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ABOUT THE COVER

A rice-based ecosystem comprises a wide range of adaptation and vulnerability. Response to environmental conditions of economically important crops, aside from rice, should not be taken for granted. The most important and basic aspect is soil quality which served as the main medium of crop performance. Being an agriculture-based country, majority of the farmers rely on food production in a typical soil that they cultivate. Preservation and sustainability of the precious ground where they grow food should be enhanced. In this issue, most of the studies tackled the performance of the crops under different soil moisture fluctuations, root development, variety yield performance, and insect pests and diseases reaction. This provides significant information on how farmers and researchers can overcome challenges in the overall crop productivity.

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RESPONSE OF LOWLAND RICE GROWN IN MINE-CONTAMINATED SOIL WITH COCONUT HUSK AND CATTLE MANURE BIOCHARS

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Abstract

A screenhouse pot experiment was conducted to study the effect of coconut husk and cattle manure biochar application on the properties of soil from mine tailing site and on the growth and yield of rice grown under lowland soil condition. Results showed that adding coconut husk and cattle manure biochar to mine-contaminated soil reduced the concentrations of heavy metals such as copper (Cu), iron (Fe), manganese (Mn), and zinc (Zn) in soil and plant tissues. Cation exchange capacity (CEC), organic carbon (OC), nitrogen (N), and potassium (K) were increased by application of both biochar. Available phosphorus (P) was also increased by applying biochar derived from cattle manure. Plant height, number of tillers, fresh and dry biomass, number of panicles per hill, harvested grain per pot, weight of 1,000 grains, percent filled grains, and harvest index were improved by adding coconut husk and cattle manure biochar with the recommended rate of fertilizer.

Keywords: *Cattle Manure Biochar, Coconut Husk Biochar, Mine-contaminated Soil, Lowland Rice, Soil Degradation, Soil Quality.*

Introduction

The Philippines is the fifth richest in copper, nickel, gold, and chromite in the world. Although mining brings socio-economic benefits, it is considered to be one of the most dangerous anthropogenic activities and one of the major causes of soil contamination.

Mining in the country began in 1521 (Flores, 2017). In Buhawen, San Marcelino, Zambales, operations started in 1975 by Dizon Copper and Silver Mines Inc. (DCSMI) and Benguet Consolidated Inc. Until 1997, the companies produced 110 M tons of tailings (Medusa Mining Ltd of Australia, 1997), which contain 0.300 g t^{-1} gold, 0.600 g t^{-1} silver, 0.074% copper, and 4% magnetite. Operations resulted in soil erosion due to movements of huge quantities of large rocks that clogged rivers and diverted water flow contaminated with non-ferrous metals and other toxins, which included cyanide (Cn), mercury (Hg), iron (Fe), copper (Cu), manganese (Mn), and zinc (Zn).

Land degradation affected approximately 50 ha of lowland rainfed rice production area with grains accumulating the contaminants. The use of biochar presents a possibility of rehabilitating contaminated lands to be productive again. Biochar is a carbon-rich product obtained when organic biomass is heated under limited or without oxygen conditions (Lehmann, 2007). It is widely accepted as a potential alternative of lime, which can address soil infertility and contamination.

Biochar has been proven to be effective in improving soil properties and increasing crop biomass. Recently, it has been used to remediate soil with heavy metal and organic pollutants (Tang, 2013) in corn. Studies also show that it can increase the growth and yield of radish planted in degraded alfisol and of cowpea cultivated in ferralsols (Chan et al., 2007 and Woolf, 2008). Further studies confirm its capacity in improving nutrient retention and cation exchange capacity (Liang et al., 2006 and Masulili et al., 2010), decreasing soil acidity (Masulili et al., 2010 and McLaughlin et al., 2009), improving soil structure, and increasing yield of rice, corn, and legumes (Lehmann, 2007). When applied on rice in poor lowland rainfed soils, biochar increases rice yield especially on unfavorable soils (Konboon et al., 2010). However, there are factors affecting the quality of biochar as soil amendment such as organic matter or feedstock, process, temperature, and charring time.

Presently, biochar is prioritized in research due to its role in mitigating environmental concerns and agronomic application (Xiapu et al., 2014).

Meanwhile, its application on soils is gaining global attention due to its potential in improving water holding and soil nutrient retention capacity. This contributes to increasing rice yield and sustaining carbon storage, which leads to reduced greenhouse gas emissions (Downie et al., 2009).

Biochar can be produced from different materials including plants or animals. Choosing raw materials depends on abundance in the locality. Coconut husk, a plant-based material produced at 4.5 t/year in the Philippines (Zafar, 2015), is often used as raw material due to its high organic carbon, surface area, and negative charge. It can resist degradation due to its high amount of fiber and it is hard to decompose. Cattle manure is considered among the animal-based materials due to its abundant supply produced at 21 t/year (Fischer, 2008). Cattle manure biochar is high in organic carbon, surface area, and negative charge. It can resist degradation and has more nutrients than plant-based biochar.

To help address soil degradation, which results in decreased crop productivity and high concentration of heavy metals in the soil, this study evaluated the effect of added coconut husk and cattle manure biochar on the properties of soil from mine tailing site, growth, dry matter partitioning, yield, and yield components of lowland rice.

Figure 1. Green Inno-technology for Negative Carbon Production (GIN-P) pyrolytic cook stove used in production of coconut husk and cattle manure biochars.

Materials and Methods

Production of Biochar

Using the Green Innotechnology for Negative Carbon Production (GIN-P) pyrolytic-cook stove (Figure 1), two types of feedstocks coconut husk and cattle manure were produced at the Agriculture Systems Institute (ASI) composting and demonstration area in the College of Agriculture and Food Science (CAFS), University of the Philippines-Los Baños (UPLB).

Coconut husk and cattle manure were air-dried for two weeks to remove excess moisture. After drying, the feedstocks were chopped approximately 2-3 cm prior to charring.

Biochars were prepared following the procedure of Pangga (2017), in which 1 kg of dried coconut husk and 3 kg of dried cattle manure were placed separately in the GIN-P pyrolytic cook stoves. The coconut husk and cattle manures were charred for 105 and 80 min, respectively. Both biochars were pulverized and sieved in 2 mm mesh, and stored in dry container before analysis and soil application.

Soil Collection, Preparation, and Potting

Soils were collected from Barangay Buhawen, San Marcelino, Zambales with geographic coordinates N 14 57’ 57.4” and E 120 19’ 19.8”. Soil samples weighing 1 kg were collected from 10 strategic spots at 15 cm depth.

Approximately 50 ha of agricultural land were covered with mine tailings. The area was the main source of food and income as it was where vegetables and rice were grown. Rice can be planted but may not be productive due to heavy metals. Farmers in the locality practice direct rice seeding. Growing other vegetable crops are also impossible due to mine tailings. Soil analysis conducted prior to this study showed that the area is contaminated with Cu, Fe, Mn, and Zn. To simulate the lowland condition, 12 plastic pots with 12-inch diameter and 12-inch height without holes were used. The mine-contaminated soil was air-dried and sieved in 2 mm mesh. Sieved soil samples (12 kg) were placed on each treatment pots.

Treatment and Experimental Design

A pot experiment was conducted in the screen house of ASI-CAFS UPLB from October 2017 to February 2018. It was laid out in Randomized Complete Block Design (RCBD) with four treatments with three replications: T1 farmers’ practice (120-30-30), T2 recommended rate (RR) of NPK (180-60-60), T3 RR + coconut husk biochar, and T4 RR + cattle manure biochar. Both biochars were applied at 40 t ha^{-1} (240g pot^{-1}) recommended rate of Bakar et al. (2015).

Cultural Management of Rice

Lowland rice (PSB Rc 18) was used as test crop for it is widely planted by the farmers in the area. Direct seeding was done following farmers' local practices. Four holes were made with approximately 10 cm between holes. Two seeds were sown in each hole at 2 cm depth. Two weeks after sowing, thinning was done by leaving only 1 rice seedling per hill or 4 seedlings per pot. From sowing to seedling, pots were irrigated once a day with 350 ml of distilled water. After seedling stage, 5 cm of water was maintained above the soil surface.

Rice grains were manually harvested 123 days after seeding. Harvested grains and plants were stored in dry place before gathering other agronomic parameters.

Biochar Characterization and Analysis

Chemical and Brunauer-Emmet-Teller (BET) Analysis was done to the produced biochars. Nitrogen (N), phosphorus (P), potassium (K), and organic carbon (OC) were analyzed.

The specific surface area, pore radius, and pore volume of the biochars were analyzed using N2 sorption isotherms run on automated surface area in the Nanotechnology Laboratory. The specific surface area and pore size distribution reading were taken from adsorption isotherms using the equation of Brunauer-Emmet-Teller (BET) physisorption analysis (Zhang, 2011).

Data Collection of Plant Parameters

Plant parameters were collected and divided into physiological and agronomical parameters. Physiological parameters include: weekly plant height and number of tillers; days to flowering and maturity; and fresh and dry biomass of roots, leaves, and stalk. Agronomical parameters include: weight of grains, number of panicles per hill, harvested grain per pot, weight of 1,000 grains, percent filled grains harvest index, and agronomic efficiency.

Harvest index was calculated by dividing the economic yield (grain dry weight in grams) with biological yield (grain and straw weight in grams) multiplied to 100 while agronomic efficiency (N, P, and K) was calculated by dividing the yield difference between biochar and control with the biochar applied multiplied to 100.

Soil and Plant Tissue Analysis

Initial sample of mine-contaminated soil and soil samples collected from each treatment after harvest were analyzed for nutrient composition. Laboratory methods used in the chemical analysis of these samples were presented in Table 1.

Table 1. Laboratory methods used for each soil analysis.

Property	Laboratory Method Used	Reference
1. pH	1:1 distilled water using pH meter	Black, 1965
2. Cation exchange capacity	Ammonium acetate	Chapman, 1965
3. Organic carbon	Walkey and Black	Jackson, 1958
4. Nitrogen	Kjeldahl	Greweling and Peech, 1960 Jackson, 1958
5. Available Phosphorus	Bray No. 2	Bray and Kurtz, 1945
6. Exchangeable Potassium	Flame photometer (Ammonium acetate extraction)	Black, 1965 Peech, 1945
7. Copper (ppm)	Atomic Absorption Spectrophotometer (AAS)	Russell et al., 1957
8. Iron (ppm)	AAS	Russell et al., 1957
9. Manganese (ppm)	AAS	Russell et al., 1957
10. Zinc (ppm)	AAS	Russell et al., 1957

Rice plant parts such as roots, leaves, stalk, and grains were subjected to chemical analysis to evaluate whether iron, copper, manganese, and zinc were absorbed by the plants and translocated to the grains (Table 2). Analysis was done at the soils laboratory of Southern Tagalog Integrated Agricultural Research Center (STIARC) in Lipa City, Batangas and ASI at UPLB.

Table 2. Laboratory method used in the analysis of plant tissues. No 2 lines.

Property	Laboratory Method Used	Reference
1. Copper (ppm)	AAS	Russel et al., 1957
2. Iron (ppm)	AAS	Russel et al., 1957
3. Manganese (ppm)	AAS	Russel et al., 1957
4. Zinc (ppm)	AAS	Russel et al., 1957

Statistical Analysis

Statistical analysis following the Analysis of Variance (ANOVA) in RCBD was done using Statistical Tools for Agricultural Research (STAR 2.0.), in which treatment means were compared using Least Significant Difference (LSD).

Results and Discussion

Chemical Properties of Collected Soil

Soil collected in Brgy. Buhawen, San Marcelino, Zambales was identified as Antipolo Series (Typic Eutrudepts). The chemical properties of this sandy loam soil are presented in Table 3. Soil was extremely acidic at 3.8 pH. Organic carbon was found to be very low at 0.36%. It is positively correlated with available N and P and negatively correlated with Fe, Mn, Cu, and Zn (Maiti and Ghose, 2005). The collected soil was low N and P but high in K. Moreover, the soil had high levels of Cu, Fe, Mn, and Zn.

Table 3. Physico-chemical properties of Antipolo sandy loam, Brgy. Buhawen, San Marcelino, Zambales.

Soil Property	Concentration
pH-H_2O	3.80
Organic carbon (%)	0.36
Cation exchange capacity ($cmol_c$/kg soil)	4.93
Total N (%)	0.03
Available P (Olsen) mg kg^{-1}	7.00
Exchangeable K (cmolc kg^{-1})	5.20
Active Cu (ppm)	390.00
Active Fe (ppm)	486.00
Active Mn (ppm)	300.00
Active Zn (ppm)	153.00
Particle size	
Sand (%)	61.00
Silt (%)	32.00
Clay (%)	7.00

Properties of Biochar

Chemical properties of coconut husk biochar (CHB) and cattle manure biochar (CMB) are presented in Table 4. The type of organic matter or feedstock and the conditions under which biochar is produced greatly affect its relative quality as a soil amendment (McLaughlin et al., 2009). CHB and CMB had very high organic carbon (OC), N, P, and K. It was observed that CMB had higher amount of OC, N, and P than CHB. However, K of coconut husk biochar is slightly greater than in cattle manure biochar.

Table 4. Chemical properties of coconut husk and cattle manure biochars.

Chemical Property	Biochar	
	Coconut Husk	Cattle Manure
Organic carbon (%)	11.65	18.94
Total N	0.60	1.37
Total P	0.32	2.79
Total K	1.82	1.81

CHB has a larger surface area than CMB measuring 107.186 m^2g^{-1} and 68.035 m^2g^{-1}, respectively. A similar pattern was observed for pore radius with 315.420 Å in CHB and 19.128 Å in CMB (Table 5). Biomass feedstock and the processing conditions are the main factors determining pore size distribution in biochar and its reactive surface area (Downie et al., 2009). Surface area of plant-based biochar was remarkably higher than animal-based due to its high aromatic carbon content (Qui et al., 2014).

Table 5. Physical properties of coconut husk and cattle manure biochars.

Biochar	Average Surface Area (m^2 g^{-1})	Average Pore Radius (Å)	Average Pore Volume (cc g^{-1})
Coconut husk biochar (CHB)	107.186	315.420	0.003
Cattle manure biochar (CMB)	68.035	19.128	0.045

Soil Properties under Lowland Soil Water Condition after Harvest

The chemical properties and levels of heavy metals of mine-contaminated soil under lowland soil-water condition after harvest are presented in Tables 6 and 7.

Cation Exchange Capacity (CEC) of the soil was significantly increased by the application of both biochars. However, higher CEC was observed on soil added with CHB (6.96 cmolc kg^{-1} soil) than in CMB (6.76 cmolc kg^{-1} soil). The improvement of the CEC can be attributed to the high surface area of the biochar, which resulted from its porous structure. Cheng et al. (2006) reported that slow oxidation of the biochar increased the number of carboxylic groups, which in turn increased the CEC of the amended soil.

No significant difference was observed in pH. However, treatments with biochar helped increase the soil pH from 3.70 to 4.14 in CHB and from 4.14 to 4.28 in CMB.

CHB and CMB increased the soil organic carbon (SOC) level by 0.64% and 0.61%, respectively. Similar with SOC, treatments added with biochars increased the total nitrogen. This supports the findings of Prommer et al. (2014), which showed that biochar amendment resulted in increased soil organic matter and enhanced total soil N concentration.

Biochar application increased the level of exchangeable K but higher increase was noted in CHB. However, in terms of available P, a significant rise was observed in the application of CMB, which was not noted in CHB. This supports the findings of Hoogwijk et al. (2003), which showed that CMB can augment available P due to the nutrient's natural presence in the treatment.

Table 6. Chemical properties of mine-contaminated soil under lowland soil-water condition after harvest.

	Treatment	CEC Cmolc kg^{-1}		pH %		OC %		N mg kg^{-1}		P Cmolc kg^{-1}		K	
		Initial	Final	Initial	Final	Initial	Final	Initial	Final	Initial	Final	Initial	Final
T1:	Farmers' practice (120-30-30)	4.93	5.38^{b}	3.8	3.80^{a}	0.36	0.28^{b}	0.03	0.024^{b}	7	3.67^{b}	5.2	42.00^{c}
T2:	Recommended rate (180-60-60)	4.93	5.31^{b}	3.8	3.37^{a}	0.36	0.23^{b}	0.03	0.019^{b}	7	4.33^{b}	5.2	41.33^{c}
T3:	Coconut husk biochar (40 t ha^{-1}) + Recommended rate	4.93	6.96^{a}	3.8	4.14^{a}	0.36	0.64^{a}	0.03	0.039^{a}	7	3.33^{b}	5.2	200.6^{a}
T4:	Cattle manure biochar (40 t ha^{-1}) + Recommended rate	4.93	6.76^{a}	3.8	4.28^{a}	0.36	0.61^{a}	0.03	0.036^{a}	7	20.00^{a}	5.2	92.33^{b}
Standard deviation			0.87		1.02		0.18		0.06		7.52		45.15
cv	(%)		6.43		7.89		11.84		11.83		20.07		25.46

Means within column with similar letter(s) are not significantly different at 5% level by LSD.

Table 7. Levels of heavy metals in soil after planted with lowland rice.

	Treatment	Cu (ppm)		Fe (ppm)		Mn (ppm)		Zn (ppm)	
		Initial	Final	Initial	Final	Initial	Final	Initial	Final
T1:	Farmers' practice (120-30-30)	390	8.52^{a}	486	111.11^{a}	300	66.61^{a}	153	4.77^{a}
T2:	Recommended rate (180-60-60)	390	8.22^{a}	486	108.64^{a}	300	65.80^{a}	153	3.71^{b}
T3:	Coconut husk biochar (40 t ha^{-1}) + Recommended rate	390	7.35^{a}	486	78.10^{b}	300	64.77^{a}	153	4.54^{ab}
T4:	Cattle manure biochar (40 t ha^{-1}) + Recommended rate	390	6.72^{a}	486	79.63^{b}	300	72.08^{a}	153	2.83^{c}
Standard deviation			2		17.05		9.22		1.08
Cv	(%)		11.82		4.58		6.8		6.8

Means within column with similar letter(s) are not significantly different at 5% level by LSD.

Though the results showed that there were no significant differences between treatments in terms of Cu and Mn, it is interesting to note that Fe level was significantly decreased with the application of CHB and CMB. These findings showed that iron toxicity can be corrected by applying biochars from coconut husk and cattle manure (Ali et al., 2017).

Data showed that Mn level was not improved by coconut husk and cattle manure biochar applications. These differences on Mn level may be due to other soil-related factors such as SOC and the presence of volatile matter (VM). However, the relationship between Mn and biochar relating to SOC and VM is still unknown (Butnan et al., 2015).

CMB significantly decreased zinc level in lowland rice. However, CHB treatment significantly increased zinc in the soil than the recommended rate of fertilizer alone.

Physiological Parameters of Lowland Rice

Lowland rice started to show significant differences on the plant height and number of tillers after 21 days and 28 days, respectively (Figure 2). Treatments with CHB and CMB significantly increased plant height and number of tillers. The highest growth increment was recorded between 7th and 8th week. This is consistent with the findings of Berek et al. (2011) and Hidetoshi et al. (2009), which showed that biochar application can enhance soil quality of mine-contaminated acidic soil and improve performance of rice.

Rice grown under lowland soil-water conditions with high concentration of iron, copper, manganese, and zinc takes longer to flower and mature. This is the result of zinc toxicity, which delays grain maturity (Dobermann and Fairhurst, 2000). However, applying CHB and CMB minimized Zn resulting in decreased number of days from sowing to flowering and from sowing to maturity (Table 8).

Application of CHB and CMB with recommended rate of fertilizer significantly increased the fresh and dry biomass of roots, leaves and stalk, and grains of lowland rice than farmers' practice and recommended rate of fertilizer alone (Figure 3). This supports the findings of Rajkovich et al. (2012), which showed that

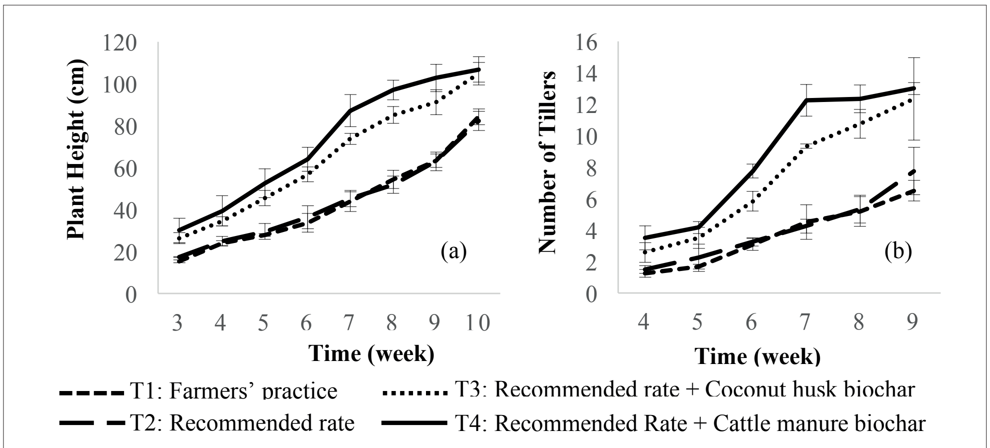

Figure 2. Comparison of weekly (a) plant height and (b) number of tillers of lowland rice grown in mine-contaminated soil added with coconut husk and cattle manure biochars.

Table 8. Days to flowering and maturity of rice grown in lowland soil-water conditions applied with coconut husk and cattle manure biochars.

Treatment		Number of Days	
		Sowing to Flowering	**Flowering to Maturity**
T1:	Farmers' practice (120-30-30)	87.33b	49.67b
T2:	Recommended rate (180-60-60)	87.33b	49.00b
T3:	Coconut husk biochar (40 t ha^{-1}) + Recommended rate	77.00a	44.33a
T4:	Cattle manure biochar (40 t ha^{-1}) + Recommended rate	78.67a	44.33a
Standard deviation		5.16	2.69
cv	(%)	2.03	0.8

Means within column with similar letter(s) are not significantly different at 5% level by LSD.

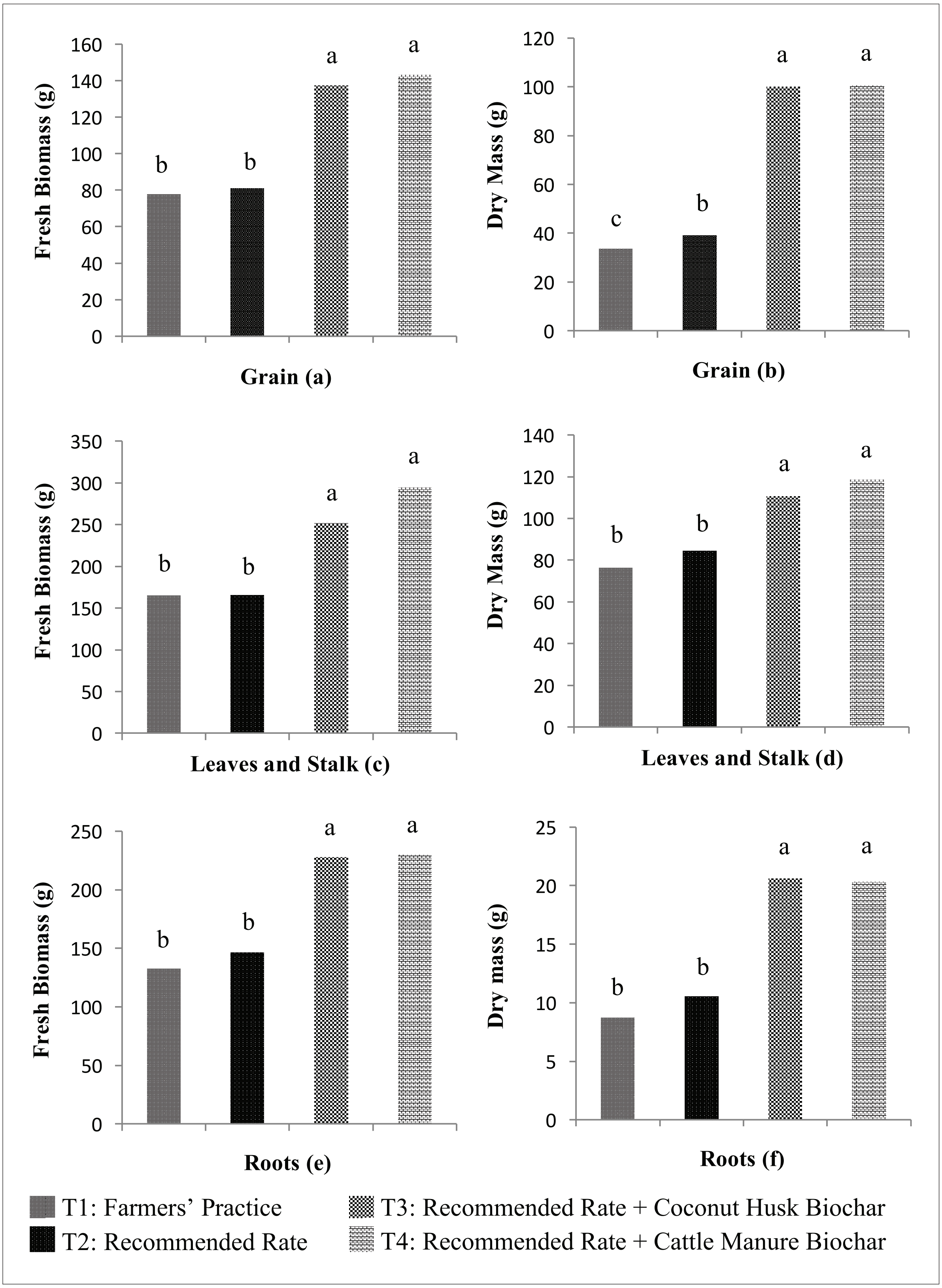

Figure 3. Fresh and dry mass of lowland rice (root, leaf and stalk, and grains) grown in mine-contaminated soil added with coconut husk and cattle manure biochars.

plant growth and yield were high when added with biochar produced at a pyrolysis temperature of 350-400°C.

