Commitment and Institutionalization, which has an equivalent point of 20 - 23.99. Level 5 is the Replication and Innovation with a corresponding point of 24 - 25. Therefore, each entry point has a ceiling score of 25 each.

Every level has descriptors serving as questions where there are three possible answers; No, Partly Yes, and Yes with equivalent scores. Policy has an overall 13 descriptors/questions, in which Levels 1 - 4 have three descriptors while Level 5 has only one. People has 27 descriptors with Levels 1 - 4 having six descriptors while Level 5 has only three. EM has 23 descriptors, in which Level 1 has only three descriptors while Levels 2 - 5 have five. PAPs have 30 descriptors, in which Level 1 has six descriptors; Level 2, eight; Level 3, 7; Level 4, five; and Level 5, four. Overall, the GMEF checklist consists of 93 descriptors with a total point of 100. As per the overall scoring, Level 1 has a point range of 0 - 30.99. Level 2 is equivalent to 31 - 60.99. Level 3 has a corresponding point of 61 - 80.99. Level 4 has a point range of 81 - 95.99 and Level 5 is equivalent to 96 - 100.

For every descriptor answerable by Partly Yes or Yes, there is a need for justification using relevant, valid, and acceptable means of verification (MOV). These MOVs are printed or electronic documentary evidences that later on are analyzed to denote the success or failure of the gender mainstreaming employed. Using the SEF, these MOVs yielded opportunities for the organization.

Surveys and key informant interviews were employed to triangulate the gathered results. Surveys were also conducted through the four types of Training Needs Assessment (TNA) Forms, which were administered before and after the five trainings. TNAs depend on the training design of the resource person. Conversion of knowledge gained to practice was also documented through the outputs of the project implementers who attended the three specialized trainings.

Thirty-five key PhilRice personnel were also interviewed on October 10, 2019 during the validation of the scores and the corresponding MOVs. The 35 PhilRice key personnel were the GAD Focal Point System members and the staff members from the Central Experiment Station and branch stations. The validation process was conducted by going through all the descriptors of the GMEF toolkit and the documentary evidence presented. The staff members from the Philippine Commission on Women (PCW) who are the validators, asked specific questions and clarifications depending on the self-rated scores. The clarificatory questions emanated from the PCW GMEF handbook to align the rating and the required MOVs.

Locale

The study focused on PhilRice with seven branch stations and four satellite stations located in: Batac, Ilocos Norte; San Mateo, Isabela; Science City of Muñoz, Nueva Ecija; Los Baños, Laguna; Sta. Cruz, Occidental Mindoro; Ligao City, Albay; Catarman, Northern Samar; Murcia, Negros Occidental; RTRomualdez, Agusan del Norte; Maramag, Bukidnon; Midsayap, North Cotabato; and San Ramon, Zamboanga City.

Study Participants

Though all internal clients became recipients of either the policies and enabling mechanisms, there were 522 internal clients and 142 external clients, totaling 664, who directly participated in the capacity development activities of this study. A 1:1 ratio of male and female participants for the capacity development was documented (male: 333; female: 331). Their ages ranged 24 - 62 years old. The internal clients were from top management, technical staff (R&D), and administrative staff.

For the purpose of this paper, internal client is characterized as someone who is currently employed in PhilRice with either permanent or service contract status. External clients are beneficiaries of the capacity development activities, projects, and enabling mechanisms that PhilRice offers. This includes farmers, extension providers, students, and other groups who played an important role in the rice industry.

Data Analysis

Using a combination of qualitative and quantitative research methodologies, the study used both thematic and descriptive statistical analyses. For the thematic analysis, documentary reviews; self-administered assessment using a checklist; and interview results were encoded. Specific themes were searched, coded, and grouped. Data were further clustered for a more detailed analysis. For the descriptive statistics analysis, frequency and averages were used.

Results and Discussion

This study argues that the gender mainstreaming applied can be considered successful when an explicit support to GAD through policies issued, reviewed, and re-issued was present; capacity development yielded change in both internal and external clients' attitude, awareness level, knowledge, and output; established EM are fully functioning; and PAPs have incorporated GAD in any stage or component of the project cycle. It is further argued that such progress has overcome challenges and opportunities offered.

The analysis consists of three disparate but closely intertwined examinations of PhilRice's experience on gender mainstreaming. The first section evaluates the progress (if there is any) of gender mainstreaming in the realm of rice R4D. This was followed by a discussion of the embedded challenges of gender mainstreaming vis-à-vis its progress in the context of R4D. The ToC model was utilized to simplify the discussion on progress and challenges. Lastly, opportunities that guide future actions was discussed using the Social Epistemology (SE) model.

Gender Mainstreaming in Rice R4D by the Numbers

The experience of PhilRice, as shown in documentary evidence and survey results, exhibited the definite success of gender mainstreaming undertaken by the institution. Relevant and mobilizing policies were issued, reviewed, and re-issued. Changed attitude, increased awareness and knowledge, and improved output were recorded as a result of capacity development for a bulk of internal clients. EMs were also re-constituted and implemented, though few in number. Full functioning of the EMs has yet to be attained. Further, only less than a quarter of the PAPs have GAD components. This is because only half of project implementers were trained on gender analysis.

People as the Actors of Gender Mainstreaming

The data to prove that capacity development activities conducted were effective in changing attitude and increasing awareness and knowledge were taken from the pre- and post-tests of the five training courses namely: Gender, Diversity, and Inclusiveness (GDI) Training in PhilRice Central Experiment Station (CES), Gender Sensitivity Training ++ (GST++) in PhilRice Negros, Gender Sensitivity Training (GST) for Management Committee (ManCom) members, Gender Analysis Training (GAT) for project implementers, and GST++ in PhilRice-Batac. While the evidence for the conversion of knowledge gained to practice were taken from the attendees' outputs from the three gender analysis training courses: GST++ in PhilRice-Isabela, GAT for project implementers, and GST++ in PhilRice Midsayap.

There was no notable change in the attitude of the participants regarding gender and development and gender relations (Table 1). This does not mean, however, that the training courses were ineffective or did not serve their purpose. It only showed that

Table 1. Attitudinal change among participants after the trainings (N=109).

GAD Perspectives	Female		Male	
	Pre-Test	Post Test	Pre-Test	Post Test
1. A mother is the "light of the home" and a father is the "foundation of the home".	2	2	3	2
2. The man should be the main breadwinner of a family.	2	2	2	2
3. If a man is able to raise sufficient income for the family, a woman should just stay at home to take care of the needs of her children and husband.	2	2	2	2
4. If a family cannot support the education of all the children, it is right to prioritize the education of the sons since daughters will be married off to other men.	1	1	2	2
5. In a family where the man is the breadwinner and the woman do household work, the man has a right to make decisions since he carries the burden of making money.	2	1	2	2
6. A woman should be blamed if she is raped because she stays late in the streets and wears sexy clothes.	1	1	2	2
7. Having more women leaders in our school/workplace ensure the protection and implementation of women's rights.	2	2	2	2
8. Women are naturally more sensitive and emotional.	2	2	2	2
9. Men are naturally more sexually aggressive than women.	2	2	2	2
10. A woman is to be blamed if she decides to stay with her abusive husband or partner.	2	2	2	2
Total	2	2	2	2

Legend: 1- Strongly disagree, 2- Disagree, 3- Agree, 4- Strongly agree the trainees or personnel already had wiring or prior knowledge and even attitude concerning GAD and its related concepts and implications.

No change on attitude and perception regarding certain gender relations were recorded in the male and female participants. In nine out of ten statements, male and female participants retained their perception after the training. However, males changed their attitude towards the gender role of mothers and fathers. Before attending the training courses, male participants agreed that mothers are the "light of the home" while fathers are the "foundation of the home". At the end of the courses, they disagreed with the arguments or perceptions. As for female participants, they became more empowered (answered from disagree to strongly disagree) and insisted that decision making does not rely solely on the provider who is the man. There are two more statements that female participants strongly disagreed while male participants only disagree. Males disagreed that education should be awarded to males during financial difficulties and that women are to be blamed in rape cases. However, women strongly disagreed in these two perspectives. The results showed that there was no significant change in the participants' attitudes and perceptions. Though there were no incorrect answers, it is important to note that convictions on agreeing and disagreeing are missing in their respective responses. The capacity development activities need to be more aligned with the goal of completely eliminating gender biases within the institution and in the community.

For the knowledge gained, four different tools were administered by different resource persons, depending on the training design prepared. Hence,

Table 2. Participants' knowledge gained from the GST++ in PhilRice Negros (N=26).

GAD Concepts	Female		Male	
	Pre-Test	Post Test	Pre-Test	Post Test
1. Sex and gender	3	4	2	4
2. GAD	3	4	2	4
3. Gender stereotypes	2	4	3	5
4. Gender discrimination	3	4	2	4
5. Gender analysis	2	4	1	4
6. Gender mainstreaming	2	4	2	4
7. Gender equality	3	4	2	4
8. Gender equity	3	4	2	4
9. Gender perspective	2	4	1	4
Total	3	4	2	4
GAD Tools				
1. 24-hour activity profile	1	3	1	4
2. Harmonized GAD guidelines (HGDG)	1	3	1	4
Total	1	3	1	4

Legend: 1- No idea, 2- Know little about it, 3- Familiar with it, 4- Confident to talk about it, 5- Can apply it to work/output deliverables

the results are discussed separately. Overall, there was an increase in the knowledge gained by the participants with regard to GAD concepts, mandates, and tools (Tables 2, 3, and 4).

Generally, the training was successful in raising the awareness and instilling knowledge regarding GAD concepts and tools among PhilRice Negros staff members (Table 2). All the participants, male and female who were familiar or somewhat knowledgeable about GAD concepts and tools, became confident to talk about these topics after the training. Male and female participants started with no idea about GAD tools. After the training. the female participants became familiar with the tools while the male participants became confident to talk about them.

Table 3. Participants' knowledge gained from the GDI in PhilRice CES (N=38).

GAD Concepts	Female		Male	
	Pre-Test	Post Test	Pre-Test	Post Test
1. Sex and gender	2	1	2	1
2. Gender bias	2	1	2	1
3. GPB	2	1	2	2
4. Strategic needs	2	1	2	2
Total	2	1	2	2

Legend: 1- Correct answer, 2- Incorrect answer

Pre- and post-test results of another GAD-related training (Table 3) showed that female participants learned the relevant GAD concepts. However, male participants had no to low knowledge gain. In a detailed analysis, male participants learned the difference between sex and gender and the concept of gender bias, yet were not able to comprehend the process of GAD Planning and Budgeting (GPB) and the different strategic needs.

Table 4 shows the pre- and post-test results of two GAD-related training courses for the top management and project implementers. Before the training, male and female participants knew little about GAD concepts. After the training, they became familiar with the concepts.

Overall, the knowledge of male and female participants on GAD policies, mandates, and tools remained unchanged (Table 4). In a detailed examination, the knowledge of male participants about the BPFA increased. Before, they had no idea about it but after the training they gained little knowledge about it. Similarly, male and female participants showed increased in knowledge on Magna Carta of Women after the training (from little knowledge to being familiar). For the tools, the training improved (from little knowledge to being

Table 4. Participants' knowledge gained from GST for the Management Committee (ManCom) and GAT for Project Implementers in PhilRice CES (N=78).

GAD Concepts	Female		Male	
	Pre-Test	Post Test	Pre-Test	Post Test
1. Sex and gender	3	4	3	4
2. GAD	3	3	2	3
3. Gender discrimination	3	4	3	3
4. Gender analysis	2	3	2	3
5. Gender mainstreaming	2	3	2	3
6. GPB	2	3	2	3
Total	2	3	2	3
GAD Policies and Mandates				
1. Convention on the elimination of all forms of discrimination against women (UN CEDAW)	2	2	2	2
2. Beijing Platform for Action (BPFA)	2	2	1	2
3. Millennium development goals and sustainable development goals	2	2	2	2
4. Republic Act 7192 (Women in development and nation building Act of 1992)	2	2	2	2
5. Philippine plan for gender-responsive development (1995-2025)	2	2	2	2
6. Republic Act 9710 (Magna Carta of Women/MCW)	2	3	2	3
7. Joint Circular 2012-01: Guidelines for the preparation of annual GPB and AR to implement the MCW	2	2	2	2
Total	2	2	2	2
GAD Tools				
1. HGDG	2	3	2	3
1. GMEF	2	2	2	3
1. Participatory Gender Audit	2	2	2	2
Total	2	2	2	2

Legend: 1- No idea, 2- Know little about it, 3- Familiar with it, 4- Confident to talk about it, 5- Able to apply/use it at work

familiar) the knowledge of both male and female participants about Harmonized GAD Guidelines while male participants demonstrated an increase in knowledge about Gender Mainstreaming Evaluation Framework (from little knowledge to being familiar).

For the last instrument, the participants were directly asked about their knowledge level about gender analysis and gender mainstreaming collectively. In a range of one to five, participants rated themselves two before the training. This self-rating increased into three after the training.

In converting knowledge into output, selected participants of the three gender analysis trainings submitted nine revised project protocols. They incorporated GAD in two of the three project cycles – implementation/management and monitoring/evaluation. They used and analyzed sex disaggregated data (SDD) and explicitly included these in the rationale, identified GAD objectives, conducted GAD-related activities, and established sex disaggregated database.

Given the usual limitations, these results show the importance of developing gender-sensitive or responsive human resources for the whole process of gender mainstreaming. Gender-focused capacity development activities develop staff to craft gender sensitive policies, establish gender equal EMs, and implement inclusive rice R4D PAPs.

Policy as a Powerful Tool in Gender Mainstreaming

Comparing the policy releases for the span of four years (2016 – 2019), there was a notable increase in 2018 and 2019 (Figure 2). Almost half of the GAD-related memoranda and administrative orders were

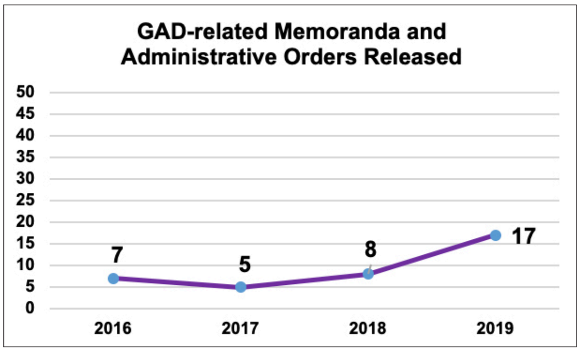

Figure 2. GAD-related policies released from 2016 to 2019.

released in 2019 (46%) while almost a quarter were released in 2018 (22%), way higher than the baseline – 2016 releases (19%). However, there was a halt on the releases in 2017 (14%).

Such increase was in response to the close working relationship of PhilRice with a number of oversight agencies and the increased consciousness within the institution on the importance of GAD in the operations, workplace, and R4D.

In terms of contents, policies released in 2016 stated directives on answering the Audit Observation Memorandum (AOM) issued by Commission on Audit, capacity development, database establishment, GFPS reconstitution, and budget allocation. In 2017, policies covered almost similar directives in 2016 except for the broad statement to support a national women-related celebration and the full functioning of an enabling mechanism – the PhilRice Day Care Center. From 2016 to 2018, policies were reviewed and re-issued together with a broad statement on the use of gender analysis to mainstream GAD in Rice R4D and establishment of new enabling mechanism – flexible working time for primary care providers. In 2019, a variety of policies were issued. This included the creation of branch stations' GFPS, series of diverse capacity developments such as GAD orientations, Gender Sensitivity and Gender Analysis training, and GPB workshops. The issuance of standard attendance sheet for centralized database, reconstitution of an enabling mechanism – Committee on Decorum and Investigation for Sexual Harassment Cases, reiteration on the use of Gender Fair Language, issuance of special laws on women, and requiring the R4D sector to incorporate GAD in all project documents like protocols were also included. It implicates that there was an aggressive release of policies encouraging the mainstreaming of GAD in all aspects of PhilRice's services, operations, and R4D efforts.

These results show the power of policy to move and pressure R4D workers in mainstreaming GAD, to sustain gender mainstreaming through the establishment of EMs, and to incorporate GAD in the planning, implementation/management, and monitoring/evaluation (M&E) of rice R&D PAPs.

Theory of Change (ToC) Model for PhilRice Gender Mainstreaming

In part A of the results and discussion, PhilRice invested money for willing and competent human capital. It is intended that these inputs are expected to yield outputs that contribute in the attainment of the outcome. The outcome would then be the basis for the evaluation of impacts as gender mainstreaming progresses. Each of the concepts – input, output, outcome, and impact – has their own innate logical relatedness to each other. Figure 3 is the ToC Model of PhilRice's Gender Mainstreaming efforts showing the relatedness of each.

The model shows that as the gender mainstreaming progresses, there is a higher possibility to meet various and difficult challenges. Given these challenges, PhilRice became more judicious in the use and disposal of resources, especially budget in the mainstreaming of GAD and attaining GAD-related outputs. There were two challenges that PhilRice faced at this phase. The first challenge is budget management. Due to the limited direct budget for GAD operations, there should be a prioritization scheme that GFPS should follow. The question as to what should be done first is critical. A preferred course of action on gender mainstreaming will either move the agenda forward or stagnate. The second challenge is to identify the strategic human resource that will accompany the GFPS to lead gender mainstreaming. The need to have champions and advocates are critical at this stage and undertaking. Willingness and competence to lead are two important qualifications need for these personnel will dictate the credibility of the people and the reliability of the message.

As its major accomplishment, PhilRice produced GAD-related outputs such as policies, capacity development activities, EMs, and PAPs. There were 37 GAD-related polices released between January 1, 2016 and July 30, 2019. Six hundred sixty-four internal and external clients were also oriented or trained on GAD concepts, mandates, and tools. PhilRice also established three major EMs namely: Committee on Decorum and Investigation (CODI), Day Care Center, and membership to Regional GAD Council. Supplementary EMs like flexible time for primary care providers and quick service provision such as priority lanes, Officer-of-the-Day, and PhilRice Text Center for internal and external clients were strengthened. Lastly, nine notable R&D PAPs became gender-sensitive/responsive.

At the output phase, more demanding challenges emerged. Quality and quantity of outputs were both at stake. Quality of output dictates the message and the conviction in the communication process. This requires that the message about the purpose of all the efforts and the specific direction of the intervention must be clear. Resource persons and internal GAD advocates should work together to analyze the audience and create an atmosphere of both learning and reflection. Quantity, on the other hand, dictates the possible maximum reach and strength as evidenced by the number. This does not refer to the number of outputs but rather on the number of recipients and their ability to cascade and share what they have learned and experienced.

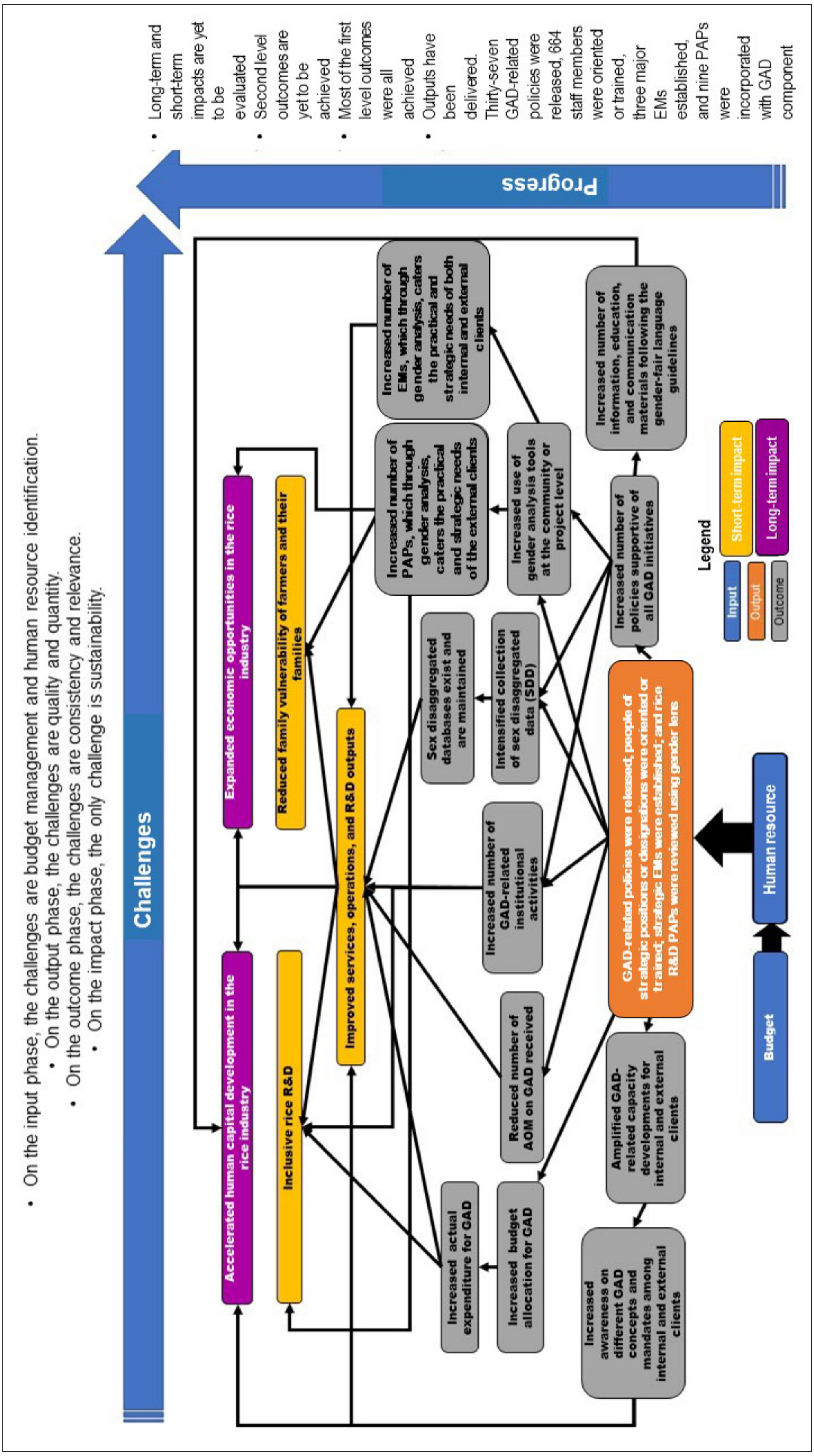

Figure 3. ToC Model for PhilRice Gender Mainstreaming.

First level outcomes are the results of the existence of the outputs. In the case of PhilRice, most of the first level outcomes were achieved. As stated in the earlier discussion, capacity development was amplified for the internal and external clients to become aware; be knowledgeable; and be able to apply GAD concepts, mandates, and tools. GAD-related activities and collection of SDD were also considered. Due to the continuous capacity development, there was an increase in the application of gender analysis initially in compliance to the GAD-related policies issued. Second level outcomes are the result of the achievement of the first level outcomes. PhilRice has yet to actualize its second level outcomes. The centralized GAD database is still on its conceptualization stage. Number of R4D PAPs and EMs, which underwent gender analysis were still few.

Except for non-achievement yet of these outcomes, other challenges like consistency and relevance are also emerging. Consistency pertains to the process by which the message is passed on from the primary recipient to the secondary recipients. Cascading and/or re-echo is a crucial aspect of gender mainstreaming. GAD is a complex concept and it is in the borderline of private and public life of a person. Understanding the audience is very important while making sure that there are no inconsistent and warring arguments. Further, relevance pertains to the amount of relative importance. In connection with consistency, the message should be of great use to the audience, who will then become the actors of gender mainstreaming. Otherwise, GAD will just become an advocacy of few people.

For PhilRice, the short- and long-term impacts are yet to be evaluated. However, these are the most relevant for rice R4D and the external clients. As the institution is just starting to learn and apply GAD, impacts are yet to be observed. However, in evaluating the impact of GAD there is a greater challenge – sustainability. It pertains to the unique and relative strategies to maintain the good practices of gender mainstreaming. Moreover, the key in sustainability is innovativeness. It is the ability to transform old practices into new practices that are relevant across time and generation.

These progress and challenges identified and the GMEF score of PhilRice support each other. After validation, PhilRice received a score of 67.1 that can be translated into a qualitative description of GAD Application. Based on the qualitative description, it is factual that PhilRice is still on its transition from Output to, at the very least, first level outcome phase of the ToC.

Social Epistemological (SE) Model of PhilRice Gender Mainstreaming

Based on the PhilRice Gender Mainstreaming Framework, there were three possibilities that could yield opportunities for achieving the outcomes and later influencing the rice R4D and the lives of the external clients specially the farmers. The social epistemological model of PhilRice's gender mainstreaming efforts (Figure 4) shows the different angles to view gender mainstreaming and its possible opportunities.

The SE model shows where opportunities are coming from. The elements of this model are the GMEF entry points and PhilRice's score in the concomitant checklist. As discussed earlier in this paper, the GMEF checklist was used to assess the level of gender mainstreaming efforts of PhilRice as a government institution that primarily do R4D on rice. It was also stated earlier that GMEF has

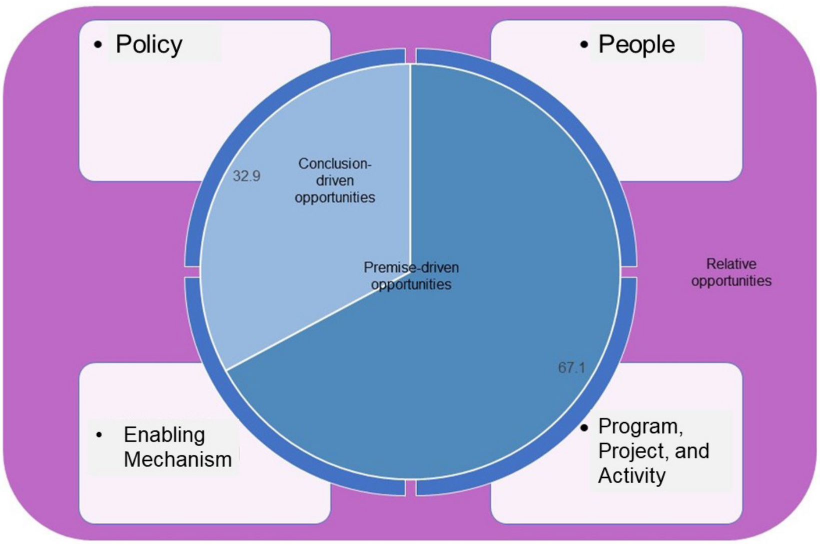

Figure 4. SE Model of PhilRice Gender Mainstreaming.

four entry points, which serve as pivotal points of the assessment. This model adopted these four entry points. Also shown in the model is a pie chart showing the score of PhilRice (darker blue) in the GMEF checklist, which is 67.1.

For SEF, the validity of the evidences (documentary review) and the acceptance of people (*i.e.,* key informant interviews/KII) are important in assessing the success of a certain innovation – gender mainstreaming. SEF also assumed that the success or failure of this innovation will yield knowledge and opportunities for improvement. There are three possibilities that SEF is assuming to occur. These possibilities are the conclusion-driven, premise-driven, and relativism. Conclusion- and premise-driven are warring possibilities while relativism is an independent one. Table 5 shows the scenarios that the assessment of PhilRice gender mainstreaming had gone through. Validity of the documentary evidences was based on the list of means of verification universal to all.

Table 5. PhilRice gender mainstreaming scenario.

Scenarios	Valid documentary evidences?	Are there objections from the key personnel?	Gender mainstreaming successful?
1	Valid	No objection	Yes
2	Not valid	No objection	No
3	Valid	With objection	Yes

During the validation sessions, not all documentary evidence was considered valid and not all valid documentary evidence was free from objections from the validation team or KII. Those valid documentary evidence both with or without objection were accepted as proof of the success of PhilRice gender mainstreaming. However, all not valid documentary evidence, even without objection, yielded unsuccessful gender mainstreaming.

Using the conclusion-driven opportunities as an argument, only those items with no valid documentary evidences yielded opportunities for the institution. Following this proposition, PhilRice should work more on perfecting its policies, PAPs, and partnership strategies to become a learning hub and be recognized and replicated by other organizations. Opportunities for gender mainstreaming exist in urging the top management to raise GAD concerns during high level meetings/discussions; developing external clients into GAD champions and endorse them for recognition; increasing the budget expenditure to 100%; making the EMs fully functional and resulted to desired impacts; conducting, completing, and reporting impact assessment of PAPs; capacitating and motivating the GFPS to function properly and be recognized by reputable organizations; establishing centralized database and making it accessible for internal and external clients; and integrating GAD in the institute's knowledge management system and open for replication.

Compared with the current status of gender mainstreaming in PhilRice, most of the opportunities identified by conclusion-driven proposition are tenfold distant. This means that more attention must be paid to these to effect deeper gender mainstreaming.

In contrast, premise-driven proposition reiterated the step-by-step process rather than look at what are and are not done. Premise-driven opportunities stated that valid and not valid documentary evidence yielded opportunities for the institution. Following this proposition, PhilRice should work first on the basics such as formulating and adopting a GAD Agenda or Strategic Framework; creating effective GAD policies that could become a model for other organizations; making sure that all GFPS members occupy strategic positions and capacitating 50% of them to become GAD resource persons to achieve full functioning and to be recognized as model of other organizations; urging the top management to raise GAD concerns during high level meetings/discussions; making the EMs fully functional and resulted to desired impacts; and making the PAPs a GAD learning hub.

For the third argument, that of relativism, success depends mainly on the holistic interpretation of scores received by each entry point. It argues that the scores are the reflection of what exists and does not relative to the performance of the institute. Using this proposition, the GMEF analysis and interpretation of scores was followed. Entry point Policy received a score of 15.84 (Table 6). This means that there was a GAD application on the issuances of policies and that GAD-related policies were released. If there is already a GAD application, then the opportunities focused only on the impacts of those policy issued, reviewed, or re-issued and continuous enhancement of these gender-sensitive or gender-responsive policies for the consumption of other organizations.