The increase in dry root biomass suggested that biochar enhances root biomass for more efficiently absorption of soil water and nutrients (Xiang et al., 2010). Jeffery et al. (2011) explained that biochar stimulates plant growth and increases the demand for nutrients and water.

Harvest index of rice grown in lowland soil condition was significantly higher in treatments with added biochars (Figure 4). It was noted that the significant increase in the grain harvest with the application of CHB and CMB resulted in increased harvest index.

Agronomical Parameters

Application of CHB and CMB significantly increased the number of panicle/hill, percent filled grains per pot and grain per pot (Table 9). This is similar to the findings of Zhang (2011) and Zhang and Chen (2011). Increase in the number of panicles resulted in higher harvest and filled grains per pot. Koyama (2016) also found that biochar significantly increased harvested grains of lowland rice. However, biochar application did not produce significant difference in terms of weight of 1,000 grains.

Agronomic efficiency of N, P, and K increased with the application of CHB and CMB (Table 10). Result is similar with Clough and Condron (2010) on increased agronomic efficiency of N and P, and Oram et al. (2014) on increased K efficiency.

Plant Tissue Analysis for Lowland Rice

Copper

Roots contain highest level of copper followed by grains, leaves, and stalk (Table 11). Application of the recommended rate of fertilizer combined with CHB significantly decreased copper concentration in root tissue. Lower concentration was also observed at recommended rate with CMB.

Biochar significantly increased copper concentration in leaves and stalk. Lower concentrations of copper were observed in grains with CMB and CHB than without biochar.

Iron

Concentration of Fe in plant tissue of lowland rice was highest in roots, then in leaves and stalk, and least in grains (Table 12). Application of biochar decreased the concentration of Fe in plant tissue (root, leaves and stalk, and grains). Least iron concentration in rice grains was observed in CMB. This may be related to the decrease of available Fe in the soil after biochar application (Ali et al., 2017).

Manganese

Results indicated that application of biochar did not significantly affect the available manganese in soil resulting in similar concentrations in the root and

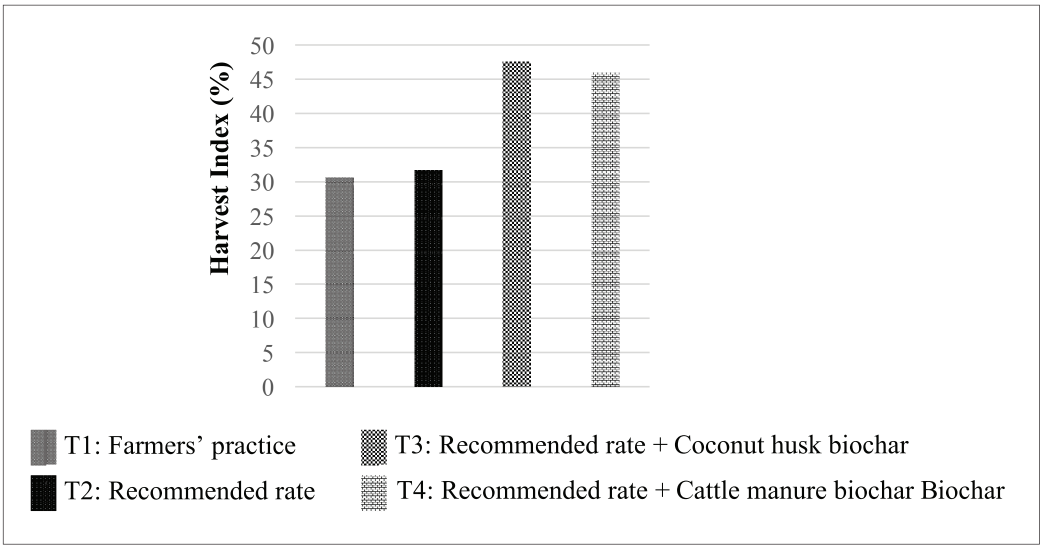

Figure 4. Harvest index of lowland rice grown in mine-contaminated soil added with coconut husk and cattle manure biochars.

Table 9. Yield parameters of lowland rice grown in mine-contaminated soil added with coconut husk and cattle manure biochars.

Treatment		Number of Panicle/ hill	Weight of 1000 grain (g)	Percent Filled Grain	Grain per pot (g)
T1:	Farmers' practice (120-30-30)	5.50^{b}	17.10^{a}	74.78^{b}	77.63^{b}
T2:	Recommended rate (180-60-60)	7.58^{b}	16.97^{a}	74.83^{b}	81.67^{b}
T3:	Coconut husk biochar (40 t ha^{-1}) + Recommended rate	13.17^{a}	23.67^{a}	85.88^{a}	137.30^{a}
T4:	Cattle manure biochar (40 t ha^{-1}) + Recommended rate	13.50^{a}	21.80^{a}	87.23^{a}	143.17^{a}
Standard deviation		3.86	8.65	6.42	33.77
Cv	(%)	16.35	13.79	2.89	13.95

Means within column with similar letter(s) are not significantly different at 5% level by LSD.

Table 10. Agronomic efficiency of N, P, and K of lowland rice grown in mine-contaminated soil added with coconut husk and cattle manure biochars.

Treatment		Agronomic Efficiency		
		N	P	K
T1:	Farmers' practice (120-30-30)	---	---	---
T2:	Recommended rate (180-60-60)	5.22^{b}	15.65^{b}	15.65^{b}
T3:	Coconut husk biochar (40 t ha^{-1}) + Recommended rate	61.42^{a}	184.26^{a}	184.26^{a}
T4:	Cattle manure biochar (40 t ha^{-1}) + Recommended rate	62.07^{a}	186.20^{a}	186.20^{a}
Standard deviation		32.63	97.91	97.91
Cv	(%)	6.35	3.79	3.75

Means within column with similar letter(s) are not significantly different at 5% level by LSD.

Table 11. Copper concentrations of lowland rice plant tissues.

Treatment		Copper Concentration (ppm)		
		Root	Leaves and Stalk	Grain
T1:	Farmers' practice (120-30-30)	236.47^{a}	7.33^{b}	13.37^{a}
T2:	Recommended rate (180-60-60)	206.50^{b}	6.97^{b}	14.77^{a}
T3:	Coconut husk biochar (40 t ha^{-1}) + Recommended rate	207.77^{b}	9.03^{a}	12.43^{a}
T4:	Cattle manure biochar (40 t ha^{-1}) + Recommended rate	125.63^{c}	9.83^{a}	9.93^{a}
Standard deviation		28.99	1.38	6.24
Cv	(%)	6.55	10.02	16.5

Means within column with similar letter(s) are not significantly different at 5% level by LSD.

leaves and stalk (Table 13). However, application of coconut husk biochar increased the manganese concentration in grains of lowland rice.

Zinc

Zinc concentration in roots was significantly higher in treatments with CHB and CMB (Table 14). However, there is a significant decrease in the accumulation of Zn in grains applied with CHB and CMB.

Conclusion and Recommendation

Biochar has gained global interest as soil amendment to positively affect properties of soil and subsequently improve crop yield. This study found that CHB and CMB can improve the quality of mine-contaminated land by increasing the levels of CEC, OC, N, P, and K and by lowering the concentration of heavy metals such as Fe and Zn in the soil. The increased in soil quality resulted in better response of lowland rice by increasing both physiological (plant height, number of tillers, number of days from sowing to flowering and to maturity, and fresh and dry mass) and agronomical parameters (number of panicle per hill, percent filled grains, grain per pot, harvest index, and agronomic efficiency) and by reducing the concentrations of heavy metals in plant tissue specifically in the grains.

Table 12. Iron concentrations of lowland rice plant tissues.

Treatment		Iron Concentration (ppm)		
		Root	Leaves and Stalk	Grain
T1:	Farmers' practice (120-30-30)	187.33^{a}	94.47^{a}	6.00^{ab}
T2:	Recommended rate (180-60-60)	187.33^{a}	91.90^{a}	6.67^{a}
T3:	Coconut husk biochar (40 t ha^{-1}) + Recommended rate	184.00^{a}	48.47^{b}	4.70^{bc}
T4:	Cattle manure biochar (40 t ha^{-1}) + Recommended rate	179.00^{b}	32.90^{c}	3.70^{c}
Standard deviation		3.91	15.12	1.32
Cv	(%)	1.14	8.17	12.56

Means within column with similar letter(s) are not significantly different at 5% level by LSD.

Table 13. Manganese concentrations of lowland rice plant tissues.

Treatment		Manganese Concentration (ppm)		
Root		Leaves and Stalk	Grain	
T1:	Farmers' practice (120-30-30)	170.17^{a}	69.60^{a}	7.53^{b}
T2:	Recommended rate (180-60-60)	180.63^{a}	60.03^{a}	6.70^{b}
T3:	Coconut husk biochar (40 t ha^{-1}) + Recommended rate	167.03^{a}	77.20^{a}	10.87^{a}
T4:	Cattle manure biochar (40 t ha^{-1}) + Recommended rate	121.13^{a}	68.63^{a}	6.77^{b}
Standard deviation		52.92	12.81	2.33
Cv	(%)	39.32	15.92	14.95

Means within column with similar letter(s) are not significantly different at 5% level by LSD.

Table 14. Zinc concentrations of lowland rice plant tissues.

Treatment		Zinc Concentration (ppm)		
		Root	Leaves and Stalk	Grain
T1:	Farmers' practice (120-30-30)	40.43^{b}	25.53^{a}	20.97^{ab}
T2:	Recommended rate (180-60-60)	38.03^{b}	21.80^{a}	24.77^{a}
T3:	Coconut husk biochar (40 t ha^{-1}) + Recommended rate	57.47^{a}	34.43^{a}	18.23^{bc}
T4:	Cattle manure biochar (40 t ha^{-1}) + Recommended rate	55.53^{a}	28.00^{a}	12.87^{c}
Standard deviation		10.47	16.05	5.25
Cv	(%)	14.24	17.39	15.57

Means within column with similar letter(s) are not significantly different at 5% level by LSD.

This study showed favorable effects of biochar on growth of lowland rice even when grown in mine-contaminated soil. However, it is recommended that further research be conducted using lower application rates of biochar because the suggested 40 t ha^{-1} recommendation may not be easy to produce and will not be economical for small farmers.

To verify the effect of biochar on remediating mine-contaminated soil, long-term research of 5-10 years is needed, including studies on effects of biochars on other heavy metals such as cyanide, lead, and nickel.

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MORTALITY OF *Corcyra cephalonica* (STAINTON) LARVAE TREATED WITH *Anamirta cocculus* (L.) WIGHT AND AM. ETHANOLIC EXTRACT

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Abstract

Corcyra cephalonica (rice meal moth) larva is a major pest that attacks rice and other stored grain crops. Chemical pesticides used to control *C. cephalonica* and other stored grain pest pose threat to humans and animals. This led to the use of plants as alternative to chemical pesticides. This research determined the potential larvicidal activity of *Anamirta cocculus* seed extract against *C. cephalonica* larvae. Ethanol extraction was used on *A. cocculus* seeds. Different concentrations (1%, 3%, and 5%) of the extract were formulated and applied to *C. cephalonica* larvae in five trials per treatment. The mortality of larvae observed after 24 and 48 h was computed and subjected to a statistical analysis. Results show that varying the concentrations of *A. cocculus* extract did not have significant effect on the mortality of *C. cephalonica*, while significant difference was noted between the 24 and 48 h intervals. This implies that larvae mortality is time dependent but not dosage dependent. Thus, *A. cocculus* seed extract possesses similar effect at par with commercially available insecticide and its effectiveness increases with time. This study also serves as baseline for further studies on *C. cephalonica* and studies on *A. cocculus* to control rice meal moth larvae in stored rice.

Keywords: *Anamirta cocculus, Corcyra cephalonica, Stored Rice Grains, Biopesticide.*

Introduction

Insect infestations in rice fields and stored rice grains produce negative impacts affecting farmers. *Corcyra cephalonica*, a major pest that mainly attacks important food such as rice, is a stored grain pest common in tropical places and semi-arid climates in Africa, North America, Europe, and Southeast Asian countries. *C. cephalonica* also strikes on wheat, maize, sorghum, groundnut, cotton seeds, coffee, spices, and cocoa beans especially in places with tropical weather (Allotey and Azalekor, 2000) and cereals in tropical and subtropical regions (Champ and Dyte, 1977).

C. cephalonica larvae cause dense webbing as they grow up (USDA, 2016). These pests leave silken threads and tubes to food commodities while feeding and moving up to the grains. Large quantities of frass and silken threads from the larvae pollute the grain causing damages (Srivastava and Sabtharishi, 2016). The moths live 1-2 weeks and each female lay about 100-200 eggs. Development period from egg to adult covers about six months during tropical climate or summer.

Prevalence of *C. cephalonica* worldwide was brought by rice trade (Durrant and Beveridge 1913 as cited in Shailaja, 2008). In Himachal Pradesh, India, *C. cephalonica* is reported to heavily infest during rainy season (Ramesh and Vaidya, 2001). Its infestation was also recorded in Kalyani, Bangladesh, which caused 6.4-25.5% damage to wheat grain and 4.7-23.4% damage to rice grain (Bandyopadhyay and Ghosh, 1999).

In the Philippines, *C. cephalonica* is a major pest in paddy, milled rice, rice bran, and maize (Caliboso et al., 1985). It was reported to be destructive to stored grain crop along with *Sitophilus zeamais*, *Sitophilas oryzae*, *Rhvzopertha dominica*, *Tribolium castaneum*, *Tribolium confusum*, *Oryzaephilus surinamensis*, and *Plodia interpuntella* (Gonzales, 1985).

Shukla and Tiwari (2011) stated that "sufficient knowledge exists on the nutritional and management in addition to the reports on influence of insecticidal agents like organochlorines, organophosphates, and a few synthetic pyrethroids" in the control of *C. cephalonica*. The use of these insecticides, however, poses threat to humans and animals.

In the Philippines, chemical insecticides are the most used pesticide, which contributes to 56% of the total pesticide trade with largest gross amount used in rice. (Tirado et al., 2008). It is generally highly toxic to fishes and tends to bioaccumulate in marine organisms. In a study conducted by Dioquino (2002 as cited in Tirado et al., 2008), almost 88% of the 273 cases of insecticide poisoning reported to the National Poison Control and Information Service (NPCIS) were due to intentional exposure. Although acute toxicity is the immediate threat to rural areas, there are also chronic health effects affecting those who consume contaminated food or water. Contamination usually leads to eye, dermatological, and respiratory problems (UNEP, 1996 as cited in Tirado et al., 2008). Comparative study conducted by Antle and Pingali (1994 as cited in Tirado et al., 2008) also found that insecticide-exposed farmers are likely to suffer from eyes, skin, pulmonary, neurological, and renal problems.

Due to the problems associated with chemical pesticides, and with the increasing resistance associated with the large-scale use of such pesticides, plants as protectants to stored grain crops are being considered as insecticidal sources because these are readily biodegradable, less toxic to mammals, and less likely to contaminate the environment (Rajashekar et al., 2012).

Farmers usually use *Anamirta cocculus* as a substitute for pesticides. They are used by farmers who could not afford to buy commercial pesticides (Bas, n.d.). *A. cocculus*, (Indian berry or Fish berry or Levant nut) locally known as Lagtang, is a climbing plant that originated from Southeast Asia and India. Lagtang, which grows in thickets in the Philippines, has a large woody vine, corky and gray bark. The flowers are yellowish while the fruit is nearly spherical (Stuart, 2016).

Several studies show the potential of *A. cocculus* seeds as biopesticide. Qadir (2014) observed the larvacidal and adulticidal effects after *Aedes aegypti* was exposed to the extract of *A. cocculus.* Qadir and Muffazer (2012). Jothivel and Paul (2008) used raw seeds of *A. cocculus* as biopesticides against fishes *Mystus vittatus* and *Clarias batrachus* (Bloch.). Both studies show that *A. cocculus* maybe used in aquaculture to eradicate unwanted fish species. Significant reduction in fecundity and hatchability of *Culex pipiens* when subjected to acetone extract of *A. cocculus* was reported by Pushpalatha et al. (2012). An ethnobotanical study in Maharashtra, India mentioned the use of *A. cocculus* as an insecticide (Sharma and Sawant, 2012). Likewise, a study titled "Ex-Situ Conservation of Medicinal Plants at University of Agricultural Sciences, Bangalore, Karnataka" indicated the use of *A. cocculus* to kill lice and other parasites (Rajkumar et al., 2011). Insecticidal activity of this plant may be attributed to the presence of picrotoxin as one of the bioactive components and wide range of phytochemicals including phenolics, saponins, volatile oils, terpenoids, and alkaloids (Jothivel and Paul, 2008; Qadir, 2014; Qadir and Muffazer, 2012). Despite possessing pesticidal activities, no mortalities as well as no toxic symptoms were produced in albino rats when administered with various dosages of *A. cocculus*. Furthermore, it was found that Lethal Dose 50 of the alcoholic extract is above 1,600 mg kg^{-1} body weight dosage (Jijith et al., 2016).

This research determined the mortality of *C. cephalonica* when treated with *A. cocculus* seed extract. It also compared the mortality of *C. cephalonica* larvae subjected to varying concentration of extracts and commercial insecticide, mortality *C. cephalonica* larvae for each group in 24 and 48 h, and interaction of concentrations and time in the mortality *C. cephalonica* larvae.

This study is a preliminary investigation of potential alternative control of *C. cephalonica* that will benefit farmers and rice dealers. Furthermore, this study serves as baseline for future studies on *C. cephalonica* as there are limited literature and studies on the potential of *A. cocculus* as protectant for stored grain crops.

Materials and Methods

Research Design

Three experimental group and a control were employed in the study using completely randomized design (CRD) with random selection and assignment of treatments. Each group had five trials. The independent variables such as the amount of extract exposure time, and *C. cephalonica* concentrations were incorporated per trial. Four hundred larvae were used with 20 larvae in each of the trial.

Collection and Extraction of Anamirta cocculus

Lagtang seeds (10 kg) were collected by a resident and delivered from Aringay, La Union to the researchers in Caloocan City Science High School. The seeds are commonly found on mountain ranges and forests. The seeds of *A. cocculus* were transferred, laid in a flat surface, and air-dried for 48 h. The mixture was filtered using a grade 1 qualitative filter paper to separate the solid residue. It was filtered twice until the liquid was clear. The mixture was kept in a semi-opaque plastic bottle and was sent to the Institute of Chemistry of the University of the Philippines-Diliman where the solution underwent rotary evaporation to remove the solvent from the sample. The water bath of the rotary evaporator was set at 40°C. After that, the extract was kept in same container at room temperature.

Formulation of Varying Concentrations of Anamirta cocculus and Control

The concentrations were formulated by placing 2.5, 7.5, and 12.5 mL of extracts in different volumetric flasks. The volumetric flask was filled to 250 mL with distilled water to obtain 1%, 3%, and 5% of *A. cocculus* extract. Sevin (carbaryl), a commercial insecticide served as a positive control, was prepared by incorporating 12.50 g of Sevin to 250 mL distilled water. Concentrations at 20 mL and positive control were transferred into the spray bottles. Twenty spray bottles were used in the experiment.

Contact Application of Anamirta cocculus and Carbaryl to Corcyra cephalonica Larvae

Larvae of *C. cephalonica* used in the test were reared by the National Crop Protection Center (NCPC) at the University of the Philippines-Los Baños. NCPC kept the larvae in a box-type container filled with rice bran. Rice bran, which the larvae fed on, has high nutrition value. The specimens were in the early larval stage ranging from one to seven days old.

The experiment consisted of plastic containers, rice sacks, rice bran, and nets. The sacks were prepared with dimensions of 17.78 x 12.7 cm. The sacks were filled with 10 g of rice bran each. The larvae were placed on the rice bran. Next, the rice sacks were folded and were placed inside a plastic container with dimensions of 5.08 x 12.7 x 17.78 cm. The net was prepared to cover the container. The setup was recommended by NCPC to imitate the way pesticide is applied on infested rice sacks. Lagtang seed extract was sprayed on the sacks.

After applying the extracts, insects were observed after 24 and 48 h. The number of dead larvae was monitored at the given time interval. Larvae were considered dead when they show no sign of movement (Rassami et al., 2016).

Statistical Treatment

The number of dead larvae was recorded to determine the mortality of rice moth larvae and the effectiveness of *A.cocculus* seeds through the use of statistical analysis and hypothesis testing. To determine the significant differences within each group, two-way repeated measures Analysis of Variance (ANOVA) was used as appropriate statistical test. Statistical Package for the Social Sciences (SPSS) was also used.

Results and Discussion

After exposure to the plant extract, 319 larvae of *C. cephalonica* showed no signs of movement after 24 h. Larvae were considered dead if there were no signs of movement after prodding and when the specimen's color turned from whitish yellow to dark yellow (Figure 1). Black spots in the body also indicated death. These reactions were seen in the treatment groups and the control.

Figure 1. Comparison of alive (left) and dead (right) *C. cephalonica* larva.

Table 1 shows the mean mortality of *C. cephalonica* when exposed to the positive control carbaryl and to different concentrations of *A. cocculus* extract after 24 and 48 h.

Table 1. Mean mortality of the treatment and control group in 24 and 48 h.

Time	Carbaryl	1% *A. cocculus*	3% *A. cocculus*	5% *A. cocculus*
24 h	16.6	15.2	15.8	16.2
48 h	17.2	15.8	16.2	16.4

Within the first 24 h after exposure, the positive control had the highest mean mortality with an average of 16.6 deaths while 1% concentration had the lowest mean mortality with an average of 15.2 deaths. The final observation was conducted 48 h after exposure to the extract with the positive control registering the highest mean mortality of 17.2. The 1% concentration had the lowest mean mortality of 15.8 average. More than half of the population died after 24 h and the mortality rate in 48 h was higher than in 24 h.

The computed p-value of time is 0.009 at 0.05 level of significance (Table 2). This means that there were significant differences among the mean mortality rates with respect to time. The results showed that exposing the rice moth for 48 h in either 1%, 3% or 5% concentration of Lagtang seed extract will yield to better mortality rate than when exposed for 24 h.

The results can be supported by several studies on *A. cocculus* used as biocontrol for pest. Pushpalatha et al. (2014) found that larvicidal activity of the *A. cocculus* extract against *Culex pipiens* increases at longer time exposure. Qadir (2014) concluded that an increased exposure, aside from increase in extract concentrations, can facilitate the probing of active

Table 2. Analysis of Variance of mortality rate of control and treatment groups in 24 and 48 h.