Table 6. PhilRice gender mainstreaming score.

Entry Point	Score	Score Description
Policy	15.84	GAD application
People	17.39	GAD application
EM	15.51	GAD application
PAPs	18.36	GAD application

Entry point people got a score of 17.39. This entails that in the realm of human resource, there was already a GAD Application. What is lacking is capacitating GFPS members to become resource persons or GAD experts within and outside the organization, customizing GAD tools relative to the organization's mandates by the GFPS, and raising GAD concerns in high level meetings by the top management.

EM received a score of 15.51. Though it is the lowest among the four entry points, the score description is still under GAD application. This means that the opportunities for PhilRice's gender mainstreaming are making the EMs able to track gender-related impacts, producing GAD-related knowledge products (KPs), and making the whole budget gender-responsive.

Lastly, PAPs got a score of 18.36. This entry point received the highest score yet it is still under the GAD Application. This means that PhilRice still needs to ensure the sustainable monitoring of its PAPs and evaluate the impacts, conduct sector-specific capacity development sessions for both internal and external clients, periodically apply and re-apply gender analysis tools to ensure integration of GAD in the PAPs, develop a sustainable action plan for GAD, make the PAPs and partnership strategy/convergence model a learning hub, and make KPs that can be accessed and referenced on by other organization.

Conclusion and Recommendations

On the progress of gender mainstreaming. Thirty-seven gender-related policies, 664 internal and external clients oriented and/or trained, three major EM established, and nine gender-sensitive and/or responsive PAPs is a commendable progress for PhilRice, given the relatively short period of evaluation. As a general rule in any other baselining activity, continuous implementation; monitoring and evaluation; and planning cycle is very important. This means that periodic self-assessment may be applicable in order to track the progress and extent of gender mainstreaming efforts of the institute. While the framework used in this paper is in the trial stage, it can be good starting point for the institute or other agencies that aim to fully mainstream gender in their institutional operations and functions.

On the challenges of gender mainstreaming. Different phases of change have different levels of difficulty. Thus, they have different challenges to face. At the onset, managing financial and human resources are the primary challenges. Critical to every course of action are the actors and their means to act. If there is no action done, there will be no result to expect. As such, prioritization can counter financial challenges and continuous effort to involve those 'willing' to be involved. Willingness is important because action is dictated by cognition and affectual state, characters that need to be considered in the selection of people to be placed and assigned to do the gender mainstreaming work.

As the stages progress, the encounters become more difficult as the quality and quantity of output are at stake. Careful transmission of message and proper identification of people that could disseminate information are important. As another step on the ladder, more difficult challenges exist. Consistency and relevance are then under question. With more people talking about and doing gender mainstreaming, variations in beliefs and practices may influence courses of action. Reiteration and re-orientation, then, are vital. On the other hand, relevance may be inquired upon or even questioned. The query on 'what is it for?' will surely recur and even stand out.

Understanding the audience/recipients of information and relating to the context are approaches that are useful. After establishing the mechanisms, sustainability will be the next challenge that must be addressed. Conditioning through proper documentation, cascading, and social mobilization is the key to maintaining gender mainstreaming efforts. Teach the r&D workers, let them internalize and reflect on it, oblige them to do it, and reward them by doing it and it will be unconsciously done repeatedly. Ultimately, this is what the Social Epistemological Framework is all about – when a practice becomes part of the operational behavior of an organization or a community.

On the opportunities for gender mainstreaming. For the PhilRice's gender mainstreaming efforts, though perceived on different angle, there are four merging points of the conclusion, premise-driven, and relativist propositions. First, PhilRice should issue, review, and re-issue effective gender policies to encourage internal clients to act, to establish EMs, and to polish PAPs towards the achievement of gender-responsive institution in which other organizations may model. Second, top management should provide an unequivocal support through being aware and knowledgeable advocates by raising gender and development concerns during high level meetings. Third, EMs should not be established just for compliance rather, there must be a conscious drive so that the mainstreaming efforts must yield the desired impacts. Lastly, gender-sensitive or gender-responsive PAPs should be rewarded. This means that PAPs that obtain such recognition must become learning hubs within and outside the organization. By doing these, gender mainstreaming would have high impact not only to the institution but also to the whole rice research and development and expectantly passed on to the community level.

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FULL PAPER

ANTHROPOMETRIC ASSESSMENT OF MALNUTRITION AMONG 0-12-YEAR-OLD CHILDREN IN RICE FARMING AREAS IN THE PHILIPPINES

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Abstract

Young children, especially in rural areas, are the most vulnerable to malnutrition. The first 12 years of children is a critical period for their physical and intellectual development; thus, the importance of proper nutrition to support this phase. This study assessed the rate and prevalence of malnutrition among 0 to 12-year-old children in 24 rice-producing provinces in the Philippines. The nutritional status of 2,472 children (1,189 boys and 1,283 girls) was measured using the most common anthropometric indicators: underweight, stunting and wasting, and overnutrition bases including overweight and obese. The indicators followed the recommended cut-off points from Child Growth Standard (CGS) and World Health Organization (WHO) Reference 2007. The Composite Index of Anthropometric Failure (CIAF) was also employed to measure overall prevalence of undernutrition. Malnutrition was common among 0 - 12-year-old children manifested mostly through stunting, which affected 46% of the children. Preschoolers were the most vulnerable to undernutrition among other age groups. Male children were also observed to be more vulnerable to malnutrition than their female counterparts. Across sample provinces, the highest magnitude of total undernourished children was observed in North Cotabato, South Cotabato, Zamboanga Sibugay, and Camarines Sur. Logit regression results showed significant association between poverty and undernutrition. Other socioeconomic factors like low educational attainment, large household size, small rice landholding, and dependence on rice income alone were also positively associated with undernutrition. Government investment and implementation of province-specific nutrition programs such as nutrition-related seminars, backyard gardening and integrated farming system, and promotion of complementary foods and diversified diet is recommended to enrich farm household's knowledge on nutrition, improve access to healthy and nutritious diet, and enhance the children's health status.

Keywords: *Anthropometric Failures, Malnutrition, Undernutrition, 0 - 12-year-old children.*

Introduction

The World Health Organization (WHO, 2020) defines malnutrition as "deficiencies, excesses, or imbalances in individual's intake of energy and/or nutrients" that continues to claim millions of lives among children. It involves two broad conditions: "undernutrition," which includes stunting (low height for age), wasting (low weight for height), underweight (low weight for age), and micronutrient deficiencies; and "over-nutrition," which includes overweight, obesity, and related non-communicable diseases like heart disease, stroke, diabetes, and cancer. Childhood malnutrition is a major global health problem contributing to increased morbidity and mortality, impaired intellectual development, suboptimal adult work capacity and increased risk of disease in adulthood (WHO, 2012). Of the annual 7.6 million deaths among children under 5 years of age, 20% was caused by underweight conditions (WHO, 2012). Malnutrition is prevalent in all countries. A joint report by the United Nations Children's Fund (UNICEF), WHO, and WorldBank (2021) revealed that 59% and 72% of all children affected by stunting and wasting, respectively, are found in lower-middle-income countries; while 45% of overweight children live in upper-middle-income countries.

In the Philippines, malnutrition among 0 - 12-year-old children is a continuing challenge. The 2019 National Nutrition Survey (NNS) of the DOST's Food and Nutrition Research Institute (DOST-FNRI) showed that 19 in every 100 Filipino children less than 5 years of age were underweight; 29, stunted; 6, wasted; and 3, overweight-for-height. Additionally, 26% of school-age children from 5 to 10 years old were underweight; 25%, stunted; 8%, wasted; and 9%, overweight or obese. The 2021 UNICEF-WHO-

WorldBank joint report indicated "high" prevalence of stunting among children in the Philippines at 28.7%. This places the Philippines sixth among the 15 countries in the East Asia and Pacific region with the highest prevalence of stunting. Meanwhile, overweight and wasting were at "low" and "medium" prevalence level, respectively, among children in the country.

According to the DOST-FNRI (2007), malnutrition is attributed to poverty and food insecurity or the failure to access nutritious food. The Social Weather Stations (SWS, 2021) reported that about 4.2 million families have experienced involuntary hunger in the first quarter of 2021. Additionally, around 16.6% (17.6 million) of Filipinos were considered poor in 2018, with their annual per capita income less than the poverty threshold. This implies that their per capita income is not sufficient to meet their basic food and non-food needs (PSA, 2019). Most of these poor families are in the rural areas where 31.6% are farmers (PSA, 2018). This shows that hunger and food insecurity, the most critical manifestation of poverty and a determinant of nutritional status, are still prevalent in rice farming areas.

It is ironic that the children of farmers who produce the food, specifically the rice that we eat, experience hunger; making them vulnerable to malnutrition. This is a pressing issue as proper nutrition is vital during the first 12 years of a child's life to support physical and intellectual development, which is foundational to growth in subsequent years. It is also during this period that children are most vulnerable to malnutrition. The study of Capanzana et al. (2018) also confirmed the prevalence of malnutrition among children aged 0 - 10 years old in households headed by farmers, forestry workers, and fisherfolks. Thus, studies focusing on the nutritional status of children in various sectors are needed for strategic interventions.

Generally, this study assessed the overall prevalence of malnutrition among 0 - 12-year-old children in rice farming areas in the Philippines using anthropometric method. Specifically, it (1) determined the prevalence of malnutrition by sex, age, and geographical areas; (2) examined the magnitude of malnutrition incidence; (3) identified the determinants of malnutrition incidence; and (4) identified policy recommendations from the results.

Materials and Methods

Source of Data

The study initially identified 3,525 rice farming households in 24 rice-producing provinces in the Philippines with an average rice yield of less than 4 t ha^{-1} and 2.5 t ha^{-1} in irrigated and rainfed areas, respectively. From this group, 1,419 households with children aged 0 to 12 years old were filtered and selected from which primary data were collected.

Children aged 0 - 12 years old including those that are in extended families and are living with the rice farming households were completely enumerated. There were 2,472 children (1,189 boys and 1,283 girls) from the 1,419 rice farming households measured by the research team. Anthropometric data such as age, sex, and physical body measurements (height and weight) were measured from each child following the standard technique. Height was taken in centimeter using a measuring tape while weight was determined in kg using a weighing or bathroom scale for children aged 3 and above. The height of children who were less than 2 years old and cannot stand independently was measured using an infantometer. These children were also weighed while being held by their mother or caregiver with the weight of the mother or caregiver subtracted from the total obtained weight. Other information including socioeconomic characteristics and income level were gathered from the rice farming households using a structured survey questionnaire. Table 1 shows the distribution of the sampled children by province.

Table 1. Distribution of 0 - 12-year-old children, sample provinces, Philippines

Province	0 - 12-year- old children		
	Girl	Boy	All Sex
All Provinces	1,283	1,189	2,472
Abra	64	78	142
Agusan del Norte	42	44	86
Aklan	45	40	85
Albay	19	7	26
Antique	7	14	21
Apayao	65	80	145
Aurora	48	50	98
Bulacan	54	53	107
Cagayan	32	45	77
Camarines Sur	91	93	184
Capiz	33	29	62
Compostela Valley	14	24	38
Davao del Norte	48	50	98
Ilocos Norte	20	32	52
Iloilo	71	73	144
Isabela	148	149	297
Negros Occidental	22	19	41
North Cotabato	106	115	221
Nueva Ecija	6	8	14
South Cotabato	19	15	34
Sultan Kudarat	140	155	295
Surigao del Norte	20	22	42
Zamboanga del Norte	29	33	62
Zamboanga Sibugay	46	55	101

Table 2 shows the distribution of 0 - 12-year-old children by age group in the sample rice farming areas. Children aged 6 - 10 years old composed most of the sample size from the sample areas (1,065), followed by preschoolers or 0 - 5 years old (928) and children aged 11 - 12 years old (479). This suggests that majority of the population are children aged 10 years and below who are at greater risk of malnutrition. Children within this age group are still in their growing period, in which high intake of protein and calories and proper nourishment is essential. For each age group, the total number of girls were quite larger than boys.

Table 2. Distribution of sample children by age group and sex, Philippines.

Sex	Age group			
	0 - 5	6 - 10	11 - 12	All (0 - 12)
Boys	451	502	236	1,189
Girls	477	563	243	1,283
All Sexes	928	1065	479	2,472

Analyses of data

Anthropometric measurement was used in the study to assess growth or nutritional status among 0 - 12-year-old children in rice farming areas in the Philippines. Three of the internationally recommended and most commonly used anthropometric indicators: low weight-for-age *(underweight)*, low height-for-age *(stunting)*, and low weight-for height *(wasting)* were used to evaluate the children's nutritional status. These indicators involve the direct measurement of a person's height and weight compared with the normal or acceptable measurement for their sex and age. Specifically, the WHO's Child Growth Standard (CGS) was used as reference data for children below 5 years old while the WHO Reference 2007 was used as reference standard for children aged 6 - 12 years old. The CGS describes the normal child growth from birth to 5 years under optimal environmental conditions, and can be applied to all children everywhere, regardless of ethnicity, socioeconomic status, and type of feeding. The WHO Reference 2007 is a reconstruction of the 1997 National Center for Health Statistics (NCHS)/WHO reference. Published in 2006, it complements the WHO child growth standards for 0 - 60 months.

To measure malnutrition, the Z-score or standard deviation (SD) system recommended by WHO was used. This system measures the three anthropometric indices and expresses the results in terms of z-scores or standard deviation units from the median of the reference standard. That is, *height-for-age z-scores* (HAZ), *weight-for-age z-scores* (WAZ), and *weight-for-height z-scores* (WHZ). Based on the internationally recognized method proposed by WHO, a child is considered undernourished if any of the indicators fall below two standard deviations ($\leq$ 2SD) of the median value of CGS and WHO Reference 2007. Since weight-for-height (WHZ) indices are not available for children older than 5 years old, the BMI z-scores were used to evaluate the prevalence of undernutrition for children aged 6 - 12 years old. Likewise, over-nourished children were also determined using the z-score system.

According to Mandal et al. (2009), the three conventional anthropometric indices (stunting, wasting, and underweight) may be misleading in providing the total number of undernourished children in a particular geographical area because they overlap. That is, a child who is underweight may also be wasting and/or be stunted and other similar combinations. Thus, the Composite Index of Anthropometric Failure (CIAF) was employed to measure the overall prevalence of undernutrition among young children more accurately. The CIAF is an aggregate indicator of the total estimate of the number of undernourished children in a population, whether they are wasting and/or stunted and/or underweight.

In evaluating CIAF, Svedberg's model that was modified by Nandy et al. (2005) was used, which includes seven groups of children (A to Y) as shown in Table 3. These groups include children with height and weight that are normal for their age (above -2 z-scores) and with no 'anthropometric failure (Group A)', and children whose height and weight for their

Table 3. Classification of children with anthropometric failure (CIAF)[a/].

Group Name	Description	Wasting	Stunting	Underweight
A	No anthropometric failure	No	No	No
B	Wasting only	Yes	No	No
C	Wasting and underweight	Yes	No	Yes
D	Wasting, stunting, and underweight	Yes	Yes	Yes
E	Stunting and underweight	No	Yes	Yes
F	Stunting only	No	Yes	No
Y	Underweight only	No	No	Yes

[a/] Nandy et al., 2005.

age are below the normal (below -2 z-scores) and thus experiencing one or more forms of 'anthropometric failure'. The CIAF excludes children with no 'anthropometric failure' (Group A) and includes all children who are wasted, stunted, or underweight, and their combinations (Group B to Y).

Additionally, the following provides a comprehensive description of the anthropometric indices used for assessing child growth or nutritional status. Stunted growth refers to low height-for-age, which is a child short for his/her age but not necessarily thin. Also known as chronic malnutrition, this carries long-term developmental risks. This measures long-term growth faltering as a result of chronic malnutrition. Underweight refers to low weight-for-age, that is, a child can be either thin or short for his/her age. It represents both inadequate linear growth and poor body proportions caused by malnutrition. This reflects a combination of chronic and acute malnutrition. Wasting refers to low weight-for-height, which indicates a deficit in tissue and fat mass due to lack of nutritious food, and is a result of acute malnutrition (WHO, 2020).

Descriptive statistics such as frequency, means, percentile, and standard deviations were used in the analysis of the survey data. Tabular analysis and logistic regression analysis using STATA were also employed to assess the determinants of malnutrition or anthropometric failures. Based on several studies (Ahmad, 2020; Capanzana et al., 2018; FNRI, 2020; Kalu & Etim, 2018), it is expected that the poverty status of the household, low educational attainment of the household head, and large household size are contributory factors to the undernutrition of 0 - 12-year-old children. Rice as only income source, households headed by females, and small farm area are also hypothesized to be additional factors of undernutrition.

Results and Discussion

Socioeconomic characteristics of rice farming households

The mean household size of the sampled rice farming households coincides with the national average of five members (Table 4). Each household had at least one young household member within the age group of 0 - 12 years old. Two-third of the household heads were male, mostly with 10 years of education or have completed secondary education and are still in their productive years with a mean age of 49 years old and with 21 years of farming experience. This means that they have been farming for almost half of their lives. Rice farming households cultivated a total rice area of 1.53 ha with 41% of them having ownership of the rice land and has one member working in the farm. Correspondingly, rice farming is their main source of income accounting for 45% of their total household income. They also engaged in other farming and non-farming activities to supplement their income from rice farming. Non-farm income has a significant share of 37% to the total household income, which is derived from employment, remittances, and business.

Considering the household income portfolio and household size, 63% of the rice farming households were considered poor, that is, their per capita income was below the poverty threshold. Additionally, 51% of the households were regarded as food poor or food insecure where their total household income was insufficient to satisfy the daily per capita food requirement of 2,000 kcal per household member (Table 4). The relatively higher incidence of poverty among farming households may be partly attributed to the selection criteria of the sample provinces whereby the yield levels are below the national average. Consequently, Mbuya et al. (2021) found that Philippine agricultural households have poor quality diet with lower protein and fruit consumption and lower dietary diversity relative to non-agricultural households. In particular, Filipino school age children (6 - 12 years old) have low diet diversity with their diet mostly composed of rice and non-nutritious food (Mak et al., 2019). On average, only four out of nine food groups were consumed by these children per day.

Anthropometric measurements of sample children

Table 5 presents the mean age, weight, and height among children by age group. The mean age in preschoolers (0 - 5 years old) was 3 years; in 6 - 10, 8; and in 11 - 12, 11. It can be observed that the mean values for weight and height increased with age, which can be explained by the normal physical growth of children. Specifically, mean height was 83.3 cm in age group 0 - 5; 114.3 cm in 6 - 10; and 131.7 cm in 11 - 12. There is also a significant mean difference in the values of weight across age groups: 11.8 kg in 0 - 5-year-olds; 20.9 kg, 6 - 10-year-olds; and 28.6 kg, 11 - 12-year-olds. Across sex, the mean values for age, weight, and height were almost the same.

Incidence of malnutrition across age groups and sex

The incidence of malnutrition varies based on sex and age group of children. However, stunting was consistently the most prevalent malnutrition problem in all sex and age groups in the study areas (Figures 1-3). This means that many of the children in rice farming households are under height for their age. Growth failure was more common among 0 - 12-year-old boys than girls. This suggests that males are at

Table 4. Household's socioeconomic characteristics, Philippines, 2010.

Item	All households
Sample size (n)	*3,525*
Household size	5
Number of children, 0 - 12-years old	1
Number of households working in the farm	1
Household head:	
Male household head (%)	67
Ave. education (in years)	10
Ave. age (in years)	49
Farming experience (in years)	21
Ave. rice area (ha)	1.53
Land ownership (%)	41
Household income composition (PhP)	
Rice farming	48,832 (45%)
Non-rice farming	18,818 (17%)
Non-agriculture	40,250 (37%)
Total	108,170 (100%)
Household's subsistence characteristics:	
Poverty incidence (<poverty threshold)	63%
Food poor (<food threshold)	51%

Table 5. Mean age, weight, and height, 0 - 12-year-old children, Philippines.

Nutrition status by sex	Children by age group (in number of years)		
	0 - 5	6 - 10	11 - 12
Boys			
Mean age (yrs.)	2.6	7.6	11.02
Mean weight (kg)	11.9	21.1	28.3
Mean height (cm)	82.7	114.5	132.0
Girls			
Mean age (yrs.)	2.6	7.6	11.04
Mean weight (kg)	11.7	20.7	29.0
Mean height (cm)	84.0	114.1	131.4
All Sexes			
Mean age (yrs.)	2.6	7.6	11.03
Mean weight (kg)	11.8	20.9	28.6
Mean height (cm)	83.3	114.3	131.7

greater risk to the different forms of malnutrition than their female counterparts, which corroborates with previous nutrition studies (Ahmad et al., 2020; Sulaiman et al., 2018; Thurstans et al., 2020) as well as the results of the 2018 NNS by the DOST-FNRI (2020). Sulaiman et al. (2018) suggested that male children are more likely to be undernourished because they spend more time outdoors than girls.

Among the sample preschoolers (0 - 5 years old), 46% were short in height or stunted for their age, 26% were underweight, and 17% were suffering from wasting or severe thinness (Figure 1). These are brought by inadequate nutrition over a short period of time. Overnutrition was also observed with 16% of the studied children in the age group being overweight.

These rates of the different forms of malnutrition among 0 - 5-year-old children were higher than the results of the 2019 NNS. This means that the prevalence of malnutrition among children in rice farming areas is higher than the overall prevalence of malnutrition among children in the Philippines. This also indicates the severity of the problem in the farming sector given that providing the nutritional requirements of infants and young children during their first 1,000 days from conception is most imperative to support their optimum health, growth, and cognitive development (DOST-FNRI, 2020). Failure to do so may result in irreversible damage to children caused by malnutrition, which could affect their growth, development, and performance as an individual in their subsequent years.

Comparably, stunting was more prevalent in children aged 6 - 10 (Figure 2) and 11 - 12 years old

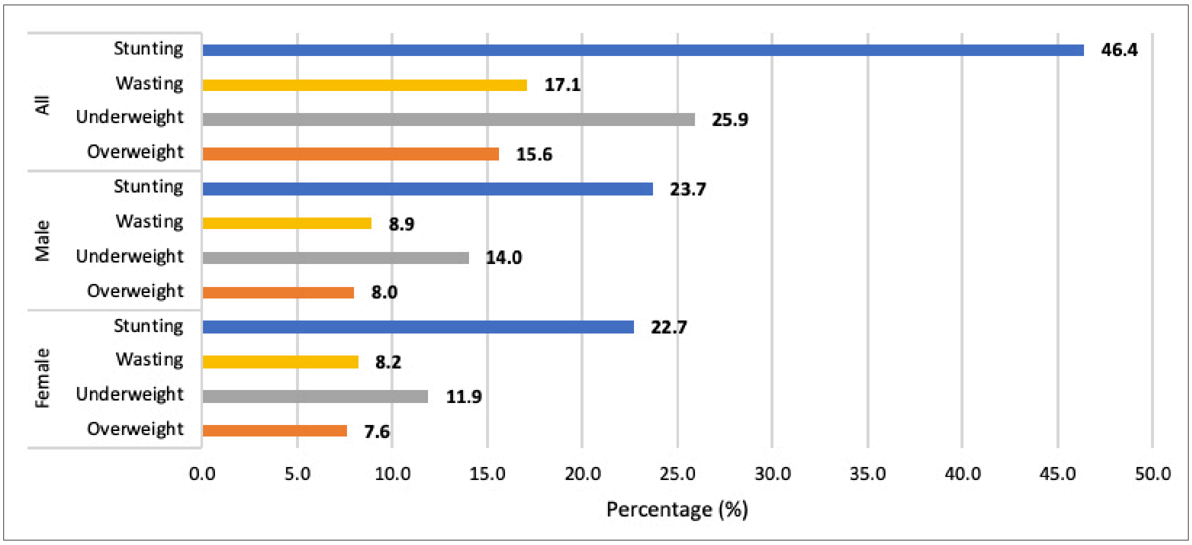

Figure 1. Prevalence of malnutrition among 0 - 5-year-old children in rice farming areas, Philippines.

(Figure 3) than in preschoolers. A very high magnitude of 62% and 57% of the children aged 6 - 10 and 11 - 12, respectively, were stunted. On the contrary, there was lower incidence of underweight and wasting in these age groups relative to preschoolers. The prevalence of overnutrition was likewise noticed although the rates were also fairly lower than in preschoolers. Obesity, though, was higher in 6 - 10-year-old children (13%) than in 11 - 12-year-olds (6%). These results were also higher than the 2019 NNS data except for underweight children indicating that malnutrition is also a severe problem among children within this age group in rice farming areas.

In a similar study, higher rates of malnutrition were also observed by Capanzana et al. (2018) among young children in households headed by fisherfolks. This shows that malnutrition is a chronic problem among children in rural areas where farming and fishing are the major sources of livelihood. This corroborates with the observations of the DOST-FNRI (2020) in its 2018 NNS, which showed consistently higher incidence of malnutrition, especially undernutrition, in rural areas relative to urban areas. This can be attributed to the level of income of households in these areas that is lower than the poverty threshold resulting in poor quality and diversity of their diet, as observed by Mbuya et al. (2021).

Incidence of malnutrition across provinces

The magnitude of malnutrition problem among 0 - 12-year-old children also varied across rice farming areas in the Philippines (Figures 4 to 6). Consistently, stunting was the most prevalent form of malnutrition across the sample provinces.

In preschoolers, a very high magnitude of chronically undernourished or stunted children was recorded in Davao del Norte (74%), followed

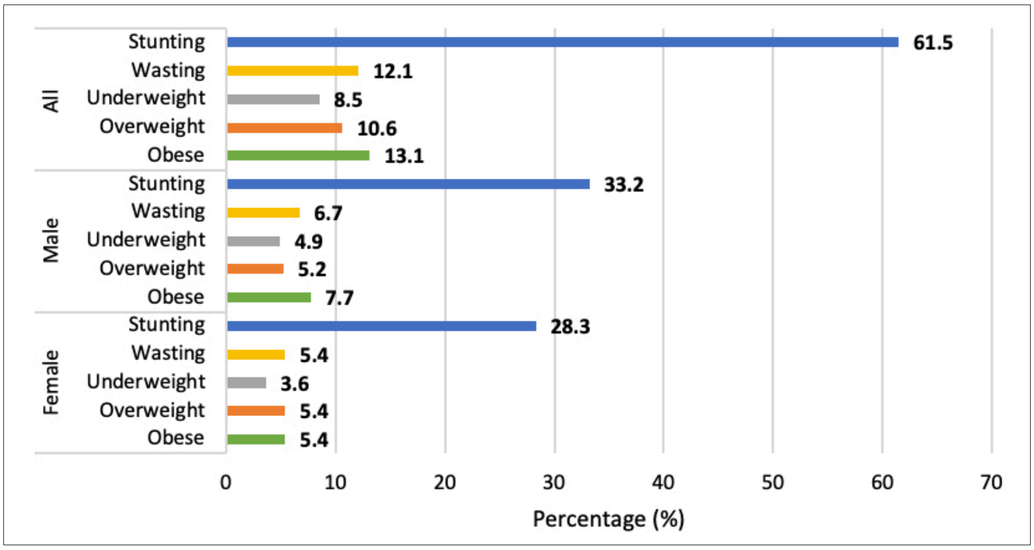

Figure 2. Prevalence of malnutrition among 6 - 10-year-old children in rice farming areas, Philippines, 2010.

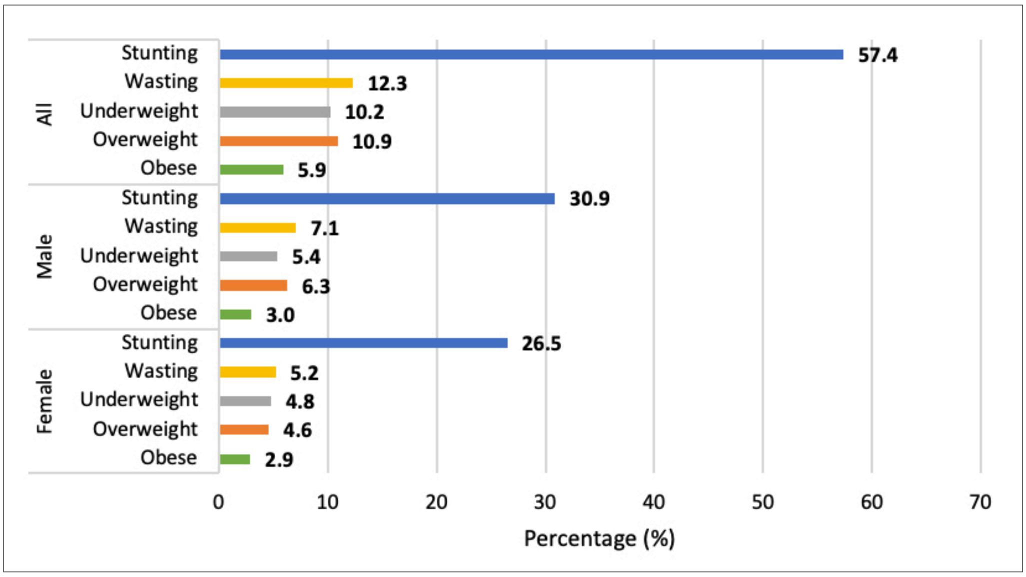

Figure 3. Prevalence of malnutrition among 11 - 12-year-old children in rice farming areas, Philippines.

by Antique, Capiz, Cagayan, Abra, Ilocos Norte, Isabela, Negros Occidental, and Albay (Figure 4). On the contrary, Nueva Ecija had the lowest magnitude of stunted preschooler among the selected provinces. Additionally, a high rate of 43% of the sample children in North Cotabato were underweight. This is trailed by Zamboanga del Norte, Camarines Sur, South Cotabato, and Abra. On the other hand, Nueva Ecija and Albay had zero incidence of underweight preschoolers. However, most numbers of overweight preschoolers were observed in Albay, Negros Occidental, Nueva Ecija, and Ilocos Norte.