Source of Variation	SS	Df	f Value	p Value	Remarks
Time	2.025	1	23.143	0.009*	Significant
Concentration	10.275	3	0.736	0.551*	Not Significant
Time x Concentration	0.275	1.931	0.595	0.570*	Not Significant

*p-value = 0.05

moieties into the body of *Aedes aegypti* larva resulting in higher mortality rate. Qadir and Muffazer (2012) and Jothivel and Paul (2008) also found that increased in exposure results in higher mortality rate of *Mystus vittatus* and *Clarias batrachus*.

Meanwhile, the computed p-value for the concentration alone is 0.551 at 0.05 level of significance. This means that there were no significant differences among the mean mortality of *C. cephalonica* in the three concentrations of *A.cocculus* seed extract. This indicates that increasing concentrations will make a slight difference but not significant enough to affect the results.

The data further indicated that there was interaction of both concentrations and time. The p-value is 0.570 at 0.05 level of significance. This shows that there was no significant difference between the mortality of *C. cephalonica* when exposed to carbaryl and when exposed to *A. cocculus* at 24 h and 48 h. The comparable effect of the various concentrations of the Lagtang extract and Sevin may be attributed to the picotroxin present in Lagtang and the active ingredient carbaryl of Sevin, which also have similar activity in the nervous system.

Carbaryl inhibits the activity of the enzyme that breaks down acetylcholine resulting in the overstimulation of nervous system, which contracts the insects' breathing muscles (Bond et al., 2016). On the other hand, picrotoxin "antagonizes the $GABA_A$ receptor channel directly, which is a ligand-gated ion channel concerned chiefly with the passing of chloride ions across the cell membrane. Therefore, picrotoxin prevents Cl^- channel permeability; thus, promotes an inhibitory influence on the target neuron, which results to uninhibited neuronal signals (DrugBank, 2020).

Conclusion

The study determined the potential larvicidal activity of *A. cocculus* (Lagtang) seed extract against *C. cephalonica* (rice meal moth) larvae. Twenty-four hours after exposure, ethanol extracts of *A. cocculus* yielded a mean mortality of 15.2 for 1%, 15.8 for 3%, and 16.2 for 5%. Meanwhile, 48 h of exposure to ethanol extracts yielded a mean mortality of 15.8 for 1%, 16.2 for 3%, and 16.4 for 5%. The positive control yielded a mean mortality of 16.6 at 24 h and 17.2 at 48 h, the highest mean mortality. Using two-way repeated measures of ANOVA, it was found that there were no significant differences among treatments and control groups. Varying the concentrations of the *A. cocculus* extract did not have a significant effect on the mortality of *C. cephalonica*. On the other hand, there were significant differences between the two-time intervals. This depicts that the mortality of the larvae is time dependent but not dosage dependent. It can be postulated that *A. cocculus* seed extract had insecticidal property comparable with commercially available insecticide that can increase mortality of *C. cephalonica*. It was also found that mortality effect was at 24 h and that effectiveness increases with time.

It is recommended that the number of trials and larvae per trial can be increased for more accurate results. It is also advised to use Soxhlet extraction method instead of ethanol extraction to produce purer extract. Other application of extracts such as ingestion, fumigant, and repellent property are also recommended. *C. cephalonica* should be used under different larval stages for thorough evaluation and Probit analysis can be used to determine lethal dose.

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EFFECTS OF *Hydrolea zeylanica* (L.) VAHL AND *Pistia Stratiotes* (L.) ON RICE GROWTH AND YIELD

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Abstract

Two experiments were conducted to determine the effects of *H. zeylanica* (HYMZE) and *P. stratiotes* (PIIST) on rice growth and yield. HYMZE at 0, 5, 10, and 15 densities/0.16 m^2 were grown under three seeding rates (24, 48, and 72 seeds/0.16 m^2) of direct-seeded rice (DSR). Young and mature PIIST, each at 0, 5, 10, and 15 densities/0.16 m^2, were also grown with transplanted rice (TPR). HYMZE-DSR competition was arranged in 3 x 4 factorial experiment in RCBD with five replications while young and mature PIIST-TPR competitions in simple RCBD each at five replications. Yield of DSR was reduced by 0.3, 6.2, and 6.1% at 5, 10, and 15 HYMZE densities/0.16 m^2. Yield of TPR, on the other hand, was reduced by 4.1% when young PIIST was grown at 15 densities while no reduction at 5 and 10 densities/0.16 m^2. Mature PIIST reduced yield of TPR by 4.2, 4.3, and 11.9% at 5, 10, and 15 densities/0.16 m^2, respectively. Higher reductions on yield of TPR was observed when mature PIIST was grown throughout the crop's growth stages.

Keywords: *Additive Design, Araceae, Hydrophyllaceae, NSIC Rc 222, Water Lettuce.*

Introduction

Weeds are group of pests considered by Filipino farmers as one of the limiting factors in achieving higher yield of cultivated rice (Beltran et al., 2016; Donayre et al., 2014; Moody et al., 1997). In a survey conducted in 10 Asian countries, weeds ranked fourth from lepidopterous leaf feeders, stemborers, and brown plant hoppers as the most destructive pests by rice farmers (Heong and Escalada, 1997). When weeds are not controlled throughout the crop's growth stages, yield of rice under irrigated-lowland, rainfed-lowland, and upland conditions could be reduced up to 55, 74, and 96%, respectively (Ampong-Nyarko and De Datta, 1991). *Hydrolea zeylanica* (L.) Vahl and *Pistia stratiotes* L are Philippine weeds that could grow, compete, and adversely affect the growth and yield of cultivated rice.

H. zeylanica (HYMZE) of Family Hydrophyllaceae, is a common weed of rice particularly in irrigated and rainfed-lowland areas (Donayre et al., 2018; Fried et al., 2017; Donayre et al., 2014; Moody et al., 2014; Moody, 1989). It is an herb in aquatic and semi-aquatic habitat and has an annual or perennial life cycle and ascending or procumbent growth habit. *H. zeylanica* has the following appearance: sparingly branched, nearly glabrous or pubescent stems; leaves lanceolate to ovate with acute base and apex and entire margin; and inflorescence and sepals covered with spreading viscid hairs. Its parts are also seen as: rarely solitary, flowers in terminal panicles or clusters at branch apices; pedicel elongating after anthesis; and calyx lobes lanceolate, pubescent or glabrous. Its characteristics include purple-blue or deep purple-green corolla; scarlet anthers; capsule, ovoid by the persistent calyx lobes; and oblong to ovoid seeds with longitudinal and transverse ridges (Pancho and Obien, 1995; Rhue-cheng and Constance, 1995; Davenport, 1988; Merrill, 1912).

In earlier times, HYMZE was only a minor problem of rice in the country (Merrill, 1912). In recent years, however, it has been considered one of the major weeds of rice in irrigated and rainfed lowland areas. Its ability to produce 1,313,000 seeds $plant^{-1}$ (average) and multiply through cut stems resulted in re-growing and forming of new seedlings, regrowth and reproduction of shoots in flooded or saturated conditions, and rapid growth; making it difficult to control in the field (Casimero et al., 2008; Morita et al., 2012; Fabro et al., 2005; Pancho, 1964). In an interference study, Donayre and Endino-Tayson

(2015) found that HYMZE can significantly reduce the yields of the transplanted rice by 18.9, 24.7, 23.8, 35.5, 51.7, and 55.9% when its densities were 6:6, 6:18, 6:30, 6:42, 6:60, and 6:120 (rice:weed ratio).

P. stratiotes (PIIST) of Family Araceae, is likewise, a weed of rice but mainly in irrigated-lowland areas where extended periods of flooding or abundant moisture takes place after harvest (Donayre et al., 2018; Fried et al. 2017; Pancho and Obien, 1995). Its genus name, *Pistia*, was derived from the Greek word "Pistos," which means watery (Bua-ngam and Mercado, 1975). It is commonly called water lettuce in English and kiapo/quiapo by Filipino rice farmers (Donayre et al., 2018; Merrill, 1912). Moody et al. (2014) described its characteristics as a free-floating, stoloniferous, and perennial plant with long feathery roots that bear off-shoots at the end of each stolon. It also has leaves that are pale green, overlapping, succulent, and covered with numerous hairs on both sides of surfaces. Its older leaves have conspicuous, ovoid swelling filled with spongy parenchyma on the lower surface; and veins resembling a fan. The weed propagates mainly by seed production or by division of off-shoots (Bua-ngam and Mercado, 1975). Its seeds germinate well underwater particularly at 10 cm depth while its off-shoot, that multiplies rapidly, could divide up to 130 individuals in two months. Thus, PIIST flourishes well in lowland rice areas with abundant water or where presence of abundant moisture is extended after harvest.

Confirming and deciding whether the weed is to be controlled or not, or allowing it to grow without compromising the yield of the crop are two of the main reasons why knowledge on crop-weed competition is very important. Earlier report confirmed that HYMZE could reduce the yield of transplanted rice by as much as 55.9% when not managed throughout the crop's growth (Donayre and Endino-Tayson, 2015). However, studies confirming its negative effects on growth and yield of direct-seeded rice are not yet known. Likewise, the negative effect of PIIST on growth and yield of transplanted rice is very limited. Thus, this study determined the a) effects of HYMZE on growth and yield of direct-seeded rice planted at three seeding rates and b) effects of young and mature PIIST on growth and yield of transplanted rice.

Materials and Methods

Location and Materials

Two rice-weed competition experiments were separately conducted at the Crop Protection Department, College of Agriculture, Central Luzon State University (CLSU), Science City of Muñoz, Nueva Ecija from November 2018 to February 2019. Registered class quality of NSIC Rc 222, obtained from the Philippine Rice Research Institute (PhilRice), was used as the test rice variety. The variety was selected as it is widely planted in irrigated-lowland areas in the country. Three-day old pre-germinated seeds for direct-seeding and 21-day old seedlings for transplanting were prepared following the standard recommendations of PhilRice (2007). Fresh, healthy stems of HYMZE and off-shoots of PIIST were collected from the fields of PhilRice. Collected weeds were brought to the experimental area of CLSU for replanting and regrowing in plastic boxes for one month. The composite soil (Maligaya soil series) used as medium for plantingwas collected from the rice fields of CLSU.

Experimental Design

Hydrolea zeylanica

An additive experimental design was utilized to determine the outcome of competition between HYMZE and direct-seeded rice (DSR) (Swanton et al., 2015). In this design, the density of HYMZE was increased while leaving the density of DSR constant. The experimental unit used was a rectangular, 0.16 m^2 plastic box (length = 48 cm, width = 34 cm, depth = 15 cm) filled with 8 kg sterilized, moist soil. Each experimental unit was seeded equidistantly with three-day old pre-germinated seeds of rice using three different seeding rates (24 seeds for 40 kg ha^{-1}, 48 seeds for 80 kg ha^{-1}, 72 seeds for 120 kg ha^{-1}). Afterwards, cut stems (with one node at the center and 5 cm in length) of one-month old *H. zeylanica* were planted at 0, 5, 10, and 15 densities/0.16 m^2 . The box, with pre-germinated seeds at different seeding rates and cut stems of the weed, were arranged in 3 x 4 factorial experiment (factor a – three rice seeding rates, factor b – four *H. zeylanica* densities) arranged in randomized complete block design (RCBD) with five replications. DSR seedlings with HYMZE were cultivated until its maturity. The plants were applied with synthetic fertilizers at 15, 30, and 45 days after direct-seeding at recommended rate of 90-30-60 NPK. Water was supplied and maintained at 5 cm level until maturity.

Pistia stratiotes L.

An additive experimental design was also utilized to determine the outcome of competition between *P. stratiotes* (PIIST) and transplanted rice (TPR). The weed was placed in a rectangular plastic box similar to the material used in planting HYMZE. Each box was planted with six 23-day old seedlings at distance of 10x10 cm. Simultaneously, two-week old young off-shoots (spread of petals = 3 cm) and one-month old mature off-shoots (spread of petals = 10 cm) of PIIST were planted separately at 0, 5, 10, and 15 densities/0.16 m^2. The boxes, with TPR seedlings and PIIST, were arranged in a simple RCBD with five replications. TPR seedlings with the weed were grown until maturity. All plants were also nourished with synthetic fertilizers using the same rate and time of application as HYMZE-DSR competition. Water was also supplied and maintained at 5 cm level until maturity.

Data Collected

Height, number of leaves and tillers, shoot-dry weights, and yield components (number of panicles plant^{-1}, number of grains panicle^{-1}, percentage filled spikelets plant^{-1}, and 1,000-grain weight) of rice were recorded. Grain yield was calculated from yield components by Yoshida (1981). Reductions on agronomic, yield components, and grain yield (GY) of rice were calculated using the equation YL (%) = $[(GY_0 - GY_1)/GY_0]*100$, where, GY_0 as the mean values at 0 weed density and GY_1 as the mean values at 5, 10, and 15 weed densities, respectively. Shoot dry weights of the two weeds were also recorded.

Statistical Analysis

Data on agronomic characteristics, yield components, and grain yields of direct-seeded relative to seeding rates and densities of *H. zeylanica* were subjected to two-way analysis of variance (ANOVA) using the Statistical Tool for Agricultural Research (STAR 201). Data on transplanted rice relative to two ages and densities of *P. stratiotes*, on the other hand, were subjected to one-way ANOVA. All treatments means were compared through Least Significant Difference at 5% level of significance (Gomez and Gomez, 1984).

Results and Discussion

Hydrolea zeylanica (L.) Vahl

Two-way analysis of variance (ANOVA) showed no significant interaction effects of seeding rate x *H. zeylanica* (HYMZE) density on growth and yield of direct-seeded rice (DSR) (Table 1). The main effect of seeding rate was significant on height per plant, number of tillers and leaves per plant, shoot-dry weight per plant, number of panicles per plant, number of grains per panicle, and 1,000 grain weight of DSR. No significant differences were observed on percentage of filled spikelets and grain yield/0.16 m^2. Post hoc analysis showed that DSR planted at 24 seeds/0.16 m^2 (40 kg ha^{-1}) significantly had higher means of height, number of tillers and leaves per plant, shoot dry weight, and number of grains per panicle than when planted at 48 (80 kg ha^{-1}) and 72 (120 kg ha^{-1}) seeds/0.16 m^2 (Table 2). The advantage of 24 seeds/0.16 m^2 over the other two seeding rates can be attributed to less intra-specific competition between DSR plants. In studying the effects of different seeding rates on growth of DSR, Gravois and Helms (1992) reported that 43 kg ha^{-1} resulted in higher grains per panicle than 85, 128, 170, and 213 kg ha^{-1} seeding rates. Ottis and Talbert (2005) also reported that 57/62 seeds m^{-2} had higher means of above ground biomass, number of panicles, and panicle weight than 114/125, 229/250, and 458/500 seeds m^{-2}. Miller et al. (1991) also observed that two rice cultivars at 120 seeds m^{-2} had higher number of spikelets panicle^{-1} than at 240, 360, 480, 600, and 840 seeds m^{-2}. DSR planted at 72 seeds/0.16 m^2 had high number of panicles basin^{-1}.

The main effect of HYMZE density, on the other hand, was significant on height plant^{-1}, number of tillers and leaves plant^{-1}, shoot-dry weight plant^{-1}, and percentage of filled spikelets box^{-1}. No significant differences were observed on number of panicles per plant and grains per panicle, 1,000 grain weight, and grain yield/0.16 m^2. Post hoc analysis showed that height of DSR was higher at 10 and 15 HYMZE densities than at 0 and 5 densities. Taller growth of DSR at higher density of the weed could be a way of plasticity in relation to competition. Although this claim is only hypothetical due to lack of evidence, Noda et al. (1968) observed that rice plants had increasing height in response to increasing density of *Echinochloa crus-galli*. The number of tillers and leaves and shoot dry weight per plant of DSR were not significantly different at 0, 5, and 10 HYMZE densities. Values of these parameters were significantly lower at 15 densities. Percentage of filled spikelets of DSR were not significantly different at 0, 10, and 15 HYMZE densities. Highest value was obtained at 5 densities. Meanwhile, no reductions were recorded on the number of leaves per plant and grains per panicle, percentage filled spikelets, and 1,000 grain weight of DSR at 5 HYMZE densities (Table 3). However, there were 0.8, 5.2, 1.0, and 4.7 reductions on height per plant, number of tillers per plant, shoot dry weight per plant, and number of panicles per plant.

Although no reductions were observed on height per plant, percentage filled spikelets, and 1,000 grain weight at 10 densities, reductions by 8.5, 4.5, 16.9, 5.4, and 3.6% were observed on number of tillers and leaves per plant, shoot dry weight per plant, and number of panicles per plant. Moreover, no reductions were noted on height per plant, number of grains per panicle, percentage filled spikelets, and 1,000 grain weight of DSR at 15 densities. However, there were 14.5, 29.6, and 8.5% reduction observed in number of tillers and leaves per plant, shoot dry weight per plant, and number of panicles per plant. Grain yield of direct-seeded rice was reduced by 0.3, 6.2, and 6.1% when the weed was at 5, 10, and 15 densities.

Table 1. ANOVA (*P*-values) for the effects of different seeding rates and densities of *Hydrolea zeylanica* on agronomic characteristics, yield components, and grain yield of direct-seeded rice.

Factors	Height (cm plant^{-1})	No. of tillers plant^{-1}	No. of leaves plant^{-1}	Shoot dry weight (g plant^{-1})	No. of panicles 0.16 m^{-2}	No. of grains panicle^{-1}	Filled spikekets (%)	1000 grain weight (g)	Grain yield (g 0.16 m^{-2})
SR	.000**	.000**	.000**	.000**	.000**	.000**	.899^{ns}	.025*	.411^{ns}
HD	.000**	.000**	.000**	.001**	.444^{ns}	.423^{ns}	.018*	.645^{ns}	.197^{ns}
SR x HD	.887^{ns}	.076^{ns}	.188^{ns}	.499^{ns}	.585^{ns}	.658^{ns}	.960^{ns}	.891^{ns}	.425^{ns}

SR – rice' seeding rate, HD – *H. zeylanica* density; * - significant, *P*<.05; ** - highly significant, *P*<.005; ns – not significant at .05 level of significance

Table 2. Agronomic characteristics, yield components, and grain yield of direct-seeded rice as affected by different seeding rates and densities of *Hydrolea zeylanica*.

Factors	Height (cm plant^{-1})	No. of tillers plant^{-1}	No. of leaves plant^{-1}	Shoot dry weight (g plant^{-1})	No. of panicles 0.16 m^{-2}	No. of grains panicle^{-1}	Filled spikekets (%)	1000 grain weight (g)	Grain yield (g 0.16 m^{-2})
SD									
24	83.8 ^{a}	5.6 ^{a}	24.0 ^{a}	12 ^{a}	65.3 ^{c}	100.7^{a}	90.2 ^{a}	24.3 ^{b}	139.4 ^{a}
48	78.1 ^{b}	3.8 ^{b}	17.2 ^{b}	4.8 ^{b}	80.0 ^{b}	79.5 ^{b}	89.9 ^{a}	24.8 ^{ab}	140.8 ^{a}
72	74.3 ^{c}	3.4 ^{c}	14.9 ^{c}	4.0 ^{b}	90.6 ^{a}	71.1 ^{b}	90.0 ^{a}	25.4 ^{a}	145.6 ^{a}
HD									
0	77.4 ^{b}	4.6 ^{a}	19.2 ^{ab}	7.8 ^{a}	82.4 ^{a}	82.4 ^{a}	88.8 ^{b}	24.6 ^{a}	146.5 ^{a}
5	76.8 ^{b}	4.3 ^{ab}	20.8 ^{a}	7.8 ^{a}	78.6 ^{a}	89.4 ^{a}	91.7 ^{a}	24.8 ^{a}	146.1 ^{a}
10	81.1 ^{a}	4.2 ^{bc}	18.3 ^{b}	6.5 ^{ab}	78.0 ^{a}	79.4 ^{a}	89.4 ^{b}	25.1 ^{a}	137.5 ^{a}
15	79.7 ^{a}	3.9 ^{c}	16.4 ^{c}	5.5 ^{b}	75.4 ^{a}	83.9 ^{a}	90.3 ^{b}	24.7 ^{a}	137.6 ^{a}

* - significant, *P*<0.05; ** - highly significant, *P*<0.005; ns – not significant at .05 level of significance

Table 3. Reductions (%) on agronomic characteristics, yield components, and grain yield of direct-seeded rice as affected by different densities of *Hydrolea zeylanica* L.

HD	Height plant^{-1}	No. of tillers plant^{-1}	No. of leaves plant^{-1}	Shoot dry weight	No. of panicles 0.16 m^{-2}	No. of grains panicle^{-1}	Filled spikekets	1000 grain weight	Grain yield 0.16 m^{-2}
0	-	-	-	-	-	-	-	-	-
5	0.8	5.2	-8.2	1.0	4.7	-8.6	-3.3	-1.1	0.3
10	-4.8	8.5	4.5	16.9	5.4	3.6	-0.7	-2.2	6.2
15	-3.0	14.5	14.5	29.6	8.5	-1.8	-1.8	-0.4	6.1

Pistia stratiotes

Simple ANOVA showed that agronomic characteristics, yield components, and grain yield of transplanted rice (TPR) at 5, 10, and 15 densities of young *P. stratiotes* (PIIST) had no significant differences from samples grown without the weed. However, there were significant differences on the number of tillers and leaves per plant (Table 4). TPR plants grown at different densities of young PIIST had less number of tillers and leaves per plant than TPR plants at 0 density of the weed. Meanwhile, growth and yield of TPR at different densities of mature PIIST were significantly different in terms number of tillers per plant, shoot dry weight per plant, number of panicles per plant, and percentage filled spikelets. TPR plants grown with mature PIIST significantly had less of tillers per plant, shoot dry weight per plant, number of panicles per plant, and percentage filled spikelets than the samples planted at 0 density of the weed. There were no significant differences in terms of height per plant, number of leaves per plant, number of grains per panicle, 1,000 grain weight, and grain yield/0.16 m^2. No significant reductions was also noted on plant height and 1000 grain weight of TPR plants at 5 densities, number of grains, and 1,000 grain weight at 10 densities, and 1,000 grain weight at 15 densities of young PIIST (Table 5). However, the young stage of the weed reduced the other agronomic and yield components of TPR plants by 0.1 to 14.5% at 5 densities, 0.7 to 15.2% at 10 densities, and 1.5 to 28.1% at 15 densities.

Grain yield/0.16 m^2 of TPR was not reduced at 5 and 10 densities of the weed. Instead, grain yield/0.16 m^2 was reduced by 4.1% when young weed was at

Table 4. Agronomic characteristics, yield components, and grain yields of rice as affected by different densities of *Pistia stratiotes* L.

PIIST Density	Plant height (cm)	No. of tillers plant^{-1}	No. of leaves plant^{-1}	Shoot dry weight (g plant^{-1})	No. of panicles 0.16 m^{-2}	No. of grains panicle^{-1}	Filled spikekets (%)	1000 grain weight (g)	Grain yield (g 0.162m^{-1})
Young									
0	99.6 ^{a}	9.4 ^{a}	28.6 ^{a}	9.6 ^{a}	31.4 ^{a}	84.5 ^{a}	90.0 ^{a}	23.6 ^{a}	53.3 ^{a}
5	101.4 ^{a}	8.1 ^{b}	24.7 ^{b}	9.6 ^{a}	29.6 ^{a}	81.7 ^{a}	88.8 ^{a}	24.3 ^{a}	53.8 ^{a}
10	98.9 ^{a}	8.0 ^{b}	25.1 ^{b}	8.8 ^{a}	30.4 ^{a}	88.3 ^{a}	88.2 ^{a}	24.1 ^{a}	56.6 ^{a}
15	98.1 ^{a}	7.7 ^{b}	25.2 ^{b}	6.9 ^{a}	30.0 ^{a}	79.6 ^{a}	88.5 ^{a}	24.0 ^{a}	51.1 ^{a}
P	.548 ^{ns}	.003 **	.036 *	.077 ^{ns}	.734 ^{ns}	.945 ^{ns}	.215 ^{ns}	.915 ^{ns}	.958 ^{ns}
Mature									
0	98.5 ^{a}	8.9 ^{a}	27.7 ^{a}	10.2 ^{a}	32.6 ^{a}	61.6 ^{a}	86.9 ^{a}	24.1 ^{a}	42.5 ^{a}
5	100.4 ^{a}	7.0 ^{b}	23.8 ^{a}	7.8 ^{b}	26.6 ^{b}	75.0 ^{a}	84.7 ^{b}	23.6 ^{a}	40.6 ^{a}
10	96.9 ^{a}	6.7 ^{b}	22.2 ^{a}	6.5 ^{b}	24.0 ^{bc}	87.5 ^{a}	84.3 ^{b}	23.4 ^{a}	40.7 ^{a}
15	99.3 ^{a}	6.3 ^{b}	21.0 ^{a}	7.1 ^{b}	22.4 ^{c}	86.9 ^{a}	81.3 ^{c}	24.3 ^{a}	37.5 ^{a}
P	.451 ^{ns}	.000 **	.117 ^{ns}	.003**	.000 **	.282 ^{ns}	.000 **	.437 ^{ns}	.942 ^{ns}

* - significant (P<0.05), ** - highly significant (P<0.005), ns – not significant at 0.05 level of significance; means with the same letters are not significantly different at 0.05 level of significance using Fisher's LSD.