Malnutrition was also prevalent among 6 - 10-year-old children in all of the studied rice-producing provinces (Figure 5). Significantly high incidence of stunting was observed in 12 of the 24 provinces led by Antique and Cagayan. These provinces had a rate of stunting that is higher than the overall rate in the country as observed in this study (61.5%). Highest magnitude of underweight children was seen in Aklan (55%) and Zamboanga del Norte (52%). Meanwhile, incidence of overweight children was lower than the recorded overall rate in the sample provinces except for Surigao del Norte.

Malnutrition was also consistently prevalent among children aged 11 - 12 years old with cases of short height or stunting recorded in 20 of the 24 provinces, led by Antique with 100% of the observed children stricken with the condition (Figure 6). Stunting among 11 - 12-year-old children were also widespread in Surigao del Norte, Ilocos Norte, Isabela, and Cagayan. High cases of severe thinness were observed in Capiz, Negros Occidental, Nueva Ecija, and Zamboanga Sibugay. Meanwhile, there were lower cases of overweight children compared to other forms of malnutrition in most of the sample provinces except in Nueva Ecija where incidence of the condition exceeded 60%.

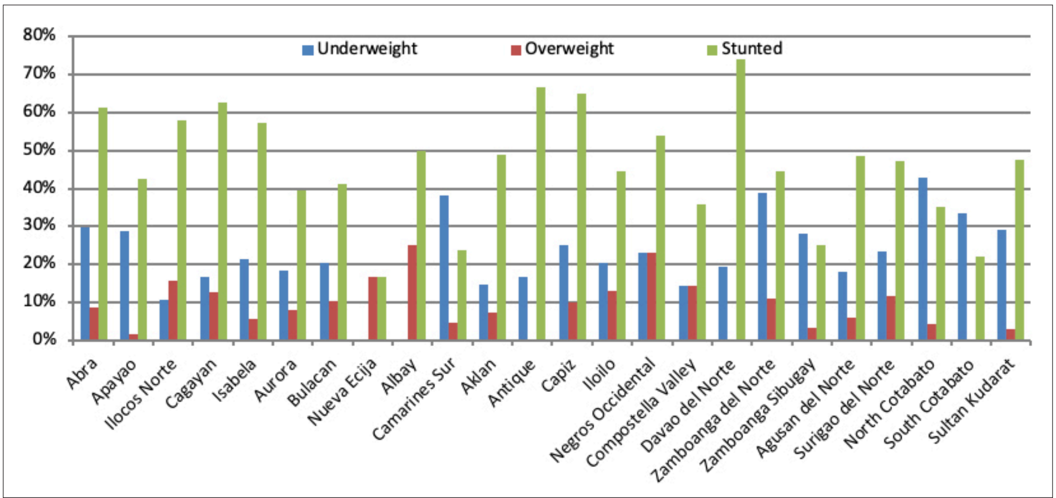

Figure 4. Magnitude of malnutrition among 0 - 5-year-old children by province.

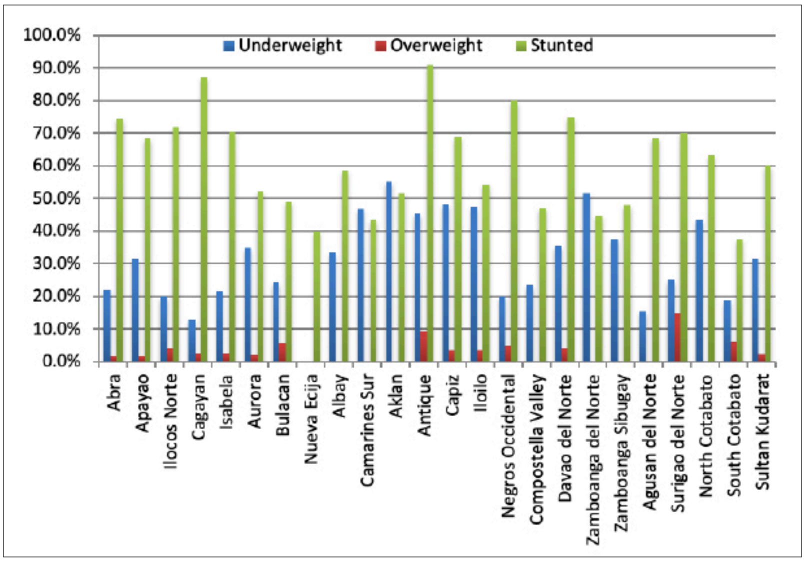

Figure 5. Magnitude of malnutrition among 6 - 10-year-old children by province.

Similar with the findings, the regional nutrition situation of the DOST-FNRI (2015) also found varying incidence of malnutrition across regions in the country. The highest incidence of stunting and wasting among preschoolers was observed in Region I and MIMAROPA region. In Central Luzon, rice-based farm households were found to have a low-quality diet with the calorie intakes of all age groups lower than the recommended calorie intakes (Abilgos-Ramos and Ballesteros, 2018). The study also found that iron and vitamin A inadequacy was highly prevalent in the area. These disparities in the nutrition situation of children may be attributed to the differences in the economic situation in each province or region as well as the level of urbanization or presence of infrastructures that affect the ease of the flow of goods or commodities, which make them more accessible to families.

Overall incidence of undernutrition

According to development economists Nandy and Svedberg (2012), the three commonly used internationally recommended anthropometric indices: stunting, underweight, and wasting may not provide an accurate estimate of the total undernourished children in a population as they overlap. As such, the Composite Index of Anthropometric Failure (CIAF) incorporates the total undernourished children in the sample provinces – be it stunted and/or wasted and/or underweight. The CIAF results showed that more than half (57%) of the total preschoolers were experiencing any form or a combination of anthropometric failure such as underweight, wasting, and/or stunting. (Table 6). This means that 6 in every 10 preschoolers were vulnerable to undernutrition. In the age group of 6 - 10 and 11 - 12 years old, 50% and 41% were determined with anthropometric failures or undernourished, respectively. Hence, undernutrition was relatively more common among preschoolers.

Vulnerability to nutrition problems, however, seems to decline as children grow older. Scientific evidence revealed that young children particularly those who are less than 5 years old are most vulnerable or are at greater risk of undernutrition as it is their critical period of growth and development. From the Centers for Diseases Control and Prevention (CDC, n.d.), the growth of a child's brain is rapid from birth up to its early childhood, which is crucial for future learning, health, and success; thus, requiring high quality diet such as high intake of proteins and calories compared with age groups of 6 - 10 and 11 - 12 years old. WHO (2000) also suggested that adequate nutrition is fundamental for children's health and development. Preschoolers, in particular,

Table 6. Total children with anthropometric failures by age group, all provinces.

Group	Age group (in number of years)					
	0 - 5		6 - 10		11 - 12	
	(n)	(%)	(n)	(%)	(n)	(%)
No anthropometric failure	403	43.4	537	50.4	282	58.9
With anthropometric failures	525	56.6	528	49.6	197	41.1

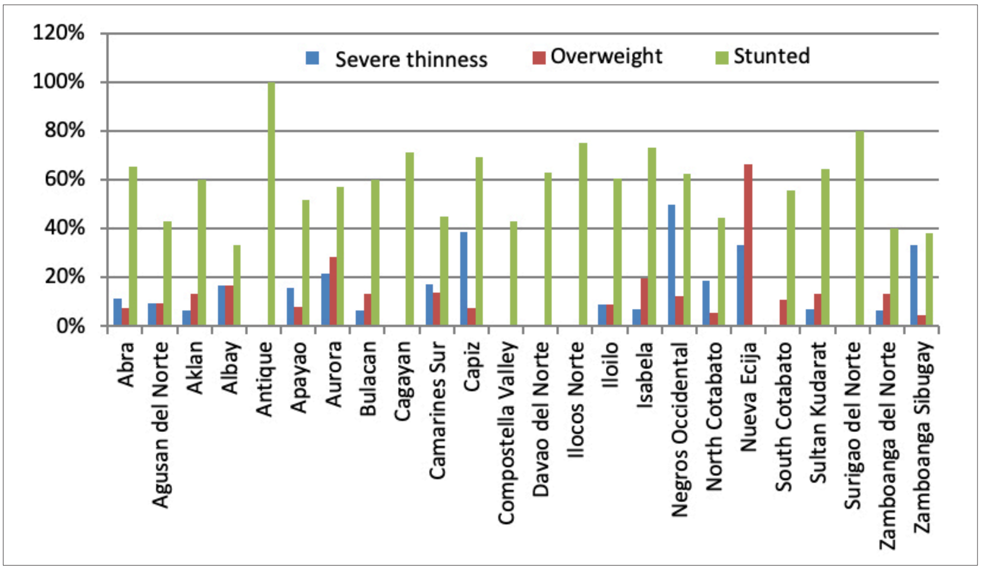

Figure 6. Magnitude of malnutrition among 11 - 12-year-old children by province.

who are rapidly growing need foods that are rich in energy and nutrients as poor diets prevent children from achieving their full genetic potential.

Geographical magnitude of undernutrition

For specific targeting of nutrition programs, the proportion of children who are suffering from any form of anthropometric failure was determined in each sample province. A relatively equal distribution of both anthropometric and non-anthropometric failure was observed in all provinces (Figure 7). The provinces with the highest rates of anthropometric failure among preschoolers include South Cotabato, Zamboanga Sibugay, Camarines Sur, and North Cotabato.

Meanwhile, 13 of the 24 sample provinces had higher magnitude of 6 - 10-year-old children with anthropometric failure relative to those without the condition. Particularly high rates of incidence were recorded in Camarines Sur, Zamboanga del Norte, Aurora, and Aklan. Additionally, 11 of the 24 provinces registered higher rates of anthropometric failure among children aged 11 - 12-year-old. Albay, Nueva Ecija, Zamboanga Sibugay, Compostela Valley, and North Cotabato showed significant rates of children with anthropometric failure.

Overall, North Cotabato, South Cotabato, Zamboanga Sibugay, and Camarines Sur, recorded the highest incidence of anthropometric failure among children in all age groups.

Determinants of undernutrition

From the study, undernutrition appears to be the more prevalent condition among 0 - 12-year children in rice farming areas in the country with stunting as the leading condition. There can be several causes of undernutrition among children. In the Philippines and in some countries, poverty is the main reason for the high incidence of undernourished children. Majority of the sample rice farming households earn incomes that are below the poverty threshold and are therefore considered poor (Table 1). To identify the determinants of malnutrition, Tables 7a and 7b show the relationships of some socioeconomic variables with the prevalence of undernourished 0 - 12-year-old children in the studied provinces.

Table 7a. Relationship of socioeconomic variables and incidence of total undernourished children.

Socioeconomic characteristics	Percent total undernourished 0 - 12 years old*
Poverty	
Poor households	54
Non-poor households	46
Educational attainment	
Elementary	52
High school	55
College	44
Household size	
4 and below	48
5 and above	52
Sources of household income	
Rice farming only	54
Diversified	46
Size of rice landholding	
1 ha and below	52
Above 1 ha	48

*Children aged 0 - 12 years old with any state of anthropometric failure.

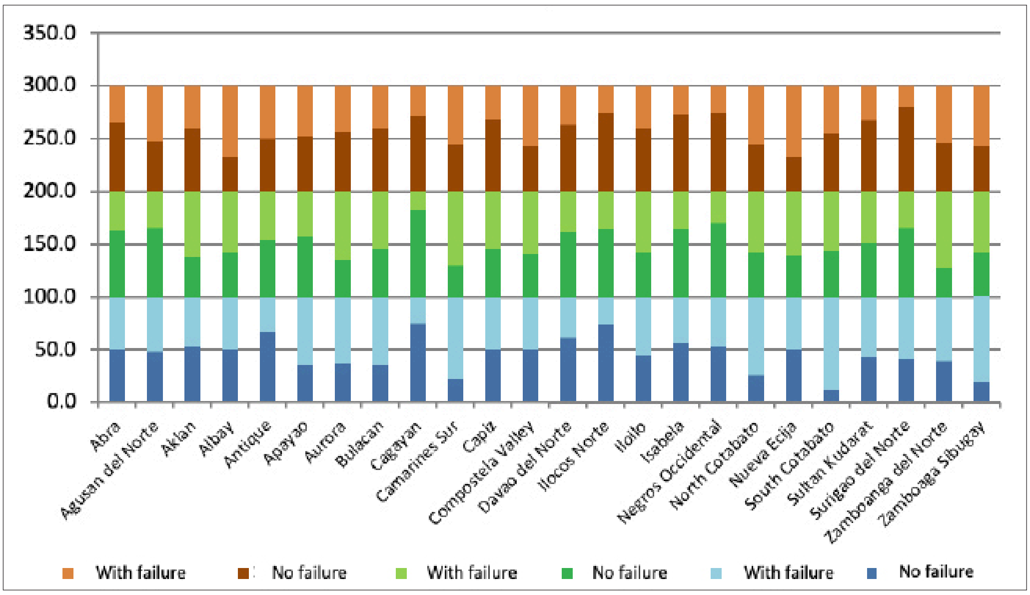

Figure 7. Percentage distribution of total children with any state of anthropometric failure by age group and province.

Table 7b. Relationship of undernutrition and socioeconomic variables, logit regression results.

Parameter	DF	Odds Ratio Estimate	Standard Error	95% Confidence Limits	
Poverty	1	1.344	0.0933	1.119	1.613
Educational attainment	1	1.091	0.0993	0.898	1.325
Household size	1	1.088	0.0897	0.912	1.297
Rice income source	1	0.928	0.1070	0.753	1.145
Size of rice landholding	1	1.065	0.0893	0.894	1.269

Hunger/Poverty. Anthropometric failures among age group 0 - 12 years old can be attributed to hunger with poverty as the main culprit. On the average, the proportion of total undernourished 0 - 12-year-old children were larger in poor households (54%) relative to their non-poor counterparts (45%). Odds ratio estimate from the logit regression indicates significant association between poverty and undernutrition. The test indicates that children belonging to poor households were 34% more likely to suffer from undernutrition as low income reduces the family's capability to acquire adequate nutrition through quality and healthy food.

Educational attainment of household head. The educational level of the household head may be another cause of undernutrition. Incidence of undernourishment among children was higher in families whose head of the household only had elementary (52%) and high school (55%) education compared with college graduates (44%). Apparently, the higher the education level of the household head, the lower the prevalence of malnutrition among their children. Children aged 0 - 12 years old whose parents did not reach college level were 9% more likely to experience undernutrition based on the odds ratio estimates.

Large household size. Higher cases of undernourished children were also more prevalent in households with more members (5 and above). This may be because the increase in the number of household members meant smaller portion of food for each member as the family was not able to augment their food supply for the household's additional member. Consequently, their food intake is reduced that increases the children's risk of being undernourished. The odds ratio estimate explains that children within the 0 - 12 years age group were 8% more likely to be undernourished if they belong to a larger household.

Rice income source only. Source of income is another factor to undernutrition in the studied provinces. Data show that the proportion of total undernourished children were quite higher among households with rice farming as the only source of income (54%). The odds ratio estimate indicates that the children in these households were 7% more vulnerable to undernutrition than in households with diversified sources of income.

Small rice farm area. More than half (52%) of the total undernourished children belong to households with less than one ha rice landholding. The odds ratio estimate implies that children from this households were 7% more likely to suffer from any form of anthropometric failures than children belonging to households with more than 1 ha rice farm area.

Conclusion

Proper nutrition is essential to support the foundational growth and development of young children and prevent malnutrition. This will help ensure their optimum growth and productivity as an individual in their later years. This study showed high incidence of malnutrition among 0 - 12-year-old children belonging to rice farming households in selected provinces in the Philippines. The different forms of malnutrition – undernutrition and over-nutrition, were observed among these children.

Among the age groups of children, preschoolers were the most vulnerable to malnutrition due to their high nutritional requirements for growth and development. Vulnerability, however, declines as children grow older. Undernutrition, particularly stunting, was the most evident nutrition problem among the studied children in the sample provinces. Specifically, 46% of the children were stunting; 26%, underweight; 17%, wasting; and 16%, overweight. Boys were also observed to be more vulnerable to malnutrition than girls.

Across sample provinces, North Cotabato, South Cotabato, Zamboanga Sibugay, and Camarines Sur, recorded the highest magnitude of total undernourished or anthropometric failure children in all age groups.

The high incidence of malnutrition among children in the studied rice-producing provinces can be partly attributed to poverty with 63% of the rice farming households living below the annual per capita poverty threshold, and 51% of them having insufficient income to provide their family's daily per capita food requirement. Other socioeconomic factors that were positively associated with undernutrition were: low educational attainment of the household head, large household size, small rice landholding, and the household's sole dependence on rice farming income.

Recommendation

Ensuring access to safe and nutritious food such as diversified diet and small and frequent meals will improve the nutrition and health status of 0 - 12-year-old children in rice farming areas in the country. The geographical disparities in the magnitude of malnutrition/undernutrition among children aged 0 - 12 years old in rice farming households suggest varying needs; thereby, requiring area-specific interventions. For example, very high magnitudes of total undernourished children were mostly noted in provinces in Mindanao and Camarines Sur. Based from the results, the following are recommended:

Improve the competitiveness of rice production by accelerating rice farm productivity growth and reducing production cost through adoption of yield-enhancing and cost-reducing technologies by farmers. This is to increase rice farm income that will raise farming households' ability to meet their dietary and nutritional needs;

Engage farming households in value adding activities in the rice value chain from production to processing and marketing of their produce that will allow them to increase their income from the added value to their products;

Encourage farming households to engage in crop diversification or profitable rice-based farming systems and link their produce to a market that will give them a sustainable, stable, and guaranteed market and reasonable income;

Capacitate household heads particularly those in charge of caring for the children by providing them with nutrition related education such as seminars on healthy diet;

Implement specific nutrition improvement program such as backyard gardening or integrated farming system in areas with high incidence of malnutrition to increase households' access to a healthy and nutritious foods. This will enhance their consumption of vegetables and fruits that will improve the health status of children;

Encourage families in the provinces to adopt a healthy and sustainable diets by promoting complementary foods and a diversified diet including healthy and nutritious homemade meals or snacks made from locally available products from animal and plant sources. In line with this, parents or caregivers must be well aware of dietary recommendations or nutritional guidelines which they may find from FNRI; and

Conduct further study on the topic, particularly, on the link between the incidence of malnourishment among children and the economic situation, and other distinct characteristics of their specific province or region to add to the literature and provide empirical evidence explaining the observed differences in the prevalence of malnourishment across provinces.

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TRADITIONAL UPLAND RICE-BASED PRODUCTION PRACTICES IN LAKE SEBU, SOUTH COTABATO, PHILIPPINES AND THEIR IMPLICATIONS ON DEVELOPMENT PROJECTS

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ABSTRACT

The importance of agrobiodiversity has been emphasized in local and international perspective. Existing indigenous knowledge systems and practices (IKSP) that safeguard agro-biodiversity would provide insights on conservation, development strategies, and sustainable use, specifically of traditional rice varieties, which have been cultivated from generation to generation. The study aimed to document the traditional upland rice-based production practices and its contribution to agro-biodiversity conservation in the pilot sites. A focus group discussion (FGD) was conducted in five villages in Lake Sebu, South Cotabato. The data was analyzed using theme identification (descriptive analysis) and theory application and development. Study results showed that rice with corn and alternate rice and corn dominated the farming system. Awot is the most planted traditional rice varieties in the villages of Klubi, Lamcade, Lamfugon, and Luhib while Dinurado is the most preferred traditional rice varieties in Tasiman. Traditional practices from seed selection to post-harvest rituals were documented while the socio-cultural importance of traditional rice among the indigenous peoples' communities were also present. For land preparation, hand tools such as shovels and hoes are preferred in sloping areas while water buffaloes are used in the rolling terrains. Preservation of rice traditional varieties and other crops and IKSP play an important role in IP culture. Thus, the need to preserve or enhance them.

Keywords: *Agro-biodiversity, Baseline Data, Dynamic Conservation, Focus Group Discussion, Indigenous Knowledge and Practices.*

Introduction

The Food and Agriculture Organization (FAO, 2013) implemented a project in line with Sustainable Development Goals agenda (SDG) primarily to mainstream and ensure institutionalization of agro-biodiversity conservation in laws and development strategies. The project on the "Dynamic Conservation and Sustainable Use of Agrobiodiversity in Traditional Agroecosystems in the Philippines" selected indigenous people (IPs) and IP communities as the primary beneficiaries because modern agriculture significantly threatened indigenous agro-biodiversity and traditional practices. Accordingly, it is paramount and urgent that existing relevant traditional agricultural practices and their contribution to agro-biodiversity be documented, disseminated, scaled-up, monitored, and evaluated.

Under Republic Act No. 8435, also known as the Agriculture and Fisheries Modernization Act of 1997 (AFMA), the Philippine government identified sustainability of the agriculture and fisheries areas with the State exerting care and judicious use of the country's natural resources as one of its guiding principles for agricultural and rural development. The law also directs the Department of Agriculture (DA) to consider biodiversity, genetic materials and environment preservation, indigenous peoples in its development project. Hence, this project appropriately addresses these concerns in accordance to the law and directives of the DA.

Specifically, the project aimed to conserve globally important agro-biodiversity of rice, mungbean, taro, yam, banana, Manila hemp, and others in traditional agro-ecosystems on selected villages in Ifugao and South Cotabato. Under this three-year project, technical assistance in production and marketing are provided to help the farmers organize and govern themselves and to receive market-based incentives for agro-biodiversity.

Agro-biodiversity resources and their management practices were documented in the pilot sites through survey and focus group discussions (FGD).

This study documented the upland rice-based production practices and how they contributed to agro-biodiversity conservation in the pilot sites. Specifically, this study: (1) classified the dominant upland farming systems in the pilot areas; (2) identified traditional upland rice-based farming practices; (3) characterized farmers' capability needs to achieve project objectives; and (4) provided recommendations to sustain the agro-biodiversity in these areas. Results from this study could be used to develop policy and laws and strategies and to ensure that these are institutionalized to safeguard upland traditional practices.

According to Viterna and Robertson (2015), modernization theorists defined the goal of development as embodying the characteristics and lifestyle of modern societies. For them, this indicated features of Western views on development in areas of society, politics, culture, and economics focusing on aggregate economic growth. This view of development is limiting for Sen (1999) who argued that the concept must be seen as a process to expand people's freedom; thus, it is both a primary end and a principal means.

Ingram (2017) agreed with Sen's view; stating that the old development paradigm did not consider the relevance of wealth distribution as certain groups benefit more on economic growth than others. He added that the better development paradigm considers the need to establish a just mechanism for wealth distribution through institutional restructuring and include other factors such basic rights, education, and the environment. To confront the criticisms on development, the United Nations (UN) focused and recognized that the people are the true wealth of a nation; thus, capacitating and enabling them is a must for development to occur and continue (UNDP, 2010). Therefore, the priority must be to increase the broader population's opportunities consequently, leading to income change or wealth distribution, and reducing inequality (Green and Zinda 2013).

By appreciating people as the means and reason for development, public participation became central in the study and implementation of development interventions. This started in the 1990s, when there was a revival of the synergy between public participation and development paradigm, which was brought into the development discourse after the concepts failed in the 1950s and 1960s (Mansuri and Rao 2013). Habermas (1991), in one of his earliest and seminal works on public participation, highlighted the significance of public participation in development as an important indicator of the democratic spirit; and this idea fostered discussions on participatory processes.

The participatory process came as a reaction to the top-down approach, which dominated development implementation (Darnhofer et al., 2012). Hence, a bottom-up approach, being in line with participatory research and extension, offered an alternative in implementing development projects (Swanson, 2008). Kemmis and McTaggart (2005) emphasized that the attributes of participatory action research can be extended and applied in differentiating a participatory development project from the traditional process identified as follows: (a) shared ownership of research projects, (b) community-based analysis of social problems, and (c) an orientation towards community action.

Tackling rural development is complicated, if not a problematic, issue and process. The notion of development is concurrent with and permeates rural development discourse. Balisacan, et al., (2006) reported that development in the Philippines should lean more towards the rural than the urban areas because more poor families reside there. Rural areas generally lag behind in development indicators than their urban counterparts (Green and Zinda 2013). Rural development has multiple pathways identified as (a) agricultural path, (b) multiple-activity path, (c) assistance path, and (d) exit path (Rivera and Qamar 2003). The agricultural path centers around modernization of agriculture with major focus on agricultural production and income (Ploeg et al., 2010). The multiple-activity path focused is equivalent to Ploeg and colleagues' (2000) rural development focused on the individual farm household especially the synergy between on-farm and non-farm income of the farmers (Ploeg et al., 2000; Rivera and Qamar 2003). Lastly, the exit path on rural development exists when the farmer migrates from rural areas or agricultural production. As a result, rural development is not necessarily equivalent to agricultural development (Roetter et al., 2007).

The SDG Agenda adopts sustainable development as the organizing principle to simultaneously focus on the combination of economic development, social inclusion, and environmental sustainability as its core dimensions (SDSN, 2015). Hence, the SDG Agenda set parameters for provision of basic services as these are intertwined with economic growth, social inclusion, equality, and poverty reduction (UN, 2018).

The SDG Agenda is consistent with Republic Act 8371 known as The Indigenous Peoples' Rights Act (IPRA) of 1997, in which the Philippine government protects the welfare of the indigenous people's community. Other provisions supporting their inclusion included determining procedures and participating in decision-making for matters that might affect them and their right to determine, formulate, and implement their own priorities for national and local development programs.

Unsustainable agriculture is identified as one of the major reasons for agro-biodiversity loss (UN, 2018). The poor are the more vulnerable and at risk (Reid and Swiderska, 2008) who bear the brunt of disruption and loss of agro-biodiversity because they are severely impacted by climate change. Hence, the SDG Agenda, particularly its objective to ensure environmental sustainability and prevent diversity loss, help ensure the survival of human species (UN, 2018). The SDG intricately linked when discussing food and nutrition security because the environment pertains to threatened resources that is interconnected with food production (Fan, 2014).

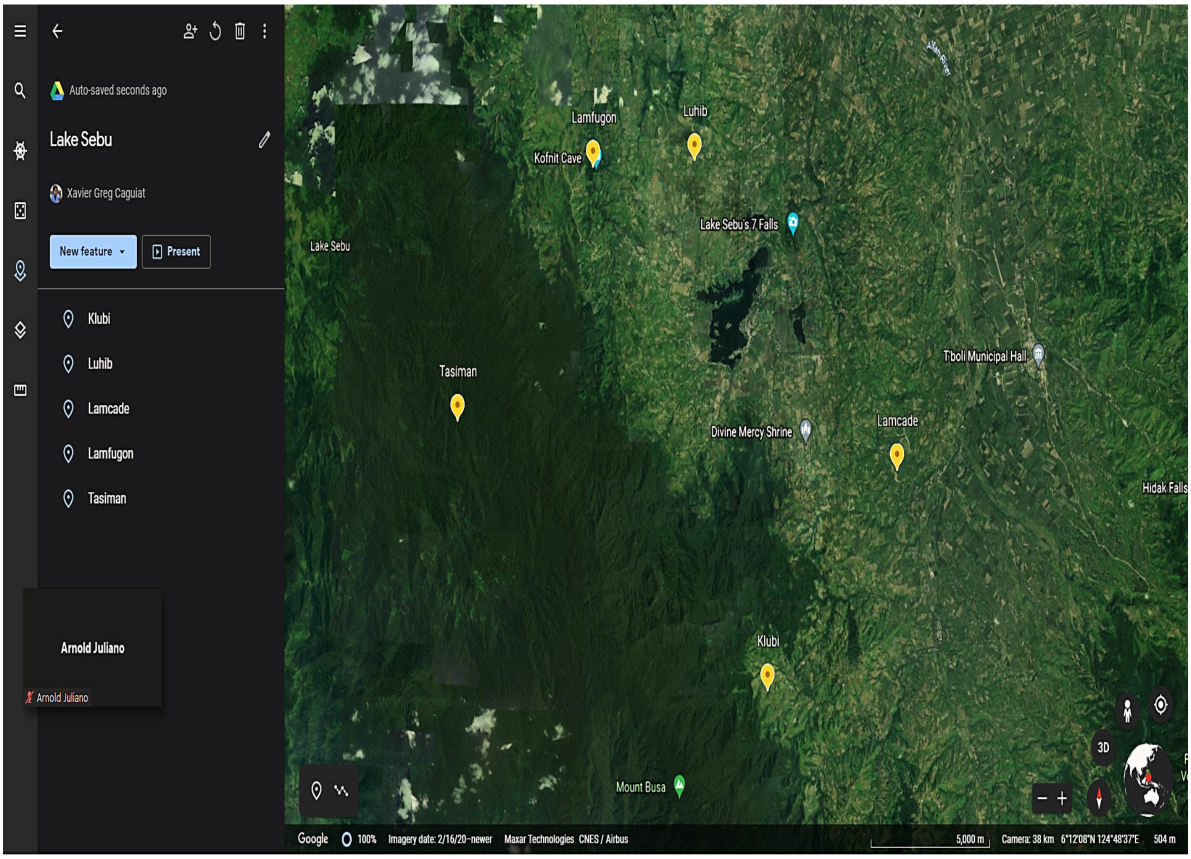
Materials and Methods

Sites and Participants

Data gathering was conducted on April 8, 2019 with the IPs from the villages of Klubi, Lamcade, Lamfugon, Luhib, and Tasiman. All villages are located in Lake Sebu, South Cotabato. Five FGDs were conducted simultaneously (one for each village) comprising of at least eight representatives. The participants were either currently or previously engaged in planting traditional varieties and must knowledgeable in the traditional rice farming practices.