Table 5. Reductions (%) on agronomic, yield components, and grain yield parameters of rice as affected by different densities of *Pistia stratiotes* L.

PIIST Density	Height	No. of tillers plant^{-1}	No. of leaves plant^{-1}	Shoot dry weight	No. of panicles 0.16 m^{-2}	No. of grains panicle^{-1}	Filled spikekets	1000 grain weight	Grain yield (g 0.16 m^{-2})
Young									
0	0	0	0	0	0	0	0	0	0
5	-1.8	14.5	13.5	0.1	5.7	3.3	1.3	-2.9	-0.8
10	0.7	15.2	12.2	8.1	3.2	-4.6	2.0	-2.1	-6.3
15	1.5	18.0	11.8	28.1	4.5	5.7	1.7	-1.7	4.1
Mature									
0	0	0	0	0	0	0	0	0	0
5	-2.0	21.1	14.1	18.4	23.6	-21.9	2.5	2.2	4.3
10	1.6	24.4	20.1	20.2	35.7	-42.3	3.4	2.9	4.2
15	-0.9	28.9	24.0	31.3	30.0	-41.2	6.4	-0.7	11.9

15 densities. Mature PIIST, on the other hand, did not reduce plant height and number of grains at 5 densities, number of grains per panicle at 10 densities; and plant height, number of grains, and 1,000 grain weight at 15 densities. However, it reduced the other agronomic and yield components by 2.2 to 23.6%, 1.6 to 35.7%, and 6.4 to 31.3% when it was at 5, 10, and 15 densities/0.16 m^2. Grain yield was reduced by 4.2-11.9% when mature weed was at 5, 10, and 15 densities/0.16 m^2.

Generally, reductions on grain yield of TPR were higher with the presence of mature than young PIIST. Mature PIIST has robust and numerous leaves and roots, which results in more adverse effects on TPR yield. Bua-ngam and Mercado (1976), on the other hand, had different results when they studied the competitive ability of PIIST against rice. They found that leaving 3-leaf stage of the weed throughout the growth stages of 10-old rice plant (IR-8) at 1:5 (rice:weed) ratio resulted in reduced grain yield of the crop by 39, 28, 11, and 9% at 0, 0.15, 0.30, and 0.45 g N kg^{-1} soil per pot. Age and variety of rice, age of the weed, ratio of rice with the weed, rate of fertilizers used, and size of the experimental unit are factors in generating different results from experiments.

Deep understanding on crop-weed competition helps confirm and decide whether a certain weed is to be controlled or not. It can also help select an appropriate weed control measures. The results of this study confirmed that HYMZE, at 10-15 densities, could reduce the yield of DSR plants by 6% when not managed throughout the crop's growth stages. These suggest that control must be implemented when HYMZE infests DSR plants at $\geq$10 densities/0.16 m^2. In a research conducted by Donayre and Endino-Tayson (2015), they found that HYMZE could also reduce the yield of TPR plants by 18.9, 24.7, 23.8, 35.5, 51.7, and 55.9% at rice:weed ratios of 6:6, 6:18, 6:30, 6:42, 6:60, and 6:120.

Gompertz and logistic models suggested that the weed should be controlled within 0-700 growing degree days (GDD) to achieve 100% relative grain yield in TPR. At 0-670 GDD and 5-580 GDD, HYMZE can reduce yield by 5 and 10%, respectively.

It is worth noting that the negative effect of HYMZE on yield of DSR was lesser than TPR. This is possibly due to the effect of high density of DSR as compared with the density of TPR against HYMZE. Chauhan (2012) pinpointed that the use of high seeding rates can also help suppress weed growth and reduce weed competition through rapid canopy closure. In a study on growth response of upland *Cyperus rotundus* L. to interference with DSR, Chauhan and Opeña (2012) found that 12 and 24 rice plants per pot (equivalent to 60 and 120 kg seeds ha^{-1}) reduced the weed's leaf area by 79 and 86%, respectively. It also reduced the shoot biomass, tuber production rate, and leaf biomass of the weed.

In another study by Cao et al. (2007), they found that weedy rice at 0, 5, 25, and 125 plants per m^2 significantly had poorer performance in the field in terms of its vegetative (plant height) and reproductive traits (panicle and seed production) when grown with DSR than with TPR. Other studies also showed that shoot biomasses of *Ammania baccifera* L., *Amaranthus spinosus* L., and *Ludwigia octovalvis* (Jacq.) Raven were reduced by 94, 92, and 98% when grown with higher seeding rates of DSR (Chauhan 2013; Chauhan and Abugho, 2012). It was emphasized, however, that the efficacy of using high seeding density to suppress weed growths depends on the kind of weeds present and the rice cultivar planted (Chauhan, 2012). For example in a study on the effects of DSR interference on growth of *Echinochloa crus-galli* (L.) Beauv. and *Cyperus iria* L., it was found that DSR interference only reduced the height of *Echinochloa crus-galli* (L.) Beauv (Chauhan and Johnson, 2010).

Stem lengths of both weeds increased when grown with more DSR plants than when grown alone. However, crop's interference reduced the inflorescences and shoot biomasses. Same trend of results was also found when *Ludwigia hyssopifolia* was grown with 4 and 12 DSR plants. The weed reduced the effects of DSR interference by increasing its leaf weight ratio, stem and leaf biomasses, and specific stem length (Chauhan et al., 2011).

PIIST can reduce yield from 4 to 11% when grown with TPR plants at 5 to 15 densities/0.16 m^2. It is remarkable that its young plantlets had less effect on crop's yield indicating that younger plants are less competitive than mature ones. The lesser capability of young PIIST can be used to take advantage on selecting practical weed control techniques such as planting tall rice varieties and using high-seeding rate. Planting tall rice varieties that have vigorous growth, good tillering capacity, and early maturing traits can help suppress weeds (Chauhan, 2013; Chauhan, 2012; Ampong-Nyarko and De Datta, 1991). High-density rate, meanwhile, suppresses weeds through rapid canopy closure.

Conclusion

This study confirmed that *H. zeylanica* at 10-15 densities/0.16 m^2 could reduce the yield of direct-seeded rice by 6%. Meanwhile, young and mature *P. stratiotes* at 5-15 densities/0.16 m^2 reduce yield of transplanted rice by 4-11%. To achieve effective, economical, and environmentally-sound weed management, further researches related to ecology and control of these weeds under field conditions are recommended. Studies on the weeds' growth responses when grown with tall rice varieties in combination with different seeding rates and fertilizer rates under direct-seeding establishment, and growth responses to manual and mechanical weeding technique can also be explored.

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IDENTIFICATION OF POTENTIAL BACTERIAL AND FUNGAL BIOREMEDIATORS IN RICE ECOSYSTEM CONTAMINATED WITH MINE TAILINGS

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Abstract

Rice ecosystem contaminated with mine tailings is one of the problems in rice production. High concentration of toxic heavy metals such as cadmium and lead are hazardous to humans, animals, plants, and environment. The study was conducted to isolate and identify potential bioremediators present in rice ecosystem contaminated with mine tailings. Isolated fungi were morphologically identified while fungi with unconvinced morphological identification were identified using internal transcribed spacer (ITS) region. Isolated bacteria were molecularly identified using 16S ribosomal RNA sequences. Cadmium and lead present in the collected soil were determined using atomic absorption spectrophotometer (AAS). The soil in the area was classified as silt loam, strongly acidic with a pH ranging from 4.26 to 4.27, and contains 0.50 mg kg^{-1} cadmium and 17.00 mg kg^{-1} lead. Four fungal species including *Penicillium janthinellum*, *Trichoderma hamatum*, *Trichoderma harzianum*, and *Curvularia lunata* along with seven bacterial species such as *Bacillus cereus, Bacillus thuringiensis, Pseudomonas gessardii*, *Lysinibacillus xylanilyticus, Lysinibacillus sphaericus,* and two species of unidentified bacteria were isolated from the contaminated soils. Occurrence of microbes in polluted soils indicates that these microorganisms are metal-resistant or metal degrading agents, which suggests further study. These heavy metal resistant microbes can be harnessed as a very useful biological tool for *in-situ* bioremediation.

Keywords: *Soil-borne Fungi, Soil-borne Bacteria, Heavy Metals, Bioremediators.*

Introduction

Intensive agricultural and industrial systems are needed to support the continuing global population with astonishing rate. However, the upward population trend causes an accumulation of waste in soil, water, and air (Philp, 2015). Controlling pollution using microbes, which could reduce the concentration and toxicity of chemical pollutants, provides solutions.

Heavy metals are naturally present in the soil, which concentrations are increased though geologic, and anthropogenic activities such as mining; harming humans, plants, and animals (Alloway, 1990). Mine tailings are deposits left after mineral extraction from mining areas that cause environmental pollution due to the presence of high concentration of trace elements. Tailings that are distributed into the ground water may cause serious environmental and health issues. Several means of extracting heavy metals were established, which include utilization of plants and microbes to remove or reduce metals from polluted sites. Studies conducted on phytoremediation proved that heavy metals such as cadmium (Cd), lead (Pb), Zinc (Zn), copper (Cu), Manganese (Mn), and mercury (Hg) can be removed from aquatic solution (Tariq et al., 2016). However, few works were conducted on the use of microbes as bioremediators.

According to Manangkil et al. (2020) biotechnology remains controversial in the development or improvement of rice varieties while it provides set of tools that have produced significant outputs in yield increase of modern rice varieties. However, the use of biotechnology in degrading pollutants has become one of the most rapidly developing fields of environmental restoration without Dua et al. (2020).

Among the heavy metals, cadmium (Cd), chromium (Cr), lead (Pb), mercury (Hg), and arsenic (As) are considered as toxic in plants and animals. Higher accumulation of toxic heavy metals in rice paddy soils may lead to health disorder to the rice plant due to continuous deposition of these elements (Shen et al., 2002). Microorganisms have developed mechanisms to resist the toxic effects of these metals (White and Gadd, 1986). Bioremediators, mostly bacteria and fungi, break down pollutants into harmless natural compounds (Abatenh et al., 2017) by using hazardous substances for their energy. As such, bioremediators degrade environmental pollutants and prevent contamination (Nascimento and Xing, 2006; Sardrood et al., 2013).

Furthermore, Abatenh et al. (2017) described that bioremediators are living organisms that break down the pollutant into harmless natural compounds. These are mostly bacteria and fungi that reproduce and grow fast in their natural habitat. Many of these microorganisms have the ability to eliminate contaminants in the soil by using hazardous substances for their energy which results in the breakdown of the targeted contaminant.

In 2002, mine tailings from Dizon Copper Silver Mines Inc. (DCSMI) in San Marcelino, Zambales, Philippines have spilled over the Mapanuepe Lake and reached the Sto. Tomas River. Rice and vegetable producing areas here were affected by the mine tailings (Philippine Daily Inquirer, 2002). Conducted 16 years after contamination, this study was implemented in a 2 ha-rice field contaminated with mine tailings in Sitio Camalca, Brgy. Buhawen, San Marcelino, Zambales (14.96917°N, 120.3131°E). Indigenous fungi and bacteria organisms were isolated from the contaminated rice paddy soils to identify potential bioremediators in the tailings-contaminated rice ecosystem. The challenge now is how to clean this environment with heavy metals to prevent hazardous effects to plants and animals. Potential bioremediators such as bacteria and fungi present in the contaminated area could be harnessed to remove or immobilize the toxic chemicals in the soil. Isolated and identified fungi and bacteria can determine the presence of heavy metals such as cadmium and lead in the soils. Knowing the microbes capable of heavy metal degradation is important in eliminating environmental pollutants in the study area; thus, providing a safer agricultural production for farmers and consumers.

Materials and Methods

Collection of Soil Samples

Study Area

Soil samples were collected in a copper and silver mines located in Sitio Camalca, Brgy. Buhawen, San Marcelino, Zambales, Philippines during the 2019 dry season. Soil samples were collected from nine sampling location points or sites. Each sampling location point or site weighs 1 kg with 50 cm depth. Collected soil samples were mixed to produce composite samples, which were placed in a clean paper bag and immediately brought to the laboratory.

Analysis Employed

Isolation and morphological identification of fungi and bacteria were conducted in Flora and Fauna Analytical and Diagnostic Laboratory. DNA extraction and PCR product for molecular identification of fungi and bacteria isolates was conducted in Tuklas - Lunas Center, Department of Biological Sciences, Central Luzon State University, Science City of Muñoz, Nueva Ecija.

Analysis of Cadmium and Lead Contents of Soil Samples

Cadmium and lead contents of soil were analyzed using Shimadzu Analytical Methods Atomic Absorption Spectrophotometry (AAS) in the CRL Environmental Corporation-Environmental Testing Laboratory in Clark Freeport Zone, Clarkfield, Angeles City, Pampanga.

Isolation of Bioremediators (Soil-Borne Fungi and Bacteria)

Serial dilution method was used to isolate the bacteria and fungi present in the soil. Soil samples weighing 10 g were added to 100 mL of sterile distilled water. The suspension was shaken well using vortex for 30 min and properly labeled. One milliliter of suspension was transferred in a 9 mL deionized water blank using sterile pipette. The dilution was repeated thrice with 1 mL of the previous suspension in a 9 mL sterile distilled water. The serial dilutions were valued as 10^{-1} through 10^{-5}.

For the isolation of soil-borne fungi, 0.1 mL of each of the five suspensions with 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, and 10^{-5} were spread in sterile Petri plates. A least three drops of Streptomycin sulfate using sterile syringe were added. Warmed sterile melted potato dextrose agar (PDA) was poured in sterile petri plates and then properly labeled. The Petri plates were incubated at ambient room temperature to allow the growth of fungal colonies within three to five days of incubation (Figure 1). Isolated and distinct fungal colonies were individually picked using sterile inoculating needle and transferred aseptically into test tubes containing slanted PDA. These tubes served as stock cultures of the fungal isolates.

One milliliter of each of the five suspensions with 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, and 10^{-5} were spread and shaken in sterile Petri plates poured with sterile warmed melted nutrient agar (NA) for bacterial isolation. Petri plates sealed with parafin were incubated at ambient room temperature within 18-24 h of incubation (Figure 2). Isolated and distinct bacterial colonies of 10^{-3} suspension were individually picked using sterile inoculating needle and were transferred aseptically into test tubes containing nutrient broth (0.1% peptone and 0.4% beef extract L^{-1}) that served as stock culture of the bacterial isolates.

Morphological Identification of Fungal Isolates

Soil-borne fungi were best observed in agar block culture also known as slide culture technique. In this method, development and structures were observed without being disturbed or constantly moved when the tissues were lifted from the glass slide.

In agar block culture, a bent aluminum foil rod on moist tissue paper held a glass slide in a sterilized Petri plates. An agar block was then cut aseptically from agar plate culture, lifted and placed on the surface of the slide. Mycelium or spores of desired organism were inoculated in the four sides of the agar block. A cover slide was centrally placed upon the inoculated agar block. The Petri dish was covered and incubated until the desired growth was obtained. Sterilized water was added to the tissue paper maintain the moisture ambient during incubation.

Cover slip was carefully lifted and placed on a drop of lactophenol on a clean slide at the sight of mycelial growth. Another drop of lactophenol was placed on the center of the fungal growth then covered

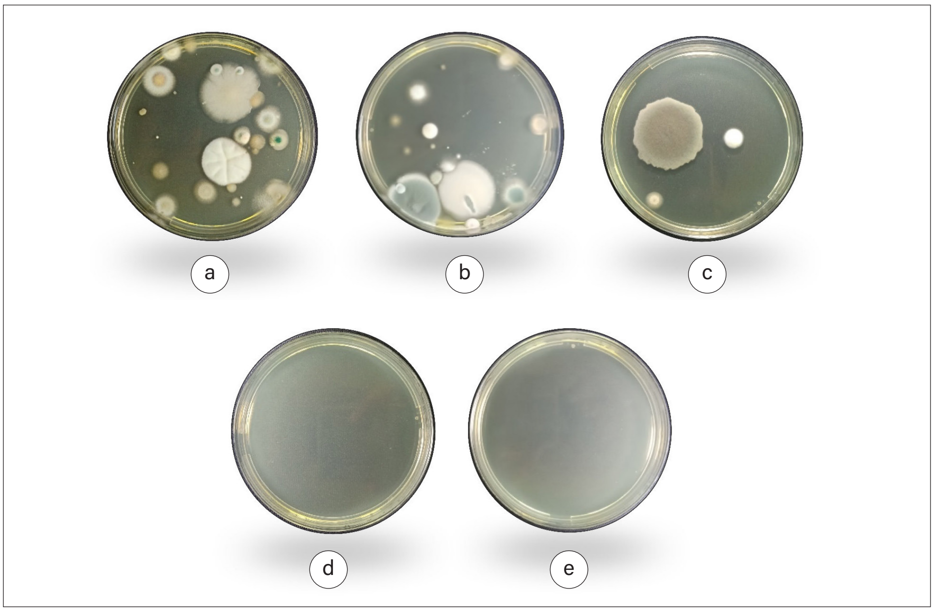

Figure 1. Fungal colonies on PDA at different dilutions, (a) 10^{-1}; (b) 10^{-2}; (c) 10^{-3}; (d) 10^{-4}; and (e) 10^{-5}.

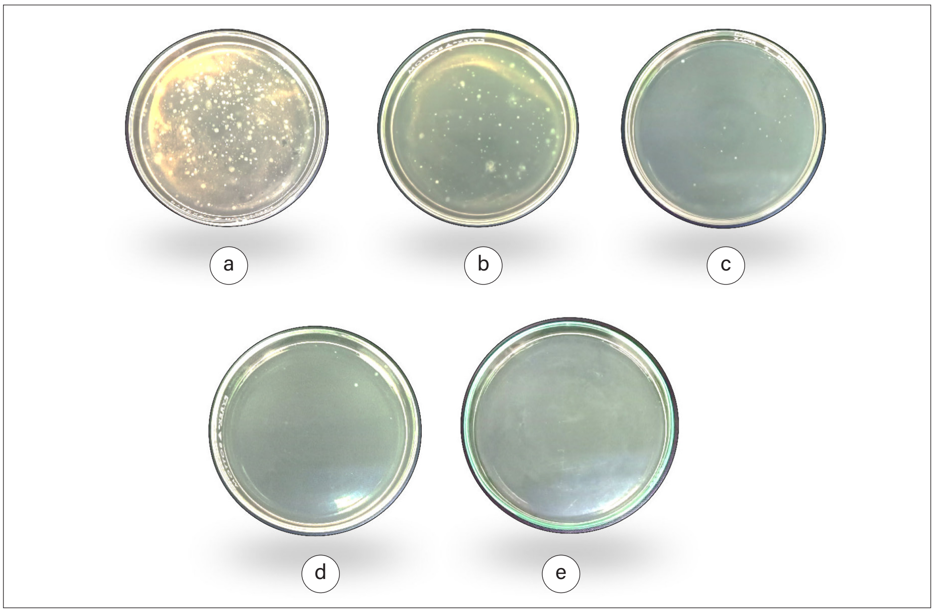

Figure 2. Bacterial colonies on PDA at different dilutions, (a) 10^{-1}; (b) 10^{-2}; (c) 10^{-3}; (d) 10^{-4}; and (e) 10^{-5}.

with a new clean cover slip. Mounted glass slides were prepared for microscopic examination for the morphological identification of the fungal isolates in accordance with protocols used by Quimio and Hanlin (1999) and Watanabe (2010).

Molecular Identification of Fungal and Bacterial Isolates

Fungal isolates that were not morphologically identified were subjected to molecular identification using internal transcript spacer (ITS) region sequencing. For DNA extraction for fungal isolates, the mycelia were mashed with 1x CTAB buffer (20 g of CTAB dissolved in 860 mL sterile double distilled water, 81.82 g NaCl, 100 mL 1M Tris with pH 8.0, 40 mL 0.5M EDTA with pH 8.1) until these become sticky. These were transferred in a 1.5 mL tube and incubated for 3 h. Fifty microliter (µl) of 20% SDS was added to the samples and mixed using vortex and was incubated at 65°C for 30 minutes to one hour using dry bath (Labnet D1200- 230V Accublock Digital dry bath). After an hour of incubation and cooling, 750 µl of chloroform was added to the samples, which were mixed thoroughly and carefully using a vortex. The tubes containing the suspensions were centrifuged for 30 min at 10,000 rpm and the upper layer was transferred into a new 1.5 mL sterile tube. Subsequently, 600 µl of ice-cold isopropanol was added and incubated overnight at -20°C. After incubation, samples were mixed gently then centrifuged for 10 minutes at 10,000 rpm. Isopropanol was decanted and the pellet was washed with 500 µl of 70% ethanol. The samples were subjected in the centrifuge for 10,000 rpm for 3 min. The ethanol was decanted and the formed pellets were air-dried until the alcohol was completely removed from the pellet. The pellet was dissolved using TE buffer with RNAse. The extracted DNA was stored in 4°C until usage. The final component concentration per 25 µl included1.0 µl of DNA extract, 2.5 µl 10x PCR Buffer (KAPA), 1.0 µl DNTP Mix (KAPA), 1.5 µl MgCl, 2.0 µl ITS, mixed 1Forward (5’-CTT GGT CAT TTA GAG GAAGTA A-3’) primer and 4Reverse (5’-TCC TCC GCT TAT GC-3’) primer (Bruns and Gardes, 1993), 16.9 µl sterilized distilled water, and 0.1 Taq Polymerase (KAPA). The PCR profile that was used to amplify the gene was made up of 35 cycles with initial denaturation at 95°C for 3 min, denaturation at 95°C for 30 sec, annealing at 51.1°C for 30 sec, extension at 72°C for 1 min, final extension 72°C for 10 min, and final hold at 4°C.

Metagenomic sequencing of bacterial samples has become the gold standard for profiling microbial populations, but 16S rRNA profiling remains widely used due to advantages in sample throughput, cost, and sensitivity even though the approach is hampered by primer bias and lack of specificity (Schriefer et al., 2018). In this respect, similar approach applicable to bacterial identification was used in this research.

Bacterial isolates in nutrient agar (NA) plates were subjected to colony PCR method using 16S ribosomal RNA gene sequencing. Single colony of each bacterial isolate was placed in tube before the dilution of final component. The final component concentration per 25 µl were 1.0 µl of DNA extract, 2.5 µl 10x PCR Buffer (KAPA), 1.0 µl DNTP Mix (KAPA), 1.5 µl MgCl, 1.0 µl 27Forward (5’-AGA GTT TGA TCM TGG CTC AG-3’) primer (Lane, 1991), 1.0 µl 1492Reverse (5’-CGG TTA CCT TGT TAC GAC TT-3’) primer (Jiang et al., 2006), 17.9 µl sterilized distilled water and 0.1 Taq Polymerase (KAPA). The PCR profile that was used to amplify the gene was made up of 35 cycles with initial denaturation at 95°C for 2 min, denaturation at 95°C for 20 seconds, annealing at 54°C, 56°C and 56.5°C for 30 seconds, extension at 72°C for 1 min, final extension 72°C for 5 min and final hold at 4°C. One (1) µl of amplification products and the 1kb DNA ladder stained with 1 µl gel red (Biotium) was run for 30 min at 100 V on 1.0% agarose gel (prepared in 1x TAE) and analyzed under gel photo documentation system (Labnet GDS- 1302 Enduro Imaging System).

Sequencing Analyses for Fungal and Bacterial Isolates

PCR products were sent to Apical Scientific Sequencing in Malaysia for PCR purification and sequencing after confirmation of the expected size of amplified fragments. The chromatogram results were evaluated using four peaks software.

Occurrence of Fungal Colonies

Percent fungal occurrence was resolved by percent occurrence formula, while bacteria present in the soil were determined by presence and absence only. Data observed included the total accumulation of microbial colony and the soil concentration of heavy metals.

Percent (%) Occurrence Formula:

$$\text{Percent (\%) Occurrence} = \frac{\text{No. of colonies of species per plate}}{\text{Total fungal population}} \times 100$$

Results and Discussion

Fungal Species Isolated from the Soil

Microorganisms present in the soil such as fungi and bacteria were collected and identified.

Four species of microfungi were isolated. Three of the fungal isolates were identified through morphological and cultural characteristics. *P. janthinellum*, *T. hamatum,* and *C. lunata. T. harzianum* strain (ACCC32889) were molecularly identified because of unconvinced morphological characteristics. Cultural and morphological characteristics of the fungal isolates include the following:

***Penicillium janthinellum* Biourge**

Class Eurotiomycetes, Order Eurotiales,
Family Trichomaceae

Colonies on Potato Dextrose Agar are velvety, pale grayish green in the obverse, and pale yellowish brown in reverse. These fungi grow 10-20 mm in 5 days of inoculation (DAI) at room temperature ambience (Figure 3a and b).

Conidiophores are hyaline, erect, brached penicillately at the apexes with verticillate metula, terminal phialides and catenulate conidia on each phialide, forming rather divergent conidial heads: phialides pen-pointed with abruptly tapered tips. Conidia are phialosporous, pale green, dark in mass, ellipsoidal or subglobose, 1-celled, smooth, and apiculate at one end (Figure 3c).

***Trichoderma hamatum* (Bonorden) Bainer**

Class Eurotiomycetes, Order Eurotiales,
Family Trichomaceae

Colonies on PDA are initially white, greenish, forming cushions distributed with age in the obverse; and pale yellowish brown in reverse. These microorganisms grow 10 to 20 mm in 5 DAI at room temperature ambience (Figure 4a and b).

Conidiosphores are developed on cushion- shaped structure, with erect hyaline, branched, bearing spore masses on alternate or verticillate phialides together with setae-like sterile elongated hyphae; while phialides are short and thick, densely arranged. Hyphae are curved setae-like, gradually tapering toward apex, and septate. Conidia are phialosphorous, hyaline, ellipsoidal or ovate, and 1-celled. Chlamydospores are pale brown, subglobose or ellipsoidal, and granulate (Figure 4c).

***Trichoderma harzianum* strain Rifai**

Class Eurotiomycetes, Order Eurotiales,
Family Trichomaceae

Colonies on PDA are needle shaped white produced in obverse, fluffy white in reverse. These microfungi were molecularly identified using ITS region sequence due to unclear and doubtful characteristics under microscope (Figure 5a and b).

Conidiosphores are hyaline, erect, branched, bearing spore masses apically at verticillate phialides;

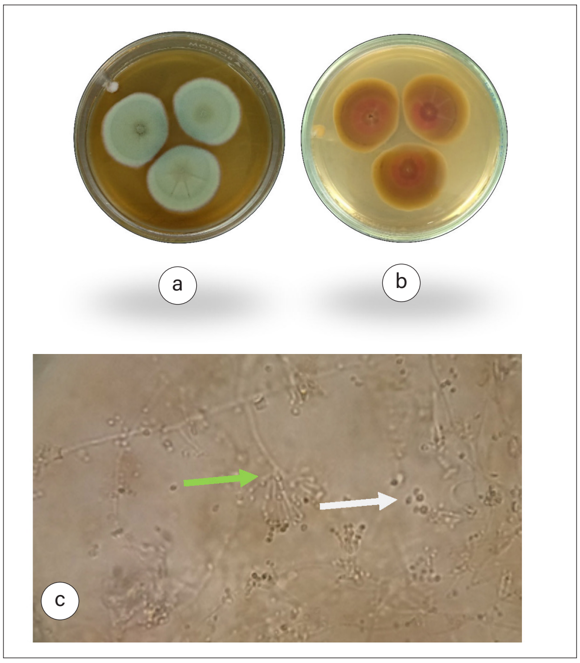

Figure 3. Cultural and morphological characteristics of *P. janthinellum* grown on PDA, 5 DAI at room temperature: (a) Obverse growth; (b) Reverse growth; and (c) Conidiophores (white arrow) and phialides (green arrow).

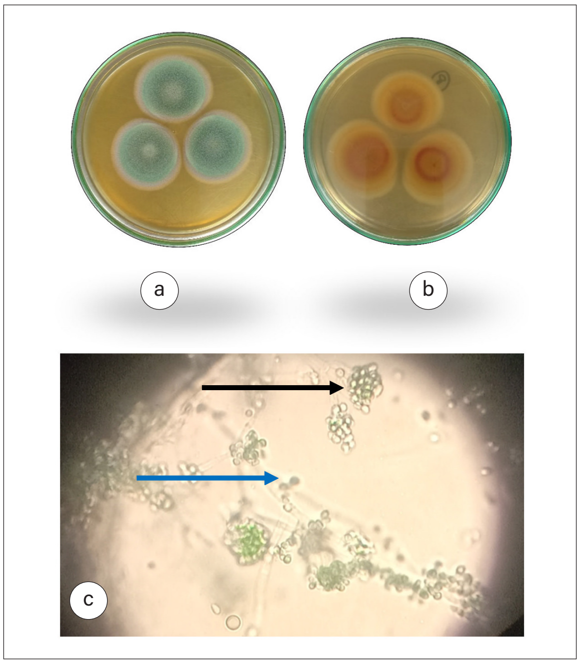

Figure 4. Cultural and morphological characteristics of *T. hamatum* grown on PDA, 5 DAI at room temperature: (a) Obverse growth; (b) Reverse growth; and (c) Conidiophores (blue arrow) and phialides (black arrow).

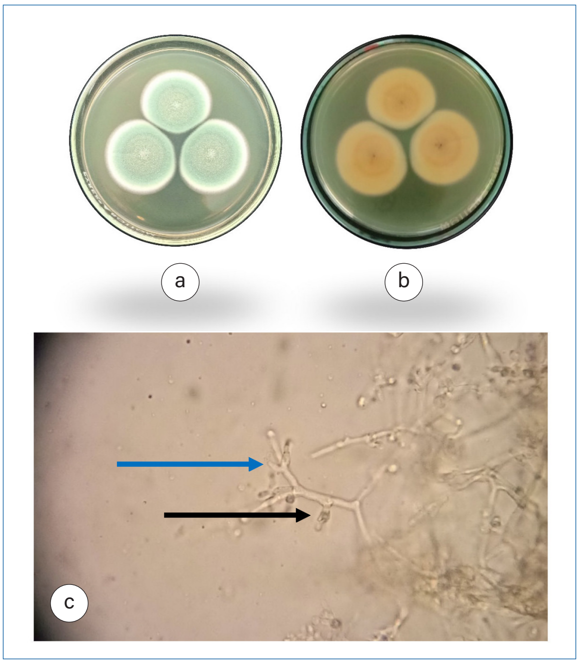

Figure 5. Cultural and morphological characteristics of *T. harzianum* strain with 99.99% identity (ACCC32889) grown on PDA, 7 DAI at room temperature: (a) Obverse growth; and (b) Reverse growth; and (c) Phialides (blue arrow) and phialospores (black arrows).

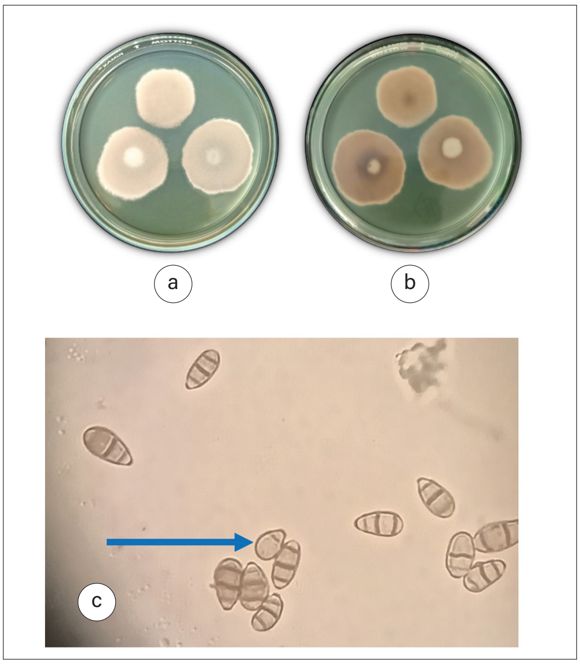

Figure 6. Cultural and morphological characteristics of *C. lunata* grown on PDA, 5 DAI at room temperature: (a) Obverse growth; (b) Reverse growth; and (c) Conidia (blue arrow).

and with short and thick phialides. Conidia are phialosphorous, hyaline, globose, subglobose or ovate, and 1-celled. Chlamydospores are brown, and subglobose (Figure 5c).

***Curvularia lunata* (Wakker) Boedijn**

Class Euascomycetes, Order Pleosporales,
Family Pleosporaceae

Colonies on PDA are brown to grey in color in obverse, tinted brown in color in reverse (Figure 6a and b).

Conidiophores are erect, brown, simple or branched, straight or curved; bear conidia apically and laterally, and conspicuous pores are left after detachment of conidia. Conidia are porosporous, subellipsoidal, mostly 4-celled, darker brown in 2 central cells, especially curved, larger in the penultimate cells, and with indistinct hilum basally (Figure 6c).

The isolated fungi are potential bioremediation agent because of the ability to thrive in an environment contaminated with heavy metals. Microorganisms can be used in remediating contaminated environment (Abatenh et al., 2017). According to Jaiswal (2011) isolated fungi from contaminated soils with mine tailings have the potential to reduce, mobilize, or immobilize the heavy metals through sorption, biomethylation, complexation, and oxidation-reduction process. Siddiquee et al. (2015) reported that fungal amendments using different *Trichoderma* strains were effective (much like known chelating agents) in mobilizing and extracting cadmium, chromium, copper, zinc, and nickel, independently of the plant species used. Mustapha and Halimoon (2015) reported that *P. simpliccium* has high cadmium absorption; *P. chrysogenum* absorbs high amount of lead; while species of *Penicillium* and *Trichoderma* were utilized as bio sorbents material. *C. lunata* cannot be used in bioremediation because it can cause plant disease.

Occurrence of Fungi in the Soil

Percent occurrence was used to measure the number of times the organism occurs in the area. Generally, *T. virens, T. santurnisporum*, and *T. gamsii* are abundant in soil contaminated with heavy metals. In this study, two species of *Trichoderma, T. hamatum* and *T. harzianum,* were found in the soil contaminated with mine tailings. Among the four isolated fungi, *T. hamatum* registered the highest occurrence with 52.78% while the lowest frequency was exhibited by *C. lunata* with a value of 5.56% (Table 1).

The occurrence of *Penicillium* spp. are common in soils and these microorganisms can also colonize different environments, especially soils contaminated with heavy metals. *P. janthinellum* was found commonly associated with soil, decaying organic matter, and in storage rots or pathogens of fruits and vegetables (Banker et al., 1997).

Table 1. Percent occurrence of different fungal isolates grown on PDA from soil contaminated with mine tailings.

Fungal Isolates	Percent Occurrence
P. janthinellum	30.56
T. hamatum	52.78
T. harzianum	11.11
C. lunata	5.56

Moreover, most species of *Curvularia* are facultative pathogens of soil, plants, and cereals in tropical or subtropical areas, while the remaining few are found in temperate zones (Knudtson and Kirkbride, 1992).

Bacterial Species Isolated from Soil

Bacterial biosorption bioremediation is essentially used for the removal of pollutants from wastes that are not biodegradable, like heavy metals. Bacteria have evolved a number of efficient systems for detoxifying metals ions; developing resistance mechanisms mostly for their survival (Mustapha and Halimoon, 2015). Although isolation, screening, and harvesting of bacteria on a larger scale may be complicated, these bioremediators still remain as one of the efficient ways of improving soils contaminated with mine tailings.

Bacterial species were isolated and molecularly identified using 16S ribosomal RNA gene sequencing (Figure 7). Ten of 12 bacteria were identified. Poor DNA amplification had caused the disconfirmation of the two bacterial isolates (Figure 7). Identified bacteria included five accessions of *B. cereus*, two accessions of *B. thuringiensis*, *P. gessardii*, *L. xylanilyticus, L. sphaericus* (Table 2).

The species of bacteria isolated from the study site have been reported to remove pollutants from contaminated environment. Sriram et al. (2011) reported that *B. cereus* is heavy metal resistant and can also be harnessed as a very useful biological tool for *in situ* bioremediation. Oves et al. (2012) concluded that in the presence of the varying concentrations (25 - 150 mg kg^{-1}) of heavy metals such as cadmium, chromium, copper, lead, and nickel. *B. thuringiensis* strain OSM29 showed an obvious metal removing potential. The biosorption of each metal was fairly rapid, which could be an advantage for large scale treatment of contaminated sites.

Table 2. Bacterial species isolated from soil contaminated with mine tailings.

Species of Bacterial Isolates	Identity	Accession Number
B. cereus	99.38%	MG206040
B. cereus	100.00%	MK894129
B. cereus	97.27%	JN206614
B. cereus	99.34%	GU369810
B. cereus	97.53%	MK346118
B. thuringiensis	98.11%	MK277453
B. thuringiensis	96.03%	FJ393313
P. gessardii	99.40%	KC903222
L. xylanilyticus	100.00%	MK388392
L. sphaericus	100.00%	JF819704
Unidentified bacterium 1	NA*	NA*
Unidentified bacterium 2	NA*	NA*

**not applicable*

P. gessardii strain LZ-E was isolated from wastewater discharge site of a petrochemical company. It degraded naphthalene and reduces Cr(VI) simultaneously. Strain LZ-E continuously remediated naphthalene and Cr(VI) at rates of 15 mg L^{-1} h^{-1} and 0.20 mg L^{-1} h^{-1} of 800 mg L^{-1} naphthalene and 10 mg L^{-1} Cr(VI) addition with eight batches in 16 days. Strain LZ-E is a potential applicant for combined pollution remediation (Huang et al., 2016).

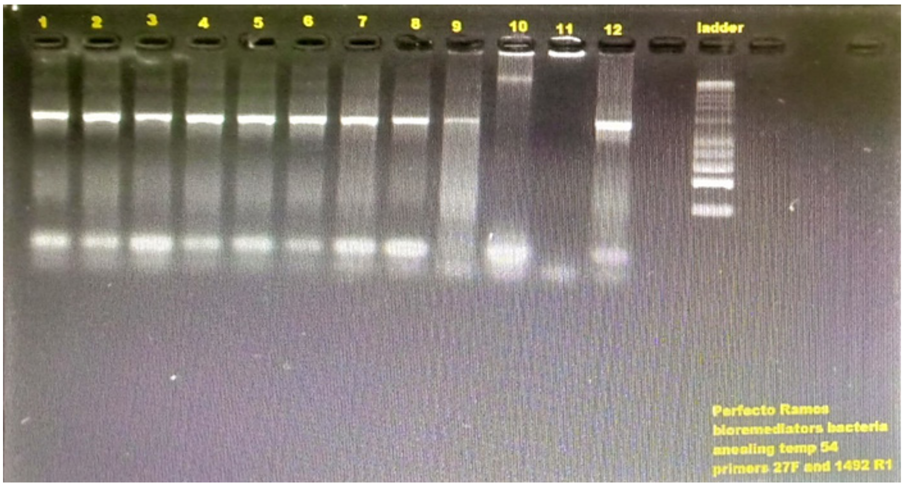

Figure 7. The DNA band sequences of bacteria extracted on agarose electrophoresis. Lanes 10 and 11 are unidentified bacteria.

Peña-Montenegro and Dussan (2013) found that *L. sphaericus* OT4b.31 (native Colombian strain) may be useful not only in bioremediation of polluted environments with heavy metals such as cadmium, zinc, cobalt, copper, nickel, chromium, and arsenic, but also for biological control of agricultural pests. Rahman et al. (2015) reported that *L. sphaericus* is significant bacterium in removing arsenics and other toxic metals from the contaminated sources. The genetic mechanisms of the isolate could be used to remove arsenic toxicity. However, no study used the bacterium *L. xylanilyticus* as bioremediator.

Based on the results of this study, the isolates are potential bioremediators because of their ability to grow in contaminated soil with mine tailings (Chen et al., 2003). As heavy metals are abundant in the environment, microorganisms have developed mechanisms to resist the toxic effects of these heavy metals (White and Gadd, 1986). A large number of microorganisms are capable of growing in the presence of high concentrations of heavy metals (Anderson and Cook, 2004).

Soil Properties and Heavy Metals Concentration

The soil in the area is classified as silt loam containing less than 70% silt and clay and not less than 2% sand. The soil is strongly acidic with a pH ranging from 4.26 to 4.27. Moreover, soil sample contains 0.50 mg kg^{-1} cadmium and 17.00 mg kg^{-1} lead. The amount of cadmium and lead obtained in this study is lower than maximal permissible addition (0.76 mg kg^{-1} for cadmium and 55.00 mg kg^{-1} for lead) of heavy metals in soils (Crommentuijn et al., 1997).

Conclusion and Recommendation

The versatility of microbes to degrade a vast array of pollutants makes bioremediation a technology that can be applied in different soil conditions. However, bioremediation is still uncommon (United States Environmental Protection Agency, 2001) despite being inexpensive and capable of reducing engineering practices through *in situ* approaches.

This study identified bacteria and fungi that can remove or reduce contaminants present in the rice field as possible candidates for bioremediation. Bioremediation systems are still limited by the capabilities of the native microbes to tolerate concentration of heavy metals.

Four fungal species namely *P. janthinellum*, *T. hamatum*, *T. harzianum*, and *C. lunata*; and five bacterial species such as *B. cereus, B. thuringiensis, P. gessardii*, *L. xylanilyticus, and L. sphaericus* were isolated from soil contaminated with mine tailings.

Only *T. harzianum* was identified using molecular approach due to its unclear and doubtful characteristics under microscope. Advances in molecular technology show great potential for the rapid detection and identification of fungi. The intervening internal transcribed spacer (ITS) regions have great potential as targets in molecular-based assays for the characterization and identification of fungi. The ITS coding regions have a critical role in the development of functional rRNA, with sequence variations among species showing promise as signature regions for molecular assays (Iwen et al., 2002).

A follow up study that will examine the two unidentified bacteria on their identity and nature is suggested to determine their ability on heavy metal elimination. The ability of the isolated bacteria and fungi to absorb heavy metals should be evaluated by growing them in a medium contaminated with lead and cadmium to determine tolerance. The concentration of heavy metals in the mycelia of fungi and bacterial cells is also recommended for further investigation. Stimulating naturally occurring microbial population in the test area by providing their needs for a faster break down of contaminants can also be considered in future studies.

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GROWTH PERFORMANCE AND YIELD OF RICE VARIETY MIXTURES (VARMIX) UNDER SOIL MOISTURE FLUCTUATION STRESS DURING REPRODUCTIVE STAGE

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Abstract

Drought stress episodes during soil moisture fluctuations (SMF) and occurrences of pest and diseases limit rice yield in rainfed lowland environment. One strategy employed to sustain higher yield in drought prone areas is the use of varietal mixtures (VarMix). This study examined the growth and yield of VarMix exposed to reproductive stage SMF. Several treatments as mono or VarMix using different sets of irrigated lowland varieties were grown and exposed to either continuously waterlogged (CWL) as control or cycles of alternate wetting and drying (CAWD) as SMF. Results showed significant interactions between water treatments and VarMix on morphological agronomic and root traits but none on water use (WU) and water use efficiency (WUE). Single varieties generally had higher yield, total dry mass, and harvest index than VarMix while some VarMix combinations showed higher values of the parameters including root lengths than monocultures under CAWD. These VarMix had five (NSIC Rc 298, Rc 216, PSB Rc 82, NSIC Rc 238 and Rc 300) and six (NSIC Rc 298, Rc 214, Rc 216, PSB Rc 82, NSIC Rc 238, and Rc 300) combinations. However, performance of VarMix including NSIC Rc 298, a direct wet seeded rice, was dependent on varietal combinations. Significant and positive relationships were found between branching index and grain weight, branching index and WUE, and WUE and grain weight especially under CAWD. Overall, results showed that right combination of VarMix, can increase branching index through compensation and facilitation mechanism for water uptake under CAWD with corresponding high WUE. Ultimately, it contributed to the increase in grain weight of VarMix under rice growing environments experiencing drought during SMF.

Keywords: *Soil Moisture Fluctuations (SMF), VarMix, Continuously Waterlogged (CWL), Cycles of Alternative Wetting and Drying (CAWD).*

Introduction

Rainfed ecosystem represents about 25% of the total rice production area (PhilRice, 2014). This ecosystem, which includes rainfed lowland (RFL), generally produces rice lower than irrigated lowland. As RFL is dependent on rainfall, soil moisture fluctuations (SMF), which is the recurrence of transiently anaerobic (flooded) to aerobic (mild drought) conditions and vice versa, is a common water stress condition (Wade et al., 1999; Fujihara et al., 2013). During SMF, episodes of drought alone can readily reduce rice yield (Singh et al., 2013). The annual rice production in the Philippines have suffered yield losses particularly in years with less rainfall (PSA, 2020).

Under rainfed lowland conditions, yield reductions can be mitigated by using varieties with good root system development that can maintain high water and nutrient uptake especially during drought conditions (Suralta, 2010). Roots play an important role in plant growth serving as anchorage of the plant and taking up water and nutrient elements (Wu and Cheng, 2014). Roots are also the first line of defense for mitigating the effect of soil abiotic stresses (Suralta et al., 2018).

Roots function is an important guarantee for increasing biological and grain yield under soil-related abiotic stresses (Lynch, 2007; Wu and Cheng, 2014). In rice, roots develop deep and extensive root systems and plasticity in lateral root to adapt to drying soil conditions (Suralta et al., 2018). When the soil surface layer dries up, roots may seek available moisture deep in the soil profile, which facilitates water uptake to sustain shoot growth (Kameoka et al., 2015) through hydraulic lift (Doussan et al., 2006). The net effect of these adaptive responses increases the surface area of the plant root system in the most important region of the soil matrix for resource capture (e.g., surface layers for phosphorus uptake and deeper layers for nitrate uptake) or secures anchorage (Atkitson et al., 2014).

Under climate change-challenged areas such as rainfed conditions, the rate of incidence of major pests and diseases is also increased (Haq et al., 2010). An increase in temperature may change the reproductive biology and the dynamics between pests and predators (Chapman and Reiss, 1992). Many pests are more tolerant to temperature increase than predators that bring in ecological imbalance, which may result in infestations. The increase of rice disease occurrence such as sheath blight (Miah et al., 1985), blast disease (Shahjahan et al., 1991), *bakanae* disease, and bacterial leaf streak (Haq et al., 2010) were related to the rise of temperatures especially during dry seasons. The brown spot disease in rice is also more prevalent in rainfed and upland rainfed rice ecosystems (Mew and Gonzales, 2002).

The development of rice cultivars tolerant to biotic and abiotic stresses has been prioritized in breeding programs. However, tolerance is always specific to a certain type of stress in space and time. For instance, under water scarce rice-growing environments, water stress may occur at any stage of growth and at different magnitudes; hence, may require different sets of quantitative trait loci (QTL) controlling for water stress tolerances. Required resistances to pests and diseases are also dependent on changing climatic conditions (Haq et al., 2011) and location specific disease strains that require different sets of QTLs controlling for resistance. As such, appropriate cultivars suited to different sets of environments may take some time to develop. This is because combining multiple QTL for tolerance and/or resistance to abiotic and biotic stresses in a single genotype through breeding is difficult complicated by the effects of genetic background and environment on trait expression.