Data Collection

FGD was an ancillary method for the project. Prior survey data from household level in the research sites were used for FGD validation of indigenous knowledge systems and practices (IKSP) on pre-planting, planting, harvest, and post-harvest rituals. According to Bloor and colleagues (2001), FGD can be used as a contemporary extension of survey activity to give aid in analyzing data. The researcher can also collect voluminous data and obtain differing views, opinions, and experiences offering in-depth information for data analysis (Hennink, 2007).



Source: Google Earth

Figure 1. Google Earth representation of the five villages: Lamcade, Lamfugon, Luhib, Klubi, and Tasiman located in the municipality of Lake Sebu, South Cotabato where the FGDs were conducted.

Data analysis

According to Hennink (2007), analyzing FGD data involves: (a) data preparation, (b) identifying themes in the data, (c) label data by themes, and (d) using the framework for analysis. In this study, there was a slight deviation from the preceding suggested process. Data analysis included: (1) responses during the Q and A were encoded, (2) open fora and collected metacards, which were translated by project staff in the community, (3) themes were identified to categorize descriptive analysis, and (4) theory application and development was applied to make inferences on the data. The themes are farming practices and traditional practices from seed to seed.

Results and Discussion

Farming Practices

Majority of the villages have upland ecosystem with no access to irrigation. The farmers practice the traditional slash and burn method. They use their own save seeds for planting in the succeeding seasons. Most of the time, they plant rice only once a year due to water scarcity and long maturity of rice crops, which takes 5 - 9 months before harvesting. They also plant taro, mungbean, ginger, and other combination of crops for their own consumption. In two of the villages, more areas are planted with abaca than rice.

Multiple farming systems exist in the farming communities (Table 1). The villages were appropriate sites for identifying traditional rice varieties as rice-corn was identified as one of the dominant farming systems. The agro-biodiversity in the area is rich with multiple crops that are permanent perennials such as: coffee, bamboo, cacao, coconut; and temporary annuals: corn, abaca, banana, tomato, bell pepper, taro, glutinous corn, sweet potato, ginger, cucumber, beans, lady fingers, onion, and turmeric. Thus, it is only proper that their agro-biodiversity be preserved or enhanced as envisioned by the UN for current and future use (UN, 1987). In introducing rice development interventions, the extension workers can consider the effects in other crops and to the communities.

Table 1. Dominant farming systems per village.

Project Sites	Dominant Upland Farming Systems
1. Klubi	Rice + corn, abaca, banana, bamboo, coffee, tomato, bell pepper, taro, and rice-fallow
2. Lamcade	Rice + glutinous corn, lunga, corn, gabi + sweet potato, cassava + sugarcane, taro, rice-fallow, tomato, banana (saba), and rubber tree + abaca
3. Lamfugon	Rice-corn, sweet potato, rice-timon or cucumber, rice-ginger, rice-segotong heet, rice-onion, rice + lemon grass, tomato, banana, Palawan, rice-beans, rice-gabi, rice-cassava
4. Luhib	Rice-corn, corn-squash, cassava + gabi + sweet potato, banana + ginger, corn-taro, coffee + pepper, cacao + coconut + sweet potato, banana + corn
5. Tasiman	Tomato, abaca, corn, rice-corn, Palawan, kadyos, cucumber, beans, lady fingers, banana, coffee, gabi, peanuts, sweet potato, onion, turmeric

Traditional practices in relation to major operations in upland rice-based farming

Rice cropping calendar. Rice is planted from March to April in all sites, which will be harvested in August. Farmers refer to this cropping period as *Halay Lowon*. Another cropping period, referred as *Halay Hesewa*, takes place from September (planting) until December (harvesting). Cropping period for *Halay Lowon* is longer than *Halay Hesewa*.

Participants also shared that they have different areas allocated for rice during *Halay Lowon* and *Halay Hesewa*, which they learned from their ancestors. This was inferred as a conservation strategy, but is still a subject for further study. As *Halay Lowon* approximately takes five months and relating it to the farmers' non-use of synthetic fertilizers, there is a chance that the land would not be suitable for second rice cropping; but could be suitable for other crops like legumes.

Seed and seed selection. The participants said that they already lost most of their traditional upland rice varieties. With the assistance of the Office of the Provincial Agriculturist and FAO staff members they are gradually recovering these lost varieties. They also tried to access seeds from their relatives in other municipalities and provinces.

Participants in Lamcade identified 16 traditional upland rice varieties in their village while there are 15 varieties in Luhib. Tasiman has more or less 25 varieties while participants in Lamfugon submitted a list of more than 50 traditional upland rice varieties. Table 2 presents the top three popular varieties commonly planted in each village.

Land preparation. In all sites, the participants considered the physical feature of their farm areas

Table 2. Top three popular varieties per site.

Project Sites	Top Three Traditional Rice Varieties
1. Klubi	Awot, Azucena, and Dinurado
2. Lamcade	Awot, Azucena, and Dinurado
3. Lamfugon	Awot, Kalimomo, and Kenumay
4. Luhib	Awot, Azucena, and Kinamomo,
5. Tasiman	Dinurado, Lehek, and Tramis

as determinant of the tools and practices they use for land preparation. For sloping areas, farmers prefer hand tools such as shovels and hoes and for the rolling areas, they use water buffaloes. These land conditions must be accounted when conducting training in the different sites. Development workers can review the secondary data available from the municipal office to ensure that interventions on land preparation are appropriate and relevant.

Major activities for land preparation include slashing (*mefos*), piling/clearing (*sentifon*), hoeing (*smangkol*), and pulverize (*kmokos*). During FGDs, the standard area used was 0.25 ha as it was easier for the participants to estimate labor, material inputs, and costs using this parameter. Four persons are needed for land preparation, and they are paid for PhP 250 each per day excluding meals.

A traditional practice they associated with land preparation was the song of the bird locally known as *tahaw*. The sound of the bird during dawn or sunrise signals time to prepare the fields. They generally avoid the drought months to prevent pest damages.

Their traditional belief of waiting for the *tahaw* to sing is a research area. It is possible that the appearance of the bird accounts for the "spring season" like in the western countries that undergo four seasons. Rainfall is sufficient in this period; making it the proper time for cultivating rice. The said "season" can also signify the presence or migration of insects or animals that prey upon rice pests. These assumptions need to be verified by a thorough study. Supposing the theory on seasonality is correct, climate change must be added in this context as this might affect the presence of *tahaw* in the area.

There is also a need to verify the sustainability of the participants' land preparation activities especially with the continued practice of swidden farming (slash-and-burn farming) for upland rice production. Although part of their traditional customs, practices, and beliefs, this activity go against the principles of biodiversity and sustainability. There might be a need to come up with an ordinance or policy to regulate and manage the practice. Swidden farming also go against the law particularly with Republic Act No. 8749 also known as The Philippine Clean Air Act of 1999. Other sustainable technologies or practices could be introduced as an alternative to swidden farming.

Crop establishment. Traditionally, farmers' source of labor for crop establishment is the *bayanihan*. Relatives help each other for free or in exchange of labor. However, laborers nowadays demand cash payment. It must also be noted that their tradition allows the division of labor along gender lines for this farm operation. Dibbling is an activity exclusively for men while seed sowing is for women. Three to four men are needed for dibbling a 0.25 ha field. The same number of women also work on seed sowing. The seed requirement for 0.25 ha was 5 - 8 *ganta*. The participants estimated that one *ganta* is equivalent to 2.2 kg. For one hectare, seeding rate is 44 kg h^{-1} - 70.4 kg h^{-1}, which is higher than the 40 kg h^{-1} recommendations.

Demsu and *But B'nek* comprise the rituals for crop establishment. *Demsu* is a prayer with dancing and chanting performed by landowners to allow them to start planting and for fortune to permit good harvest. On the other hand, *But B'nek* is an exclusive area for rice crops planted solely for the benefit of the landowner and family members. These rituals are performed only for traditional rice varieties but not with modern varieties. They also believe that the best time to plant is during full moon (*T'ngel*) when there are lots of stars in the sky.

Water management. It is inherent in the definition and nature of upland rice cultivation for the participants to depend on rainfall for source of water. They stated that as long as they follow the cropping schedule based on their traditional practice (e.g., wait for the *tahaw* to sing), the chance that they may encounter excessive or insufficient water is quite low.

Nutrient management. The participants stated that they do not apply any synthetic fertilizers and depend only on the available nutrients inherent in the soil. They also allow weeds to decompose in the rice field as fertilizer.

Pest management. The common pests across all the villages are weeds, rice bugs, birds, and rodents. Weeding is a task exclusive for women and they do this twice every cropping season. The task requires at least 15 women to manually weed an area of a quarter hectare. They use a small digging knife (*surot*) to remove weeds. They usually encounter rice bugs in July but they said that these are easy to manage if they follow the proper timing for planting as recommended by their elders. They also use lemon grass and *cosmos*, a local plant, as insect repellants. The participants also recognized the importance of synchronous planting to manage pests.

Harvest and postharvest. Farmers use panicle harvesting (*muta*) for seed collection and purification. They select the rice with long panicles and with filled grains. However, they use a sickle to harvest the rice allocated for food and marketing. The fresh *palay*, including the individual stalks were then placed in a bamboo basket (*abi*), which they carry like a backpack. They place the *palay* in the bark of trees for drying (*lihub*). They practice foot threshing (*knusu*) to separate the grains from panicles. Grains are then placed inside bamboo hollow tubes (*tidol*) for storage. Other traditional practices related to harvesting include tying the rice for seed selection (*hemules*) and hanging selected seeds in the hearth (*mete halay*) as they perform thanksgiving rituals (*kmuhom* and *kemini*). Their main problem during harvesting is low production; thus, they do not have surplus rice for marketing. Most of the harvest are allotted for seeds and consumption.

Capability needs to achieve the project objectives

One concern from this study is determining which agro-biodiversity practices would be enhanced, sustained, and expanded. Swidden farming, crop establishment innovations, and *bayanihan* can be viewed as cases. For the first case, it is recognized that swidden farming is part of the traditional practices of Indigenous Cultural Communities/Indigenous People (ICC/IPs) but such practice may threaten agro-biodiversity. The challenge would either be to totally stop the practice or look for an opportunity for enhancement, sustainability, and expansion.

For crop establishment innovations, assuming that a new technology will be introduced in this farm activity that would displace women participation on seed sowing, would this threaten agro-biodiversity? *Prima facie*, there seems to be no direct relationship between women's engagement in seed sowing in relation to preservation and conservation of agro-biodiversity. What can be gleaned here is the safeguarding and perpetuation of the local non-material culture specifically, tradition. Hence, the tension is between induced innovation and the loss of tradition, in which women will no longer perform their traditional role in seed sowing. However, given that it is the tradition and not agro-biodiversity that is being threatened, does this permit project implementers to impose innovations? It is submitted that this is a false dilemma. There is a need for the participants to be informed before giving their consent or whether they agree to the innovation as provided under the Indigenous People's Rights Act of 1997? The implementers should also be transparent on the potential effects of the intervention to the local customs, traditions, and ecology. An alternative to the dilemma is applying the precautionary principle in relation to the innovation and its effects (Agcaoli, 2012).

Bayanihan. The participants also expressed that the *bayanihan* spirit is dying with laborers no longer working in exchange for labor but for wages instead. Participants expressed sentiments to revive this culture within their communities; however, they admitted it is challenging in a modernizing world. The laborers' social relations and exchanges do not only revolve among members of the community but are also dictated by the structure of the whole economy. In the case of the project, this economic activity is not necessarily a threat to agro-biodiversity unless it forces farmers to abandon traditional rice upland cultivation.

Given the results, there is a need to theorize the development thrust of the project for future implementation.

Conclusion and Recommendations

The study identified the traditional rice-based practices in the uplands of Cotabato and reflected on their implications on development projects.

Multiple dominant farming systems exist in the study sites, which show the natural, financial, and cultural capital available in each IP community. The major farm operations also involve a rich culture and traditions, however, participants recognized that these are gradually declining due to modernization. Not all traditional practices and the planned interventions uphold the principles of agro-biodiversity, and that innovations might even disrupt social cohesion. Using the participatory process and precautionary principle, the implementers can verify their actions to ensure that they bring development to the communities based on the shared understanding with the participants.

In relation to characterizing the capability needs of the participants to achieve the project objectives, the data posed a challenge for the implementers on how the communities would share their resources and how to institutionalize such sharing where all benefits from the process. Hence, there is a need to continue to strengthen the capabilities of the participants to sustain the progress after the project ends. More importantly, these institutionalizations of interventions must be carefully in line with the culture, existing institutions, norms, participatory process, and the precautionary principle.

Through the project, participants have recovered many traditional varieties they have lost. The project also heightened their interest in preserving their traditional rice varieties, which they now recognize as an asset for their development aspirations.

As implied from the results of the study, there is the need to consider issues regarding planned social change and conservation of agro-biodiversity amidst a modernizing culture, traditions, market forces and structure, and the seemingly inherent contradictions of the project to enhance, sustain, and expand agro-biodiversity. Material and non-material cultures are subject to change; thus, there must be a need for the implementers to establish parameters to determine the extent of the interventions that can be made.

The implementers should also include the concept of precautionary principle when introducing interventions in the project sites. The principle states that "when human activities may lead to the threats of serious and irreversible damage to the environment that is scientifically plausible but uncertain, actions shall be taken to avoid or diminish that threat", (Agcaoli, 2012). This can be done by including it in a memorandum of agreement with the participants, ordinances implemented by the villages, orders coming from the municipal or provincial offices, or the researchers must present to them how the precautionary principle is applied during project implementation.

The main theory revolves around the participatory process of development, and the precautionary principle. It is suggested that the concepts be considered and embedded in the agro-biodiversity development framework that will be developed. In this way, there will be guiding principles governing the project implementation. It will be easier for the implementers and the participants to evaluate their progress and the strategies being done to achieve shared goals.

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GENETIC CHARACTERIZATION OF SELECTED PHILIPPINE WILD RICE (*ORYZA* SPP.) USING SSR MARKERS

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Abstract

Wild rice relatives (WRR) act as reservoir of genes needed to develop varieties that are resistant to biotic and abiotic stresses. Thus, preliminary diversity analysis is imperative. This study assessed the genetic diversity of WRR collected from natural habitats using molecular markers. Genetic diversity was determined using 14 highly polymorphic microsatellite Simple-sequence repeat (SSR) markers with one sample of *O. sativa* as check. Overall diversity observed among the entries was 58% indicating moderately diverse. Polymorphism information content (PIC) values ranged from 0.24 (RM24843) to 0.60 (RM11) with an average of 0.42. The UPGMA cluster analysis grouped the 15 entries into two major clusters at 42% similarity coefficient, in which Cluster I consisted of the two *O. rufipogon* samples while Cluster II had *O. minuta, O. meyeriana,* and *O. sativa*. Other species formed subclusters at 70% similarity coefficient: *O. sativa* (IIA), *O. meyeriana* (IIB), and *O. minuta* (IIC), in which two samples of *O. minuta* (h and i) had 100% similarity. This study shows the current diversity and further analysis is required to generate information relevant to the breeding program.