Variety mix or VarMix is an alternative strategy to improve productivity in areas with high incidence of pest and diseases. It refers to mixtures of cultivated varieties growing simultaneously on the same parcel of land with no attempt to breed for phenotypic uniformity (Mundt, 2002). The use of seed mixtures of varieties may increase grain yield compared with the average of component varieties in pure stands (Kiær et al., 2009). Previous studies reported increased resistance to biotic and abiotic stresses and stochastic events through increased genotypic diversity in plant communities (Hughes and John, 2004; Reusch et al., 2005; Hughes et al., 2008). The allelopathic effects of different genotypes in VarMix inhibits the pathogen (diseases) and pests' expansion (Finckh et al., 2000); thus, provide an effective disease management (Mundt, 2002).

Generally, seed mixtures of varieties with diverse characteristics can increase and stabilize crop yield across diverse environments (Smithson and Lennė 1996; Finckh et al., 2000; Kiær et al., 2009, 2012). In *Zostera marina* L., a mixture of six genotypes under high temperature produced 30% more than monocultures indicating a significant impact of using a diverse population in terms of maintaining productivity under abiotic stress (Reusch et al., 2005; Tooker and Frank, 2012). Under drying soil, VarMix may mitigate drought by hydraulic lift (Doussan et al., 2006), the passive transport of water through the roots of deep rooting genotypes from deeper, wetter soil layers to drier, upper soil layers (Sekiya et al., 2011). Water then is consequently redistributed and taken up by shallow rooting genotypes to sustain its growth (Izumi et al., 2018).

The usefulness of VarMix whether multilines or cultivar mixtures for disease management has been well demonstrated for rusts and barley powdery mildews of cereals (Finckh et al., 2000; Mundt, 2002). Multilines are mixtures of genotypically identical lines (near- isogenic lines) that differ only in a specific disease or pest resistance gene (Browning and Frey, 1981). Multiline cultivars of rice are widely used to prevent the breakdown of resistance against blast in Japan, where the first registered rice multiline was released in 1995 (Koizumi, 2001).

Contemporary research on rice VarMix focuses on disease resistance. Few researches have been conducted on assessing the impact of varietal mixtures on productivity under water stress. In this study, researchers hypothesized that some VarMix combinations can produce higher yield than monoculture or single variety under rice environments prone to SMF. Thus, this study quantified the growth and yield of VarMix culture in the reproductive stage SMF condition. The VarMix combinations used in the study were mixtures of selected released variety with similar agronomic traits but with genetic dissimilarities, and possess diversified function to mitigate the effect of both biotic (pest and diseases) and abiotic (water) stress.

Materials and Methods

Plant Materials

Twelve VarMix combinations and corresponding monocultures consisting of irrigated lowland varieties including NSIC Rc 298, Rc 214, Rc 216, PSB Rc 82, NSIC Rc 238, and Rc 300 were used (Table 1). Yield of these varieties under favorable conditions ranged from 5.3 to 6.4 t ha^{-1}. Other morphological agronomic traits and reactions to pest and diseases are presented in Table 2 (www.pinoyrice.com).

Table 1. Description of planting cultures and morphological characteristics of monocultures used in the study.

Designation	Code	Ratio
Monoculture (NSIC Rc 298)	A	1
Monoculture (NSIC Rc 214)	B	1
Monoculture (NSIC Rc 216)	C	1
Monoculture (PSB Rc 82)	D	1
Monoculture (NSIC Rc 238)	E	1
Monoculture (NSIC Rc 300)	F	1
VarMix (NSIC Rc 298: Rc 238)	AE	3:3
VarMix (NSIC Rc 298: PSB Rc 82)	AD	3:3
VarMix (NSIC Rc 298: Rc 238: Rc 300)	AEF	2:2:2
VarMix (NSIC Rc 298: Rc 214: Rc 216)	ABC	2:2:2
VarMix (NSIC Rc 214: Rc 216: PSB Rc 82)	BCD	2:2:2
VarMix (NSIC Rc 214: PSB Rc 82: NSIC Rc 238)	BDE	2:2:2
VarMix (NSIC Rc 298: Rc 214: Rc 216: Rc 238)	ABCE	1:1:3:1
VarMix (NSIC Rc 298: Rc 216: Rc 238: Rc 300)	ACEF	1:3:1:1
VarMix (NSIC Rc 298: Rc 216: PSB Rc 82: NSIC Rc 238)	ACDE	1:3:1:1
VarMix (NSIC Rc 298: Rc 214: Rc 216: Rc 238: Rc 300)	ABCEF	1:1:2:1:1
VarMix (NSIC Rc 298: Rc 216: PSB Rc 82: NSIC Rc 238: Rc 300)	ACDEF	1:2:1:1:1
VarMix (NSIC Rc 298: Rc 214: Rc 216: PSB Rc 82: NSIC Rc 238: Rc 300)	ABCDEF	1:1:1:1:1:1

Experimental Design and Treatments

A pot experiment under greenhouse condition was conducted during 2017 dry season at the Philippine Rice Research Institute-Central Experiment Station (PhilRice-CES), Science City of Muñoz, Nueva Ecija, 3119, Philippines (15º 40’N, 120º 53’E, 57.6 masl). The average daily solar radiation, minimum and maximum temperatures, and relative humidity during the conduct of the experiment were 17.87 MJ/m^2, 23.25ºC, 31.85ºC, and 79.35%, respectively.

The experiment was arranged in split-plot design in Randomized Complete Block Design (RCBD) with three replications. The soil moisture treatments were assigned as main plots while VarMix combinations as subplots. Pre-germinated seeds from each variety were sown in a seedling tray. Seedlings were transplanted in the pot (47 cm diameter x 100 cm height) filled with 80 kg of dry loam soil 16 days after sowing (DAS). Six seedlings in monoculture or VarMix were transplanted according to different treatment combinations (Table 1).

The plants were subjected to two soil moisture conditions: continuously waterlogged (CWL; control) and cycles of alternate waterlogging and drought (CAWD) condition as SMF. In CWL, the water level was maintained at 5 cm above the soil surface (approximately 35% soil moisture content (SMC), V/V) throughout the whole duration of the experiment. In CAWD, the plants were subjected to initial waterlogged condition for 14 days then watering was stopped. SMC was allowed to dry down until it reached 15%. Thereafter, rewatering was done to bring back the moisture level similar in CWL. SMC was monitored by weighing the pots through digital weighing balance. SMC in each pot was calculated using the formula:

$$\text{SMC} = \frac{\text{FWsoil (kg) - DWsoil (80 kg)}}{\text{DWsoil (80 kg)}}$$

where:
SMC- Soil Moisture Content (%)
FW- Fresh Weight
DW- Dry Weight

Rewatering was done to replace the amount of water lost and maintained the desired SMC. In CAWD, the cycle was repeated until maturity. Fertilizers were applied with complete (14-14-14) at the rate of 17.14 g pot^{-1} 10 DAS and with Ammosul (21-0-0-24s) at the rate of 5.71 g pot^{-1} 30 DAS.

At maturity, the plants from each pot were collected for agronomic measurements. At harvesting, the stalk and leaves were separated from grains and oven dried at 70ºC for 48 h prior to weighing. Grain weight was recorded for each pot and adjusted to 14% grain moisture content. Harvest index (HI) was computed as the ratio of grain weight to total shoot dry matter including grains.

The root systems were carefully extracted from each pot and washed thoroughly to remove soil and other dirt. Thereafter, the root systems from each pot was temporarily stored in 95% alcohol for further measurements. The nodal root number (NRN) at the base of each plant was manually counted. The roots systems were scanned at 600 dpi (EPSON v700). After scanning, the root samples were oven dried at

Table 2. Characteristics of rice varieties used in the study (adopted from PhilRice Binhing Palay).

Variety	Average Yield	Maturity (DAS)	Plant Height (cm)	Grain Shape (mm)	Amylose Content (%)	Blast Induced			Bacterial Leaf Blight Induced					Tungro Induced				Tungro Modified			Brown Plant Hopper	Green Leaf Hopper	Stemborer								
																							Dead Heart	Dead Heart		White Head					
																							White Head	White Stemborer	Yellow Stemborer	White Stemborer	Yellow Stemborer				White Head
						A	B	C	D	G	A	B	C	G	A	D	G	A	C	G				C	B	H	A	D	I	J	Yellow Stemborer
NSIC Rc 298	5.3	104	93	3.2 S	19.5 I	S	S	S	S	I	I	I	I		S	S			S	S	MR	I				S	MR				
NSIC Rc 214	6	116	106	3.5 S	21.9 I					I				I			S			S	MR	MR									R
NSIC Rc 216	5.5	112	96	3.2 S	20.5 I	S					I				S			S			MR	MR									MR
PSB Rc 82	5.4	110	100	3.2 S	21.5 I					R				I			S				I	MS	I								
NSIC Rc 238	6.4	110	104	3.2 S	21.0 I	S	R		I	I	I	I	I		S	S			S	S	I	MR		MR	R	MR	MS	R	MR		
NSIC Rc 300	5.7	115	98	2.9 I	20.4 I	S	R	R	I	I	S	I	I		S	S			S	S	MR	MR				S	I				

S-susceptible; MS-moderately susceptible; I-intermediate; MR-moderately resistant; R-resistant
A - PhilRice-CES; B - PhilRice-Isabela; C - PhilRice-Midsayap; D – UPLB; E - DA-CVIARC; F – VSU; G – IRRI; H - PhilRice-Agusan; I – BIARC; J – WESVIARC

70°C for 48 h before recording the root dry weight (RDW).

Scanned images were analyzed for total root length (TRL) and total lateral root length (TLRL) using WinRhizo v. 2016d (Régent Instruments, Québec, Canada) at a pixel threshold value of 175. The total lateral root length (TLRL) was classified as roots with a diameter of ≤0.2mm according to the classification of Yamauchi et al. (1987). The total nodal root length (TNRL) was computed as the difference between the TRL and TLRL. Branching index (BI) was computed as the ratio of TLRL to TNRL.

The total plant dry weight was computed as the sum total of the dry weights of the shoot, grains, and roots.

Statistical Analyses

Two-way ANOVA was performed using the STAT Excel to investigate the effects of soil moisture treatments and VarMix on different growth parameters. Tukey's Honest Significant Different (HSD) post hoc test ($P < 0.05$) using water treatment and VarMix as independent variables and shoot and root growth parameters as the response variables was used to examine statistically significant differences between means. The relationships among selected traits were analyzed using Pearson Correlation Analyses with significance on the coefficient value determined either at 5 or 1% level of significance.

Results

Growth and Yield

The ANOVA showed significant interactions between water treatments and VarMix cultures on yield, total dry mass, and harvest index (Table 3). Under CWL condition, monocultures especially C showed significantly higher yield, total dry mass, and harvest index than most of the VarMix except ACDEF (Table 3). Under CAWD, VarMix involving five (ACDEF) and six (ABCDEF) combinations had heavier grain weight by 15.2 and 14.4 %, respectively, than monocultures. Total dry mass and HI of VarMix were not significantly different from monocultures regardless of soil moisture conditions (Table 3).

Root System Development

There were significant interactions between water treatments and VarMix on root lengths and branching index (BI) (Table 4). Generally, the total nodal root length (TNRL) and mean nodal root length (NRL) under CWL were significantly higher than under CAWD. In contrast, total root length (TRL), total lateral root length (TLRL), and BI were significantly higher in CAWD by 10.4, 17.8, and 35.0%, respectively, than in CWL. Furthermore, the VarMix had no significant differences in any of the root traits relative to monocultures regardless of soil moisture conditions.

Relationship Between Yield, Branching Index, and Water-use Efficiency

Positive and significant relationship between BI and grain weight was computed in the CWL ($r = 0.46$, $p<0.01$) and CAWD ($r = 0.56$, $p<0.01$) conditions (Figure 1). The magnitude of the grain weight under both soil moisture conditions were strongly influenced by the extent of the roots' BI. There was also a strong positive and significant relationship between grain weight and WUE ($r = 0.85$, $p<0.01$) (Figure 2) and between WUE and BI ($r = 0.68$, $p<0.01$) (Figure 3) under CAWD.

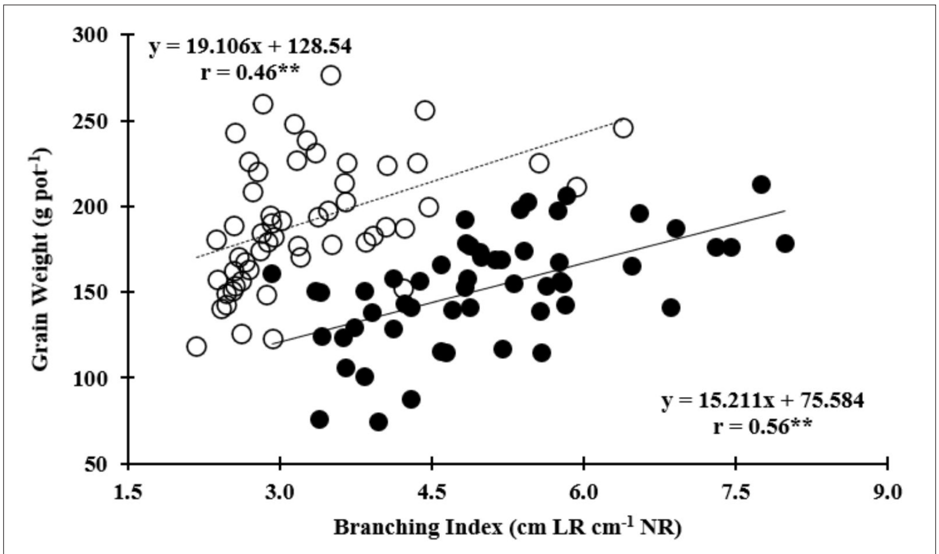

Figure 1. Relationship between branching index and grain weight under continuously waterlogging (CWL, unshaded) and continuous cycles of alternate waterlogging and drought condition (CAWD, shaded). NR- nodal roots. **, significant at P<0.01. LR- lateral roots.

Table 3. Grain weight, total dry mass, and harvest index of mono or VarMix cultures under continuously waterlogged (CWL) and continuous cycles of alternate waterlogging and drought condition (CAWD).

Water Treatment	Mono/VarMix Cultures (Code/Ratio)	Grain Weight (g pot^{-1})	Total Dry Mass (g pot^{-1})	Harvest Index
CWL	A	208.4^{ab}	535.9^{a}	0.39^{abc}
	B	183.1^{b}	535.0^{a}	0.34^{c}
	C	248.5^{a}	524.7^{ab}	0.47^{a}
	D	174.6^{b}	457.8^{bcd}	0.38^{bc}
	E	190.4^{b}	503.3^{abc}	0.37^{bc}
	F	181.1^{b}	419.3^{d}	0.42^{abc}
	AE (3:3)	184.7^{b}	476.0^{abcd}	0.39^{abc}
	AD (3:3)	167.6^{b}	417.1^{d}	0.41^{abc}
	AEF (2:2:2)	191.6^{b}	463.4^{bcd}	0.41^{abc}
	ABC (2:2:2)	199.9^{b}	478.7^{abcd}	0.41^{abc}
	BCD (2:2:2)	188.2^{b}	481.0^{abcd}	0.39^{abc}
	BDE (2:2:2)	187.1^{b}	479.8^{abcd}	0.39^{abc}
	ABCE (1:1:3:1)	178.2^{b}	455.0^{cd}	0.39^{abc}
	ACEF (1:3:1:1)	182.3^{b}	458.1^{bcd}	0.39^{abc}
	ACDE (1:3:1:1)	179.7^{b}	439.5^{cd}	0.41^{abc}
	ABCEF (1:1:2:1:1)	193.7^{b}	458.6^{bcd}	0.42^{abc}
	ACDEF (1:2:1:1:1)	202.5^{ab}	457.4^{bcd}	0.44^{ab}
	ABCDEF (1:1:1:1:1:1)	198.0^{b}	502.5^{abc}	0.39^{abc}
CAWD	A	164.8^{a}	419.6^{abc}	0.39^{ab}
	B	114.2^{b}	430.7^{abc}	0.26^{e}
	C	168.5^{a}	377.5^{c}	0.45^{a}
	D	170.4^{a}	435.1^{abc}	0.40^{ab}
	E	172.9^{a}	469.4^{a}	0.37^{abc}
	F	157.5^{ab}	383.7^{bc}	0.41^{ab}
	AE (3:3)	168.4^{a}	430.7^{abc}	0.39^{ab}
	AD (3:3)	138.6^{ab}	404.0^{abc}	0.35^{bcde}
	AEF (2:2:2)	142.3^{ab}	393.5^{bc}	0.36^{bcd}
	ABC (2:2:2)	152.7^{ab}	448.1^{ab}	0.34^{bcde}
	BCD (2:2:2)	157.9^{ab}	422.6^{abc}	0.37^{abc}
	BDE (2:2:2)	114.6^{b}	415.4^{abc}	0.27^{de}
	ABCE (1:1:3:1)	139.6^{ab}	418.6^{abc}	0.34^{bcde}
	ACEF (1:3:1:1)	140.8^{ab}	378.6^{c}	0.37^{abc}
	ACDE (1:3:1:1)	166.1^{a}	407.3^{abc}	0.41^{ab}
	ABCEF (1:1:2:1:1)	115.2^{b}	394.8^{bc}	0.29^{cde}
	ACDEF (1:2:1:1:1)	178.4^{a}	440.4^{abc}	0.41^{ab}
	ABCDEF (1:1:1:1:1:1)	177.2^{a}	444.4^{abc}	0.40^{ab}
Water Treatment (A)		**	**	**
Mono/VarMix (B)		**	**	**
A x B		**	**	**
CV %		9.14	5.14	7.67

A) NSIC 2012 Rc 298, B) NSIC Rc 214, C) NSIC Rc 216, D) PSB Rc 82, E) NSIC 2011 Rc 238, and F) NSIC 2012 Rc 300.
In a column, means followed by the same letter (s) are not significantly different at 5 % level by LSD. ** significant at 1% level.

Table 4. Mean total root length (TRL), total lateral root length (TLRL), total nodal root length (TNRL), mean nodal root length (MNRL), and branching index (BI) of mono or VarMix cultures under continuously waterlogged (CWL) and continuous cycles of alternate waterlogging and drought condition (CAWD).

Water Treatment	Mono/ VarMix Culture (Code/Ratio)	TRL (m pot^{-1})	TLRL (m pot^{-1})	TNRL (m pot^{-1})	MNRL (cm)	BI (cm LR cm^{-1} NR)
CWL	A	3077.1^{ab}	2246.2^{ab}	831.0^{ab}	37.2^{a}	2.7^{ab}
	B	3620.6^{a}	2888.7^{a}	731.9^{abcde}	30.7^{abcd}	3.9^{ab}
	C	2866.5^{ab}	2164.7^{ab}	701.8^{abcde}	32.9^{abcd}	3.1^{ab}
	D	3032.9^{ab}	2242.3^{ab}	790.6^{abc}	28.3^{bcd}	2.8^{ab}
	E	3107.7^{ab}	2301.4^{ab}	806.3^{abc}	34.5^{abc}	2.9^{ab}
	F	2521.2^{ab}	1759.1^{b}	762.1^{abcd}	36.7^{a}	2.4^{b}
	AE (3:3)	3231.1^{ab}	2368.8^{ab}	862.3^{a}	32.3^{abcd}	2.8^{ab}
	AD (3:3)	2544.5^{ab}	1836.2^{b}	708.3^{abcde}	29.1b^{cd}	2.7^{ab}
	AEF (2:2:2)	3040.9^{ab}	2278.1^{ab}	762.8^{abcd}	32.6^{abcd}	3.0^{ab}
	ABC (2:2:2)	2633.7^{ab}	2109.8^{ab}	523.9^{f}	25.7^{d}	4.5^{a}
	BCD (2:2:2)	2980.7^{ab}	2315.4^{ab}	665.3^{bcdef}	28.0^{cd}	4.1^{ab}
	BDE (2:2:2)	2780.6^{ab}	2184.3^{ab}	596.4^{def}	26.1^{d}	4.2^{ab}
	ABCE (1:1:3:1)	2856.0^{ab}	2211.9^{ab}	644.1^{cdef}	32.7^{abcd}	3.5^{ab}
	ACEF (1:3:1:1)	3097.6^{ab}	2301.2^{ab}	796.4^{abc}	32.7^{abcd}	2.9^{ab}
	ACDE (1:3:1:1)	2699.9^{ab}	1992.9^{ab}	707.1^{abcde}	30.1^{abcd}	2.9^{ab}
	ABCEF (1:1:2:1:1)	2458.3^{b}	1877.1^{b}	581.3^{ef}	28.3^{bcd}	3.4^{ab}
	ACDEF (1:2:1:1:1)	2940.5^{ab}	2282.7^{ab}	657.8^{bcdef}	31.3^{abcd}	3.7^{ab}
	ABCDEF (1:1:1:1:1:1)	3088.9^{ab}	2362.8^{ab}	726.2^{abcde}	35.4^{ab}	3.5^{ab}
CAWD	A	4305.4^{a}	3699.8^{a}	605.6^{abcde}	31.0^{a}	6.5^{a}
	B	3398.3^{abcdef}	2850.1^{abcde}	548.3^{abcdef}	21.9^{cd}	5.6^{ab}
	C	2503.3^{f}	2056.5^{e}	446.8^{ef}	19.7^{d}	5.2^{ab}
	D	4015.6^{ab}	3308.1^{ab}	707.5^{a}	19.3^{d}	5.0^{ab}
	E	3558.6^{abcdef}	2936.5^{abcde}	622.1^{abcd}	24.8^{abcd}	5.0^{ab}
	F	3416.4^{abcdef}	2812.8^{abcde}	603.6^{abcde}	24.2^{abcd}	4.9^{ab}
	AE (3:3)	2856.0^{cdef}	2346.2^{bcde}	509.9^{cdef}	25.4^{abcd}	5.1^{ab}
	AD (3:3)	3761.7^{abcd}	3120.9^{abcd}	640.9^{abc}	24.9^{abcd}	5.6^{ab}
	AEF (2:2:2)	2743.6^{def}	2255.2^{cde}	488.4^{cdef}	25.1^{abcd}	4.8^{ab}
	ABC (2:2:2)	2626.3^{ef}	2200.4^{cde}	425.8^{f}	21.6^{cd}	5.8^{ab}
	BCD (2:2:2)	2789.1^{cdef}	2217.3^{cde}	571.8^{abcdef}	24.6^{abcd}	4.1^{b}
	BDE (2:2:2)	3381.1^{abcdef}	2755.0^{abcde}	626.1^{abcd}	22.6^{bcd}	4.7^{ab}
	ABCE (1:1:3:1)	2774.6^{cdef}	2248.2^{cde}	526.4^{bcdef}	23.1^{bcd}	4.7^{ab}
	ACEF (1:3:1:1)	2627.0^{ef}	2163.2^{de}	463.8^{def}	21.1^{cd}	4.9^{ab}
	ACDE (1:3:1:1)	3090.2^{bcdef}	2511.6^{bcde}	578.6^{abcdef}	22.9^{bcd}	4.6^{b}
	ABCEF (1:1:2:1:1)	3318.7^{abcdef}	2665.4^{bcde}	653.4^{abc}	24.3^{abcd}	4.6^{ab}
	ACDEF (1:2:1:1:1)	3859.9^{abc}	3162.7^{abc}	697.2^{ab}	29.9^{ab}	4.8^{ab}
	ABCDEF (1:1:1:1:1:1)	3656.2^{abcde}	3021.9^{abcde}	634.3^{abcd}	27.4^{abc}	4.9^{ab}
Water Treatment (A)		**	**	**	**	**
Mono/VarMix (B)		**	**	**	**	ns
A x B		**	**	**	**	**
CV %		12.14	13.70	9.20	9.01	15.29

A) NSIC 2012 Rc 298, B) NSIC Rc 214, C) NSIC Rc 216, D) PSB Rc 82, E) NSIC 2011 Rc 238, and F) NSIC 2012 Rc 300.
In a column, means followed by the same letters are not significantly different at 5 % level by LSD. **, significant at 1% level.

Discussion

The study showed that VarMix had similar agronomic traits and overall root system development under CWL but not in CAWD conditions. Under CAWD, VarMix of five (ACDEF: NSIC Rc 298, Rc 216, PSB Rc 82, NSIC Rc 238, and Rc 300) and six (ABCDEF: NSIC Rc 298, Rc 214, Rc 216, PSB Rc 82, NSIC Rc 238, and Rc 300) combinations produced higher yield than monocultures indicating that mixing multiple varieties can help mitigate the effect of water stress occurred during reproductive stage. In root system development, root components such as TRL, TLRL, TNRL, and MNRL did not show influence on grain weight due to large similarities of the VarMix traits under CAWD condition.

Under drought- and SMF-prone conditions, dry matter production and yield are results of water use (WU) and water-use efficiency (WUE) (Suralta et al., 2018); WUE being related to root trait development while WUE to shoot trait responses. Water use is often link to root system development and function in response to moisture stress. Root system plasticity in terms of branching of lateral roots at the shallow soil layer, especially in situations when the soil conditions limited deep root growth, rewatering, and whole root system during transient waterlogged-to-drought conditions can contribute to the overall promotion of root system development. These factors also affect maintenance of leaf water status, dry matter production (Suralta et al., 2018), and yield through sustained development and activity of yield components (Kato et al., 2008; Quinones et al., 2017). On the other hand, WUE is often used to highlight the relationship between crop growth development and the amount of water used (Sinclair et al., 1984). An increase in water

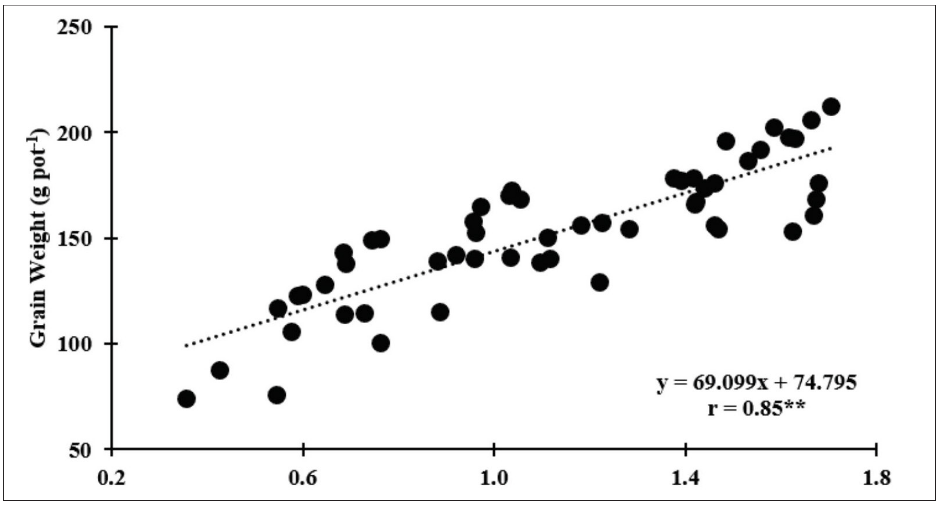

Figure 2. Relationship between water use efficiency and grain weight under continuous cycles of alternate waterlogging and drought condition (CAWD, shaded). **, significant at P<0.01. SDW- shoot dry weight.

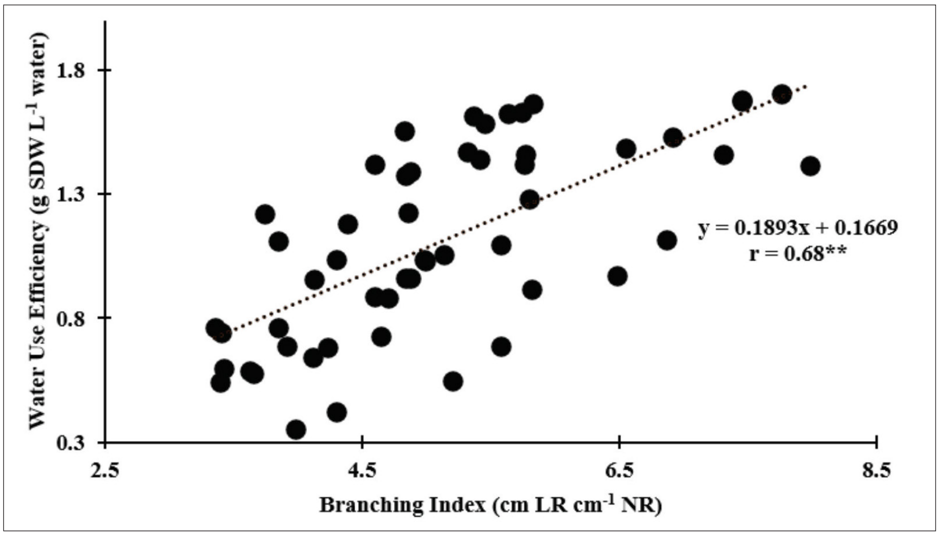

Figure 3. Relationship between branching index and water use efficiency under continuous cycles of alternate waterlogging and drought condition (CAWD). **, significant at P<0.01. LR- lateral roots; NR-nodal roots.

productivity ameliorates yield grains while reducing losses of irrigation water applied (Playán and Mateos, 2006).

This study recorded heavier grain weight in some VarMix combinations due to WUE. Yield differences was due to the inherent ability of VarMix to produce higher shoot dry mass and yield per unit amount of water taken up from the drying soil during reproductive stage. WUE is also more important than WU because the varieties used in this study were bred for transplanted irrigated lowland conditions except NSIC Rc 298, which was developed for direct-seeded irrigated lowland system. Most of the irrigated lowland varieties are bred based on their high yield potential, pest and disease resistance, and grain quality (Peng and Khush, 2003). Further, BI ability (length of LR per unit length of NR) generally increased under CAWD, and also had significant relationship with WUE.

VarMix, with proper identification of most suitable combination, can help optimize resources better than monocropping systems, which would lead to improve grain yield and harvest index under water-limited condition (Li et al., 2018). In water-limited environments, mixtures tend to conserve soil water before stem elongation, ensuring the availability and efficient utilization of resources that translates into greater water-use efficiency and higher yield than in pure stands. VarMix reduces the impacts of abiotic stresses by buffering yield through more efficient resource use including soil moisture that are particularly evident when mixtures comprise complementary physiological traits that influence WUE (Gyamfi et al., 2015).

Results indicated that NSIC Rc 298, a direct seeded irrigated lowland rice variety, showed root plastic developmental response (increase in trait value under CAWD relative CWL) based on TRL in response to CAWD. This was partially attributed to its plasticity in TLRL under the condition indicating the key role of the degree in branching of the lateral root as key response to water stress conditions (Suralta et al., 2018). Thus, root system development and physiology are closely associated with above-ground plant biomass (Suralta et al., 2018). NSIC Rc 298 also produced the greatest water use and shoot dry weight in monoculture under CAWD.

VarMix characteristic underground mechanism may partially intensify the root uptake area by using available assimilates during drought. In this study, the presence of NSIC Rc 298 in some of the VarMix triggered the increase in mean root plasticity in response to CAWD, although it did not increase total water use. This result indicates that combined compensation (Yadav et al., 2009) and facilitation (Garcia-Barrios, 2002) mechanisms resulted in high yield gained by VarMix.

NSIC Rc 298 as monoculture produced high grain yield, TRL, and water use under CAWD. This variety when combined with appropriate varieties in VarMix may have worked through these steps. First, it expressed high root plasticity in response to CAWD, which may have resulted in the increase of its root water uptake from deeper soil layer through hydraulic lift (Doussan et al., 2006; Sekiya et al., 2011). Second, the water taken up by the roots of NSIC Rc 298 may have been redistributed in the upper drier soil layer (Neumann and Cardon, 2012) and initially take up by neighboring roots of other varieties to continue their shoot growth (Izumi et al., 2018). And third, part of the redistributed water may have been used by the other varieties to improve their root system as evidenced by the marked increase in the average BI particularly in the two VarMix such as five (NSIC Rc 298, Rc 216, PSB Rc 82, NSIC Rc 238, and Rc 300) and six (NSIC Rc 298, Rc 214, Rc 216, PSB Rc 82, NSIC Rc 238, and Rc 300) combinations. Ultimately, this led to their balanced water use to maintain above ground dry matter production and yield via increase in WUE. The targeted combination of physiological traits, which are the main drivers for yield under water stress through VarMix, could be further explored as a strategy to improve yield and stability of yield in water deficit environments (Gyamfi et al., 2015). The above mechanisms will be subjected for further studies contrasting VarMix and monocultures.

Conclusion

VarMix, given right combination of varieties, can collectively increase branching index and can maintain optimal water use but high WUE during water deficit conditions. Increase in WUE under CAWD significantly increased the grain weight in some VarMix combinations. The high performing VarMix may express their full potential over monocultures when grown under real field conditions where the varieties are not only exposed to SMF but also to pest and disease pressures.

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CHANGES IN THE TOTAL PHENOLIC CONTENT OF RICE AS INFLUENCED BY YELLOW STEMBORER (*Schirpophaga incertulas* WALKER) INFESTATION

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Abstract

Phenolics are secondary metabolites with important role in protecting plants from insects. This study was conducted to further understand the biochemical response of the rice plant to stemborer infestation in terms of phenolics and support the development of resistant rice cultivars for sustained yield increase. It evaluated the changes in the total phenolic content (TPC) in resistant (TKM6) and susceptible (TN1) rice varieties when infested with yellow stemborer (YSB). Highest TPC in infested leaves was observed during the day of larva introduction, which was 10.9% higher than in the control. A 14.0% and 8.1% higher TPC in infested leaves than the healthy leaves was observed 3 and 5 days after larvae introduction, respectively. Lower phenolic concentration in infested leaves than in the control was observed 7 days after larvae introduction. TPC of both healthy and infested TKM6 stems were generally comparable. TPC of TN1 leaves peaked during the day of larvae introduction but was comparable with that of the control. Phenolic content of infested TN1 stems were generally comparable with the control. Initial liquid chromatography (LC) profiling of phenolic compounds in TKM6 was also conducted. Using the optimized high-performance LC (HPLC) conditions and mobile phase system, chromatographic peaks of the seven phenolic standards were observed, with serotonin eluting first, followed by tryptamine, 4-hydroxybenzoic acid, vanillic, syringic, p-coumaric, and trans-cinnamic acid. Vanillic and p-coumaric acid were detected in the diluted TKM6 extract. This study further measured the changes in total phenolic concentration of resistant and susceptible checks and the initial qualitative and quantitative determination of secondary metabolites in rice when infested with yellow stemborer.

Keywords: *HPLC, Phenolics, Resistance, Stemborer, TKM6.*

Introduction

Rice remains as staple food for more than half of the world's population particularly in Asia as it plays a primary role in the current and future world food security (Chauhan et al., 2017). Increasing rice productivity to meet the demand of the growing global population is a formidable challenge, and one of these challenges is dealing with insect pest infestation. Over 175 species of insects are known to attack rice at different growth stages while 20 species significantly affect rice yield.

Stemborer is among the major insect pests of rice (Singh and Tiwari, 2019). The life cycle of stemborers that attack rice shares several features. Egg masses are laid on leaf blades or in between the leaf sheath and stem of rice plants, which hatch usually in the morning with a few minutes' interval. Within 2 h upon egg hatching, almost all the larvae will have either entered the leaf sheaths of the plant on which they enclosed themselves or will have dispersed to other plants. After a week, some larvae move to other plants or other tillers of the same plant. Early instars feed on leaf blades or on leaf sheath that often leave characteristic scars and lesions while later instars bore into rice stalks. The larva passes through 4 or 5 instars and a pupal stage in the stem in 4-5 weeks (Chen et al., 2012; Cohen et al., 2000, Pathak and Khan, 1994). Severe damage is inflicted to the plant during larval feeding. Subsequent penetration of larva to the tiller results in severed tillers or deadhearts during the vegetative stage and damaged panicles or whiteheads during maturity (Nayak et al., 2019; Devasena et al., 2018). In rice-growing countries in Asia, the most devastating genera of *Scirpophaga* that cause significant yield loss are the yellow stemborer *Scirpophaga incertulas* (Walker), dark-headed stemborer, *Chilo polychrysus* (Meyrick), and pink borer *Sesamia inferens* (Walker) (Singh and Tiwari, 2019; Ane and Hussain, 2016; Pathak and Khan, 1994: Saxena, 1986; Pathak et al., 1971). This monopahagous insect can damage the rice plant during early tillering stage in a low percentage of dead hearts; however, every 1% of whitehead may account for 1-3% yield loss (Pathak et al., 1971). Yield losses from stemborer infestation were caused by deadheart (30%) and whitehead (>20%) (Litsinger et al., 2011; Muralidharan and Pasalu, 2006; Chieng, 1985).

In the Philippines, two species of stemborers are common: yellow (*Scirpophaga incertulas)* and white (*Scirpophaga innonata).* Yellow stemborer (YSB) is most prevalent in Luzon while white stemborer (WSB) is common in the Visayas and Mindanao (Litsinger et al., 2011; Litsinger et al., 2006; Sebastian et al., 2000). According to Litsinger et al (2011), the average yield loss per crop was estimated in each rice ecosystem from stemborers in the Philippines to be 6% in irrigated wetland (Zaragosa, Nueva Ecija; Guimba, Nueva Ecija; Koronadal South Cotabato; and Calauan, Laguna), 11% in rainfed wetland (Oton, Iloilo; Solana, Cagayan; and Manaoag, Cagayan) but none in rainfed dryland (Siniloan, Quezon: Tanauan, Batangas, Claveria, Misamis Oriental; and, Tupi, South Cotabato). This was based on time-series sampling in farmer's fields from 1976 to 1991.

When an herbivore insect attacks, plants naturally respond to defend themselves using direct and indirect defense mechanisms. Plants' direct defense mechanisms include morphological traits such as hairs, trichomes, thorns, spines, and thick leaves or through toxic chemical production that influences feeding, growth, and survival of insect herbivore. Indirect defenses involve quick activation of specific signal molecules that release volatile organic compounds that can attract natural enemies, act as repellant, or act as antibiosis or antixenosis to insect (Fürstenberg-Hägg et al., 2013; War et al., 2012; War et al., 2011; Spiteller, 2008; Lattanzio and Cardinali, 2006; Hartmann, 1996; Kogan and Paxton, 1983).

Phenolic compounds are secondary metabolites constituting one of the most common and widespread group of substances in plants (Amsagowri et al., 2018). Phenolic compounds are released in response to environmental stresses such as the presence of an herbivore or pathogen, physical injury, unfavorable temperature and pH, saline stress, heavy metals stress, and UVB and UVA radiation (Sanchez et al., 2018; Lattanzio and Cardinali, 2006). In addition, concentration of plant phenolics and other defensive compounds has been shown to increase when pre-existing compounds are insufficient to stop infestation (Kumar et al., 2014).

Understanding the biochemical changes in the rice plant during the stemborer infestation will help establish the basic information relating to plant defense mechanisms against the stemborer. Development of resistant genotypes becomes easier with the identification of biochemical factor of resistance and its association with other genetic mechanism (e.g., morphological and physiological characteristics). Furthermore, the biochemical evaluation may be included in the screening process for potential parentals in breeding for resistance against stemborer (War et al., 2011a; War et al., 2011b; Heinrichs, 1986; Pathak and Dale, 1982).

However, limited information and the complexity of the behavioral and physiological responses of insect herbivores to plant biochemicals make identification of the cause of resistance difficult. Detailed analysis is also a prerequisite in various aspects of insect-host plant interaction while identification of the biochemical factor of resistance is often intricate and requires adequate bioassays (Saxena, 1986; Pathak and Dale, 1982).

In this study, the changes in total phenolic concentration of TKM6 (resistant) and TN1 (susceptible) genotypes was measured as infested by yellow stemborer. HPLC profiling of phenolic compounds was also made using the optimized method to provide initial qualitative and quantitative information on secondary metabolites for resistance.

Materials and Methods

Plant Materials and Experiment Location

Two check cultivars namely, TKM6 (the universal donor for stem borer resistance identified by the IRRI as resistant check) and Taichung Native 1 or TN1 (susceptible check), were selected for this study (Table 1).

The experimental set-up was established from February to May 2019 at the screenhouse of the Central Experiment Station of the Philippine Rice Research Institute (PhilRice CES). There were two treatments in the experiment: (1) healthy (control) and (2) infested with yellow stemborer (YSB). The treatments were laid out in three replications. Ten data collection points were established: 5, 3, and 1 day before YSB-larva introduction (DBI); day of introduction (0 DI), and; 1, 3, 5, 7, 9, and 11 days

Table 1. Rice cultivars used in the study.

Rice Cultivars	Pedigree	Seed Source Origin	Country of Origin	Maturity
TKM6	CO 18 / GEB 24	IRRI-PH	India	115-120
TN1 (Taichung Native 1)	DEE GEO WOO GEN / TSAI YUAN CHUNG	IRRI-PH	Taiwan	125-130

after larva introduction (DAI). Overall, 120 pots were established and randomized.

Insects

YSB larvae were reared at the screenhouse at PhilRice CES. The 2-day old laboratory-incubated larvae were introduced using camel's hair brush on the leaf sheath of the plants during booting stage or when TN1 and TKM6 reached 72 and 61 days, respectively. Three sets of infestations (one set per replicate) were conducted on April 16 (1st infestation), April 18 (2nd infestation), and April 22 (3rd infestation).

Sample Collection

Three tillers were cut from a hill. The leaf and stem were separated, placed in properly labelled glassine bags, and stored in ice-chest to preserve the integrity of the samples during transport to the laboratory for storage. Samples were stored in a -80 °C freezer for about a month before sample preparation

Sample Preparation and Extraction

One leaf and one stem from each infestation event were pulled and collected. Collected leaves and stems were freeze-dried for at least 48 h, pulverized, and stored in a freezer. Phenolics from powdered samples (0.1 g) were extracted with 10 mL of 85% methanol, placed in a shaker for overnight shaking (12-14 h) at 300 rpm at room temperature, and centrifuged at 3,000 rpm for 15 min at room temperature. Supernatant was collected and stored at 4°C prior to analysis.

Determination of Total Phenolic Content (TPC) and Phenolic Compounds Used

TPC of samples were evaluated according to the method of Singleton et al. (1998) with minor modifications. Diluted extracts (0.5 mL) were added with 2.5 mL of 10% Folin-Ciocalteau reagent and mixed using a vortex. After 15 min, 2 mL of 7.5% sodium carbonate (Na_2CO_3) was added. Samples were mixed, incubated at dark conditions for 1 h, and read at 765 nm wavelength using UV-Vis spectrophotometer. Results were expressed as mg gallic acid equivalents per g of sample (mg GAE/g) based on a standard calibration equation obtained from the absorbances of known concentrations of gallic acid standard solutions.

Statistical Analysis

ANOVA test was performed and means were compared using Tukey's$_b$ test at $p<0.05$. Comparison of means (control and infested samples) were made using paired sample *t-test*. Statistical analyses were made using 2011 IBM SPSS Statistics for Windows, Version 20.0.

Optimization of High-Performance Liquid Chromatography (HPLC) Method for Phenolic Identification

Optimization of chromatographic procedure was conducted using Waters Alliance e2695 HPLC module equipped with Waters 2998 photodiode array (PDA) detector and EMPOWER 3 PDA Software (Waters, Milford, MA 01757, USA). Peak separation was executed using a BRISA LC2 C-18 reverse-phase HPLC column (250 mm × 4.6 mm × 0.46 µm; Teknokroma, Barcelona, Spain).

Powdered TKM6 leaf samples (0.5 g) was used for extracting the phenolics by 85% methanol (v/v) and was shaken overnight (12-14 h). The sample was centrifuged at 3,000 rpm for 15 min at room temperature. Extract of which was filtered using 13-mm syringe filters (0.45 PVDF). Different concentrations of standard solution (10, 25, and 100 ppm) containing all the phenolic standards were prepared from 1,000 ppm stock solutions of each of the phenolic standards. Seven phenolic compounds observed to increase in the leaves of rice when infested with stemborer, namely, vanillic acid (Sigma-Aldrich, Switzerland), 4-hydroxybenzoic acid (Sigma-Aldrich, USA), *p*-coumaric acid (HWI Group, Germany), serotonin (Sigma-Aldrich USA), syringic acid (Sigma-Aldrich, China), and tryptamine (Sigma-Aldrich, Switzerland), were used in this study as standards (Table 2). Uniform solvent matrix in the blank, sample, and standards was ensured during dilution. Sample (20 µL) was injected by an autosampler and eluted through the column with mobile phase consisting of solvent A (2:98 v/v

Table 2. Phenolic compounds found present in stemborer-infested rice leaves.

Phenolic Compounds	Stemborer Species	Rice Variety	Reference
o-hydrobenzoic acid Vanillic acid Syringic acid *p*-coumaric acid Cinnamic acid	Striped stemborer (*Chilo suppressalis*)	TN1	Usha Rani and Jyothsna (2010)
Tryptamine Serotonin	Yellow stemborer (*Scirpophaga ncertulas*)	-	Ishihara et al. (2008)

acetic acid:water) and solvent C (acetonitrile) at a flow rate of 1 mL/min. Different elution conditions at column temperature of 30°C were conducted until appearance and separation of chromatographic peaks corresponding to each phenolic standards were attained. Chromatographic peaks were obtained at 245 nm wavelength. Total run time was 55 min.

Results and Discussion

Measurement of Total Phenolic Content (TPC) in TKM6 and TN1 Leaf and Stem Extracts

As shown in Table 3 and Figure 1, TPC of healthy (control) TKM6 leaves ranged from 7.30 to 11.19, which peaked at 9 days after larvae introduction (DAI). On the other hand, TPC of infested TKM6 leaves ranged 7.71-10.71, which peaked at 0 DI (day of introduction). During 0 DI, the difference in phenolic content between infested and healthy plants was 10.9% with the infested plants having higher phenolic content. This finding could be attributed to the induced response of the plants when YSB larvae were introduced (Sanchez et al., 2018; Spiteller, 2008) in the leaf sheath where biosynthesis begins and unknown molecules are released. It is a classic example of an induced defense mechanism of plants to alleviate the immediate biological stress and to decrease the chance of adaptation of the attacking insects to the induced chemicals (War et al., 2012). Thus, feeding by insect can quantitatively change the phenolic content in a rice plant. The increase in plant phenolic acids post-infestation was previously observed in *Scirpophaga incertulas*, *Cnaphalocrocis medinalis,* and *Nilaparvata lugens*-infested rice plants (Usha Rani and Jyothsna, 2010). However, Lattanzio and Cardinali (2006) noted that accumulation of defensive compounds is a complex process requiring more resources from the host plant for the synthesis of these biochemicals. This became evident 7 DAI, in which lower phenolic concentration was observed in infested leaves than in healthy leaves.

For healthy leaves, the high TPC in 9 DAI may be attributed to age-related plant changes. This is similar to the results of the study by Gowda and Palo (2003), that there were lower concentrations of total phenolics in younger leaves than in mature ones. TPC of TKM6 leaves may differ significantly up to 3.7% without the influence of YSB larvae as observed before infestation. It should also be noted that there is an increase in TPC in infested leaves compared with the healthy leaves at 3 (14.0%) and 5 (8.1%) DAI. These increases could be an indication of TKM6 resistance against YSB by continuously increasing its defensive compounds. The increase of TPC after 3 DAI may be related to the first boring and feeding of YSB larvae in plants during post-introduction (Pathak et al., 1971). Additionally, the above process may lead to the changes in genetic expression of resistant check in response to the larval attack. Similar observation was found by Gutbrodt et al. (2011) who detected increased resistance of the plant used as resistant check after 3-4 days.

TPC of healthy TKM6 stems ranged from 3.08 to 3.62 mg GAE g^{-1} and were generally comparable except during 0 DI and 9 DAI, when phenolic content peaked; and at 11 DAI, when the lowest concentration of TPC change was observed. Among infested stems, highest phenolic content was observed at 7 and 9 DAI. The increase could be attributed to the downward larvae movement and staying in the pith for feeding.

Table 3. Total phenolic content of healthy and YSB-infested TKM6 plants.