Keywords: *Diversity, Oryza meyeriana, Oryza minuta, Oryza rufipogon, Simple-sequence repeats (SSR), Wild Rice Relatives (WRR).*

Introduction

The wild species of the genus *Oryza* is composed of two domesticated (*O. sativa* and *O. glaberrima*) and 22 wild species (Vaughan, 1994), representing 15 - 25 million years of evolutionary diversification. In the Philippines, four wild rice species were native namely: *O. rufipogon*, *O. meyeriana*, *O. minuta,* and *O. officinalis*. These wild species of rice represent a rich source of genes for rice improvement, including genes for disease and insect resistance and tolerance to abiotic stresses such as drought and salinity. These wild *Oryza* species are, in fact, grass-like plants, which are phenotypically inferior in agronomic traits such as poor plant type, low grain yield, poor grain type, and are shattering in nature (Ali et al., 2010). The wild species exhibit tremendous diversity in morphological traits, height, tillering, flowering, growth habit, panicle, leaf, culm, and seed characteristics, and adaptation to different habitats and agronomic traits. The wild plant species of rice are valuable resources and serve as a virtually untapped reservoir of genetic diversity that can be used to improve rice through breeding programs.

Previous evaluations show that wild relatives of rice are sources of resistance. Sources of resistance to striped stem borer have been found in 13 species, brown planthopper in 11 species, and bacterial blight in 8 species (Heinrichs et al., 1985; Ikeda et al., 1990; Khan et al., 1991). Limited sources of resistance to sheath blight in cultivated rice hinders the development of host resistance. Some accessions of *O. nivara*, *O. barthii O. rufipogon*, *O. minuta*, and *O. latifolia* have resistance or moderate resistance to sheath blight (Amante et al., 1990). *O. officinalis* has sources of resistance to yellow stem borer, brown planthopper, white-backed planthopper, zigzag leafhopper, and green leafhopper (Khan et al., 1991; Heinrichs et al., 1985). The tetraploid species *O. minuta* has been used as a source of blast and bacterial blight resistance (Amante-Bordeos et al., 1992; Sitch et al., 1989). In addition, assessment of pathogens showed similarly high levels of resistance in wild germplasm. Sixty-six of 98 accessions of *O. rufipogon* were shown to be resistant to six Philippine races of bacterial blight (Ikeda et al., 1990). *O. nivara* is a major dominant gene donor for resistance to the grassy stunt virus biotype 1 (Khush and Ling, 1974). A resistance gene to one of the strains of grassy stunt virus (GSV1) was found only in wild rice *O. nivara* (IRGC Acc. 101508) (Khush and Ling, 1974).

All the wild species in the *Oryza* genus serves as a gene pool that can be utilized to expand the genetic background of cultivated rice in breeding programs conducted in wide hybridization for rice improvement. Attention given to the wild relatives of rice has increased as evaluation of this germplasm revealed new sources of resistance to pests and diseases. Techniques to transfer genes from wild rice to cultivated rice are also becoming more available.

Wild rice species have evolved to survive droughts, floods, and extreme temperatures and become adapted to cope with natural hazards (Borromeo et al., 1994). The presence of *O. officinalis, O. meyeriana, O. minuta,* and *O. rufipogon* in the Philippine rice ecosystem serves as genepool for breeding programs. For instance, *O. rufipogon* has been used in breeding *O. sativa* to provide resistance against stem rot disease caused by *Magnaporthe salvinii* (Tseng and Oster, 1994), resistance against rice tungro viruses (Angeles et al., 1998), and submergence tolerance (Mandal and Gupta, 1997). In addition, it is also resistant to rice blast (RB) caused by *Magnaporthe grisea* (Reimers et al., 1993) and bacterial leaf blight (BLB) caused by *Xanthomonas oryzae* pv. *oryzae (*Sun et al., 1992; Kaushal and Sidhu, 1998). Likewise, *O. officinalis* has resistance to brown plant hopper and white backed planthopper. *O. minuta* has resistance against brown planthopper, green leafhopper, white backed plant hopper, blast and BLB.

Unfortunately, wild rices are at risk of extinction due to natural and man-made changes such as land conversion and adverse effects of climate change (Borromeo, 2000). Their vulnerable position is compounded by the fact that rice fall between the agricultural and conservation agenda; agriculture looks at tended lands and conservation does not focus on agricultural resources. Genetic erosion of the wild species gene pool has occurred in many areas. Populations of wild rice are continually being destroyed especially in the rapidly developing countries in Asia where urban conglomerations are spreading into rural areas. Forest habitats of shade-loving *Oryza* species are also disappearing at an alarming rate.

Wild rice populations also face extreme pressures from the advancement of developmental projects. Without efforts to rescue and preserve the dwindling wild rice populations in the Philippines, they will become extinct. Rescue and conservation strategies include but not limited to protection of wild habitats and *ex-situ* conservation as what IRRI-Genetic Resources Center put up. The opportunity lies in some wild rice relatives that could be vegetative propagated and can also be used for cryopreservation. Therefore, conservation of this vast wild rice germplasm will be an opportunity for rice breeders to explore a number of useful traits not present in the cultivated rice for rice improvement (Borromeo et al., 1994).

Materials and Methods

Polymerase chain reaction (PCR) Analysis of Wild Rice in the Philippines

Fourteen WRR were collected: three samples of *O. meyeriana,* nine *O. minuta,* and two *O. rufipogon.* Genetic diversity was determined using 14 highly polymorphic microsatellite SSR markers with one sample of *O. sativa* as check. Total genomic Deoxyribonucleic acid (DNA) was extracted from the leaves using modified Cetyl Trimethylammonium Bromide (CTAB) method. The purity and concentration of DNA were determined using spectrophotometer at 260 nm and 280 nm while the quality of the DNA was determined using agarose gel electrophoresis. The DNA samples with Tris-EDTA (TE) buffer were diluted with sterile distilled water for the amplification of SSR primers and analysis of genetic polymorphism. Polymerase Chain Reaction (PCR) was conducted in a reaction of 5.6 uL volume containing 5x PCR buffer, 5 µM deoxynucleotide triphosphate (DNTPs), 25 mM of MgCl2, 10 mM of forward and reverse primer, 5 units of Taq DNA Polymerase, and the template DNA. The PCR was amplified using a thermal cycler, following the cycle profile; initial denaturation at 94°C for 5 min, followed by 35 cycles of denaturation at 94°C for 1 min, annealing at 60°C for 1 min, elongation at 72°C for 2 min, and final extension at 72°C for 7 min. PCR-amplified products were subjected to electrophoresis in 8% polyacrylamide gel in 1x Tris/Borate/EDTA (TBE) buffer at 100 volts with a running time of 75 min. The gels were stained with gel red for 10 min. DNA bands were visualized under UV light using the AlphaImager gel documentation system.

Data Analysis

Amplified products from microsatellite analysis were scored for the presence (1) and absence (0) of each marker allele genotype combination. The genetic index (Table 1) for each SSR marker including major allele frequency, genotype number, allele number, gene diversity, heterozygosity, and polymorphism information content (PIC) were determined using the PowerMarker software version 3.25 (Liu and Muse, 2005). The analysis and dendrogram construction were performed using the Unweighted Pair Group Method with Arithmetic Average (UPGMA) through the NTSYSpc software version 2.10x (Applied Biostatistics, Inc).

Table 1. Genetic index of 14 SSR markers used in the genetic characterization of 15 *Oryza* spp.

Marker	Major Allele Frequency	Genotype Number	Allele Number	Gene Diversity	Heterozygosity	PIC
RM11904	0.76	3.00	3.00	0.39	0.00	0.35
RM25934	0.59	3.00	2.00	0.48	0.24	0.37
RM19754	0.71	3.00	4.00	0.47	0.29	0.44
RM27233	0.44	4.00	3.00	0.61	0.88	0.53
RM24843	0.85	4.00	3.00	0.26	0.06	0.24
RM447	0.71	3.00	3.00	0.44	0.00	0.38
RM11	0.50	4.00	4.00	0.65	0.53	0.60
RM334	0.53	3.00	3.00	0.60	0.00	0.53
RM44	0.65	4.00	4.00	0.52	0.12	0.47
RM495	0.47	3.00	3.00	0.62	0.00	0.55
RM162	0.71	3.00	3.00	0.44	0.47	0.38
RM259	0.71	3.00	3.00	0.44	0.12	0.38
RM6051	0.76	2.00	2.00	0.36	0.00	0.30
RM328	0.71	2.00	2.00	0.42	0.00	0.33
Mean	**0.65**	**3.14**	**3.00**	**0.48**	**0.19**	**0.42**

Results and Discussion

Fourteen of the 300 SSR primers were used to generate marker profiles among which 14 succeeded in amplifying the 14 wild rice relatives and one *O. sativa* species, producing 45 alleles with an average of 3.2 alleles per chromosome. The number of alleles indicates the richness of the population. The gel picture shows the banding pattern of 14 wild rice relatives using RM27233 marker (Figure 1). The highest genetic diversity (0.60) was recorded in marker RM11 while the lowest genetic diversity (0.24) was recorded in RM24843.

The observed genetic diversity index varied from 0.03 to 0.46 averaging 0.58 with genetic similarity (GS) coefficient of 0.42, reflecting a moderate level of genetic diversity (Figure 2). UPGMA cluster analysis showed two major clusters consisting of two entries of *O. rufipogon* and the other cluster had the other *Oryza* spp. including *O. sativa*.

Four sub-clusters separated the difference species at 70% coefficient with three sub-clusters IIA with *O. sativa*, IIB with the three *O. meyeriana* entries, and IIIC with the nine *O. minuta*. *O. minuta* (0.30) had the highest diversity within species while the least diversity was observed between the two populations of *O. rufipogon* (0.03).

The WRR from the Philippines has an average of 3.2 alleles per locus higher than the observed 2.4 alleles per locus as reported by Singh et al. (2013) who only focused in two wild rice *O. rufipogon* and *O. nivara.* The level of polymorphism observed among the WRR were highly informative for four markers (PIC>0.5) and were moderately informative (0.5>PIC>0.25) for nine markers as observed based on Dang et al. (2015) classification of PIC values. The genetic diversity of each SSR locus appeared to be associated with the number of alleles detected per locus. In accordance with the observation of Lapitan et al. (2007). The higher the PIC value of a locus, the higher the number of alleles was detected. This study showed RM24843 with 3.00 alleles per locus at 0.24 PIC value as compared with RM 211 with 4 alleles per locus at 0.60 allele per locus. Interspecies diversity was also observed and could infer no significant variation. In case of *O. minuta*, the sources of samples were collected in the same riverbank from upstream to downstream, which indicates low diversity (0.10). Two entries may also be clones as they are 100% similar in characteristics. *O. meyeriana* entries also had low diversity (0.13) but slightly higher than *O.*

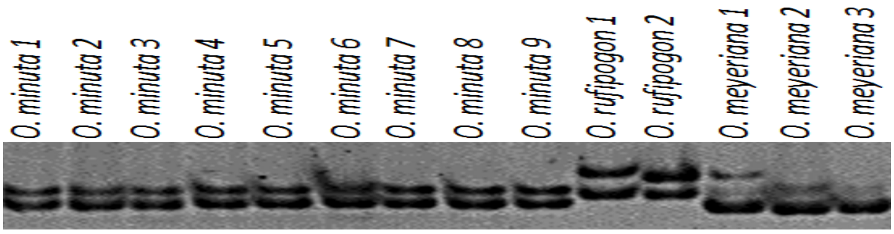

Figure 1. Representative 8% non-denaturing polyacrylamide gel electrogram showing banding polymorphic patterns of wild rice relatives using RM27233.

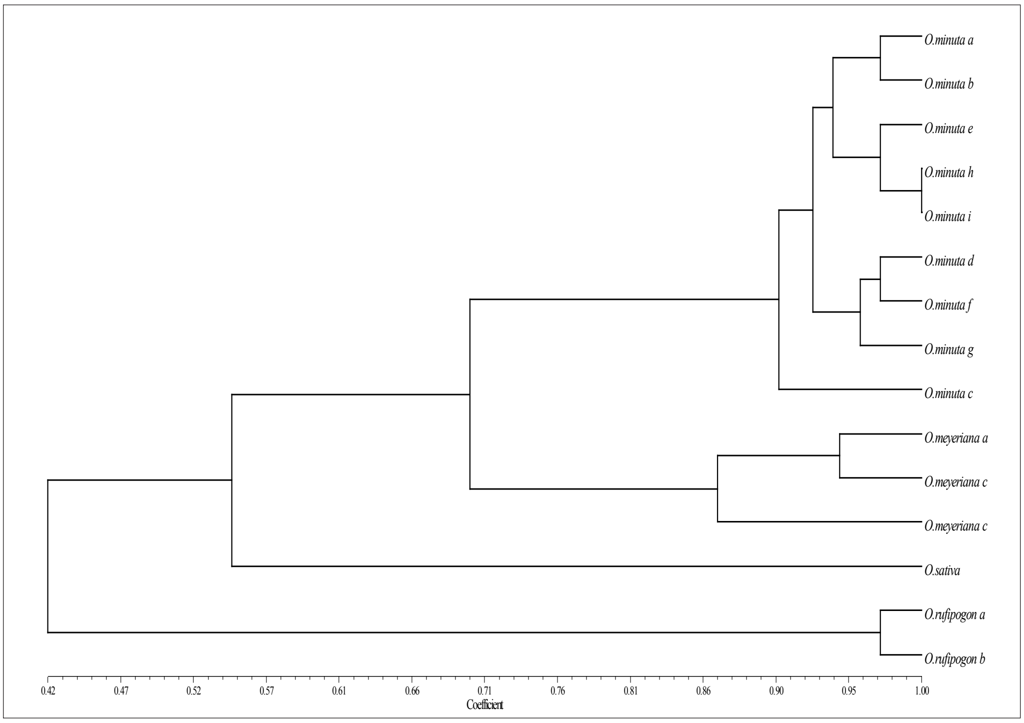

Figure 2. Dendrogram of 15 *Oryza* species derived from UPGMA cluster analysis using NTSYS v2.0 based on 14 polymorphic SSR markers.

minuta. Seeds of *O. meyeriana* are highly shattering and could attract birds or wind leading to their spread.

On the other hand, the observed similarity between *O. minuta* and *O. rufipogon* wild rice relatives ranged 35 - 43% while *O. rufipogon* and *O. meyeriana* ranged 49 - 59%. This indicates that *O. minuta* and *O. rufipogon* are most likely related wild rice relatives in the Philippines also shown in previous findings (Borromeo et. al., 1994).

Conclusion

The genetic diversity within species is low while diversity between species is high based on 14 polymorphic SSR markers. The number of samples per population may have contributed to this initial result; thus, the number of collection sites should be increased in future studies. Further analysis such as phenotyping and gene-specific molecular markers may be conducted to generate information for breeding programs.

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