Day[3]	Total Phenolic Content, mg GAE g^{-1}					
	Leaf			Stem		
	Healthy[1]	Infested[1]	Diff. (%)[2]	Healthy[1]	Infested[1]	Diff. (%)[2]
5 DBI	7.30±0.10^{f}	7.71±0.17^{h}	5.7	3.22±0.11^{c}	3.55±0.03^{a}	10.4*
3 DBI	8.59±0.07^{d}	8.91±0.02^{e}	3.7*	3.29±0.12^{bc}	3.37±0.06^{ab}	2.2
1 DBI	8.69±0.05^{d}	9.06±0.20^{de}	4.2	3.27±0.04^{bc}	3.32±0.01^{c}	1.4
0 DI	9.66±0.08^{bc}	10.71±0.05^{a}	10.9**	3.62±0.05^{a}	3.33±0.05^{c}	-8.1*
1 DAI	8.29±0.06^{de}	8.59±0.26^{e}	3.6	3.22±0.09^{c}	3.43±0.11^{ab}	6.5
3 DAI	8.10±0.17^{e}	9.23±0.08^{cde}	14.0*	3.26±0.03^{bc}	3.49±0.09^{ab}	7.3*
5 DAI	9.36±0.05^{c}	10.12±0.04^{b}	8.1**	3.08±0.09^{c}	3.54±0.11^{a}	15.1**
7 DAI	9.71±0.25^{bc}	8.20±0.13^{g}	-15.5**	3.30±0.16^{bc}	3.45±0.08^{ab}	4.5
9 DAI	11.19±0.13^{a}	9.38±0.05^{cd}	-16.2**	3.52±0.11^{ab}	3.29±0.03^{c}	-6.4
11 DAI	9.82±0.36^{b}	9.51±0.02^{c}	-3	2.83±0.12^{d}	3.40±0.07^{ab}	20.3*

[1]Means followed by the same letter within a column are not significantly different at *P*>0.05 based on Tukey's$_{b}$ test (n=3).
[2]Significant differences (paired t-test) between healthy and infested plants (n=3) are indicated by single (*P*< 0.05, significant) and double (*P*<0.01, highly significant) asterisks; no asterisk means not significant; negative values indicate higher TPC of control than the infested.
[3]DBI = days before larva introduction; DI =day of introduction; DAI = days after introduction

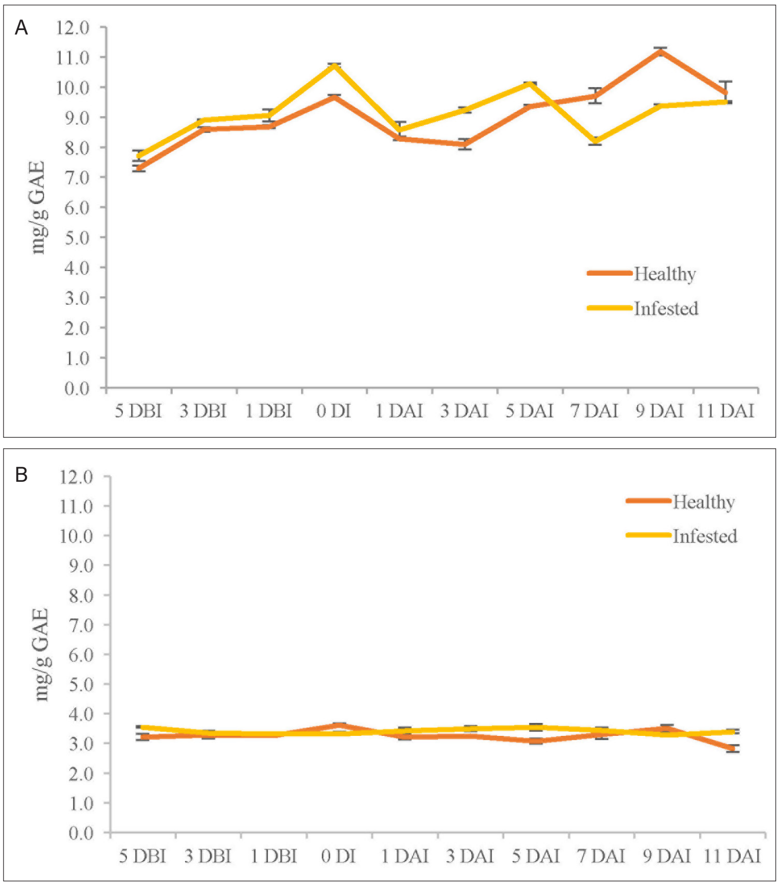

Figure 1. Total phenolic content of (A) leaf and (B) stem of healthy and YSB-infested TKM6 plants

The plant signals were activated during those days; thus, higher TPC was observed after larvae introduction. Compared with the control, the increase in TPC among infested plants was 15.1% higher but still generally comparable, except the day before and during introduction and 9 DAI.

The lowest phenolic concentration was 0 DI (-8.1%). During the same time, the leaves of TKM6 also recorded the highest phenolic content (10.9%). This finding supports the sink-to-source dynamics hypothesis in plants wherein the plant organ needing support increase its defensive compounds — in this case the leaves, has to be the stronger sink. In this study, the stem was the source of resources delegated to the leaves (Schaller, 2008). It can also be observed that the highest increase in TPC (20.3%) in comparison with the control was observed 11 DAI. This result indicates that there might be a need to increase the duration of sampling to see if high phenolic concentration shall hold for some time. If this is possible, it may shed a light on the likelihood of accumulating phenolic-derived defensive compounds in the stems such as lignin and tannins. If not, then it may reinforce the hypothesis that age-related phenolic changes may have been causing the increased TPC at 11 DAI.

The healthy leaves of TN1 with TPC peaking on 7 DAI, as well as that of TKM6, may be attributed to age-related phenolic changes. TPC of TN1 leaves may differ significantly up to 9.3% without the influence of YSB larvae as observed during the days of pre-larvae introduction (Table 4 and Figure 2). Therefore, the peak of phenolic content observed during the day of larvae introduction may not be influenced by stemborer introduction in plants. This is in contrast with the response of infested TKM6 leaves during the same time, and the phenolic content value was also not significantly different to that of the control. As there was higher phenolic content without larvae introduction, it is supposed that the response of the susceptible TN1 to infestation was more of a constitutive defense mechanism, rather than induced, whereby the defensive chemical had always been there prior to herbivory-induced infestation.

TPC values of infested TN1 stems were generally comparable with the control, except 7 and 9 DAI of YSB larva, when infested TN1 stems were 13% higher in TPC than in control. By 7 and 9 DAI, the larvae could already be actively feeding in the stem, resulting in higher TPC as a defensive response by the susceptible TN1.

Table 4. Total phenolic content of healthy and YSB-infested TN1 plants.

Day[3]	Total Phenolic Content, mg GAE/g					
	Leaves			Stem		
	Healthy[1]	Infested[1]	Diff. (%)[2]	Healthy[1]	Infested[1]	Diff. (%)[2]
5 DBI	10.11±0.05[bcde]	10.65±0.12[bc]	5.3*	3.64±0.10[a]	3.75±0.03[a]	2.9
3 DBI	10.02±0.12[de]	10.95±0.08[ab]	9.3*	3.36±0.17[b]	3.29±0.10[b]	-2
1 DBI	10.02±0.19d[e]	9.80±0.04[f]	-2.3	3.07±0.00[c]	3.20±0.11[bc]	4
0 DI	10.50±0.25[abc]	11.21±0.18[a]	6.8*	3.18±0.09[bc]	3.12±0.04[bcd]	-2
1 DAI	10.08±0.08[cde]	10.49±0.03[cde]	4.1*	3.09±0.06[c]	3.03±0.13[cd]	-1.9
3 DAI	9.27±0.34[f]	10.58±0.08[cd]	14.2*	2.98±0.06[cd]	2.97±0.06[d]	-0.3
5 DAI	10.55±0.08[ab]	10.28±0.25[de]	-2.5	2.85±0.03[de]	3.10±0.11[bcd]	8.7
7 DAI	10.68±0.14[a]	10.42±0.09[cde]	-2.4*	2.72±0.07[e]	3.08±0.03[cd]	13.1*
9 DAI	10.37±0.12[abcd]	10.16±0.08[e]	-2.1	3.17±0.10[bc]	3.58±0.01[a]	13.1*
11 DAI	9.88±0.09[e]	10.39±0.15[cde]	5.1	2.77±0.09[de]	2.98±0.09[cd]	7.9

[1]Means followed by the same letter within a column are not significantly different at *P*>0.05 based on Tukey's$_b$ test (n=3).

[2]Significant differences (paired t-test) between healthy and infested plants (n=3) are indicated by single (*P*< 0.05, significant) and double (*P*<0.01, highly significant) asterisks; no asterisk means not significant; negative values indicate higher TPC of control.

[3]DBI = days before larva introduction; DI =day of introduction; DAI = days after introduction

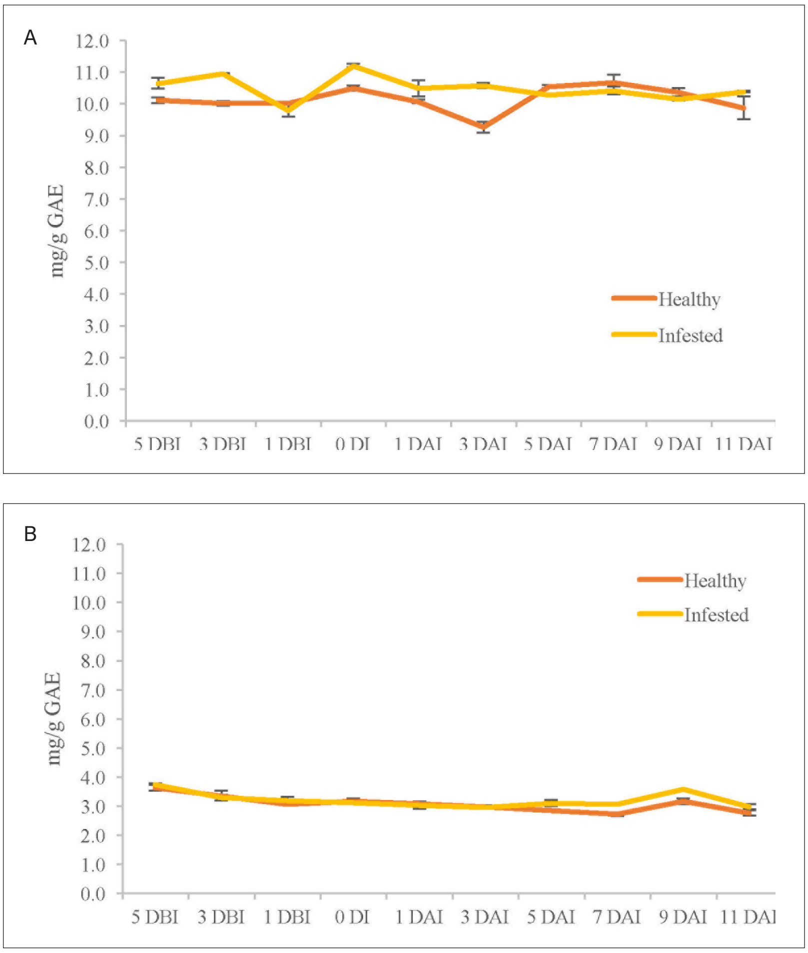

Figure 2. Total phenolic content of (A) leaf and (B) stem of healthy and YSB-infested TN1 plants

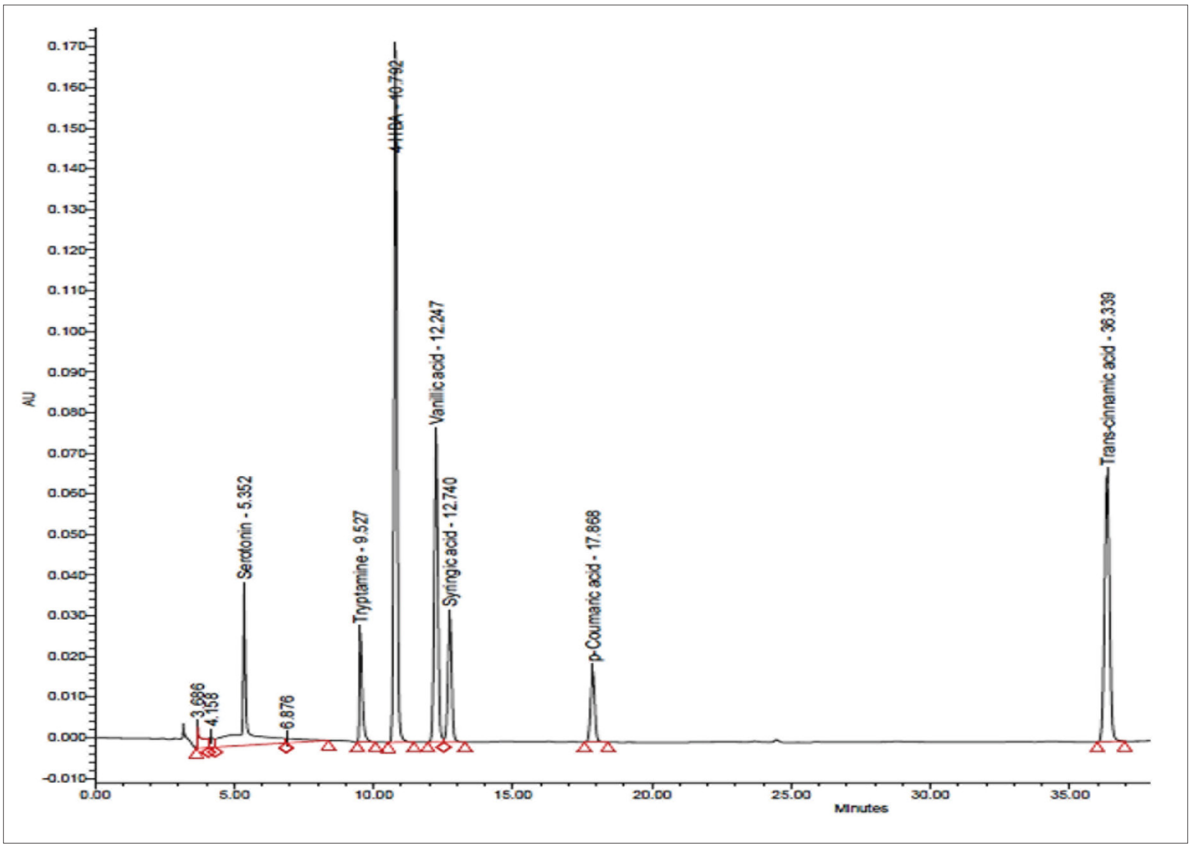

Figure 3. HPLC chromatogram of phenolic standards (100 ppm) at 254 nm: serotonin, tryptamine, 4-hydroxybenzoic acid, vanillic acid, syringic acid, p-coumaric acid, and trans-cinnamic acid.

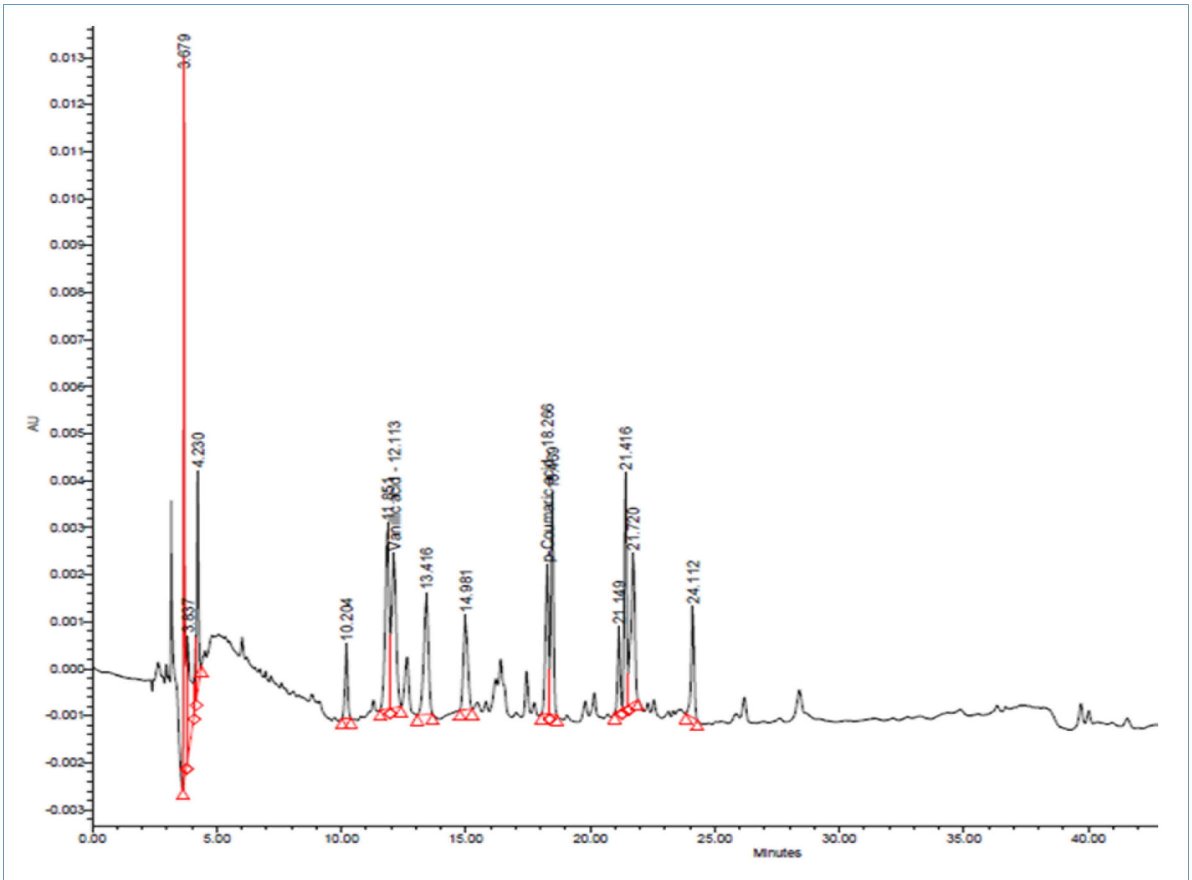

Figure 4. HPLC chromatogram of TKM6 extract (20 df) at 254 nm.

Generally, there were higher TPC in the leaves of both TKM6 and TN1 than in their stems (Tables 3 and 4). These same results were also observed in stemborer-infested resistant and susceptible maize varieties (Praveen et al., 2013) and in another study looking at the TPC in the leaves and stems of *Monsonia burkeana* (Mamphiswana et al., 2010). Surprisingly, TPC of the susceptible TN1 was generally higher than that of the resistant TKM6, both for the infested leaves and stems. It is hypothesized that the resistant genotypes have higher TPC than the susceptible. In contrast, the results obtained in the current study is different from the results of Praveen et al. (2013) on maize varieties and Punithavalli et al. (2013) on rice genotypes against leaf folder. With lower TPC than the susceptible, TKM6 showed promising significant phenolics-induced defense mechanism. This mechanism may hint on the possible biochemical mechanism of resistance of TKM6 amidst biological enemy such as YSB larvae, which can be explored in succeeding studies. In contrast, TN1 had high TPC even before larvae introduction; thus, the slight increase in TPC may not be necessarily attributable to YSB infestation but to other biological stress, which the plant detected during the experiment.

LC Conditioning and Adjustments for Identification of Peaks and Compounds

Different mobile phase system and column temperatures were tested to determine the optimal chromatographic conditions to separate analytes. The peak of serotonin standard tends to elute very early while the peaks of trans-cinnamic acid tend to elute very late, and often does not appear using other elution conditions. Some peaks also tend to co-elute, which is probably due to the same retention properties, such as in the case of vanillic acid and syringic acid. Peak of trans-cinnamic acid was observed, and peaks of vanillic acid and syringic acid were slightly separated at 254 nm wavelength using the optimized mobile phase system used (Table 5).

Table 5. Optimized mobile phase system.

Minutes	% Absorbance	% Concentration
0	95	5
5	85	15
10	85	15
20	75	25
30	75	25
35	60	40
45	75	25
50	95	5
55	95	5

Identification of Peaks in Standards and Leaf Extracts

In RP-HPLC, polarity of phenolic compounds determines the elution order with the most polar one eluting first followed by the less polar ones (Zhang et al., 2013). Most polar compounds eluting first were serotonin (5.35 min), followed by tryptamine (9.53 min), 4 hydroxybenzoic acid (10.79 min), vanillic acid (12.25 min), syringic cid (12.74 min), p-coumaric acid (17.87 min), and lastly, trans-cinnamic acid (36.34 min) (Figure 3).

Vanillic acid (12.113 min) and p-coumaric acid (18.266 min) were detected at 254 nm wavelength in the diluted TKM6 extract (Figure 4). It is plausible that these phenolics found in TKM6 may be among the biochemical natures for its resistance against YSB. Both vanillic and p-coumaric acid are well-studied phenolics in relation to plant resistance and insect herbivory. In a study conducted on castor plant by Usha Rani and Jyothsna (2014), vanillic acids delayed the egg laying of *Achaea janata* L. and *Spodoptera litura* F. On the other hand, a decrease in *A. janata* and *S. litura* larval body weights was evident in coumaric acid treatments confirming the antifeedant effects of the phenolics tested. In maize, the amount of free p-coumaric acid was correlated with the resistance level. Higher quantities of p-coumaric in the pith could contribute to general resistance to stemborer attack. Jointly with ferulic acid, p-coumaric acid could provide resistance mechanisms through cell wall fortification and lignification. The vanillic acid showed a decreased tendency after silking, when maize is most attractive for *Sesamia nonagrioides*, suggesting this acid could act as a chemoattractant for *S. nonagrioides* larvae or adults (Santiago et al., 2005). The dietary exposure to vanillic acid led to enhanced activities of detoxifying enzymes, β-glucosidase, carboxyl esterase, glutathione S-transferase, and glutathione reductase in the midgut tissues of all the larval instars of *Spodoptera litura*, indicating the toxic nature of these compounds in *Capsicum annum*.

Conclusions and Recommendation

This study shows the importance of evaluating the chemical mechanism of resistance in rice against stemborer using analytical techniques in acquiring qualitative and quantitative information from secondary metabolites. Preliminary results showed potential in revealing the mechanism of resistance of rice plant against pests. As shown in resistant rice genotype, TKM6, increase of phenolics may be the plant's plausible induced-defense mechanism as a response to YSB infestation. The induced response of resistant and susceptible genotype to

YSB varied in terms of released phenolic compound. The resistant genotypes exhibited multiple defense mechanism in terms of higher expression of phenolic compound particularly when plants perceived threat following YSB infestation. Moreover, the optimized biochemical screening method from this study, in addition to existing breeding methods, can aid in the selection of materials to be used in the development of lines/variety that are resistant to stemborer in particular. However, correlation of results with the parallel studies on morphological (trichome density, orientation, and distribution) and physiological factors (lignin thickness and content) is necessary to explain the ways rice cope and to provide a complete picture plants protecting themselves from insect pest infestation such as stemborer.

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FULL PAPER

ROOT DEVELOPMENT AND YIELD RESPONSES OF RICE TO APPLICATION OF COMBINED INORGANIC AND ORGANIC FERTILIZERS UNDER DROUGHT

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Abstract

Rice production is negatively affected by nutrient deficiency and drought stress caused by climate change. The application of nitrogen (N) fertilizer can mitigate the effect of drought stress; however, ensuring environmental sustainability remains a challenge. Greenhouse experiment was conducted to assess the impact of different water continuously waterlogging (CWL) and progressive drought (DRT) and fertilizer treatments [no fertilizer, NF; inorganic fertilizer, IF (120-60-60 kg ha^{-1}); organic fertilizer, OF (10 t ha^{-1} vermicompost); and combination of inorganic and organic fertilizers, IF/OF (60-30-30 kg ha^{-1} + 5 t ha^{-1} vermicompost) on the root system development and yield of NSIC Rc 226. Results showed that shoot dry weight (SDW) of IF/OF under DRT at maximum tillering (MT) and maturity stage (MS) was significantly highest among fertilizer treatments. Highest grain yield (GY) and root system development based on total root length were obtained from both the IF and IF/OF, regardless of water treatments. The relationships between root traits with SDW at MT and MS, and with GY were both positive and significant. This indicates that dry matter production and GY were strongly dependent on root growth and development as influenced by fertilizer application using either IF/OF or IF especially under water-limited conditions. Compared with using pure inorganic fertilizer (IF alone) especially under DRT, substituting a portion of inorganic fertilizer requirements with organic fertilizer could reduce water requirements by 5.71 (30.1%) without yield penalty.

Keywords: *Continuously Waterlogging, Progressive Drought, Inorganic, Organic Fertilizer.*

Introduction

Rice is one of the most important food grains in the world, especially in developing countries. It is geographically distributed in North and South America, and Asian countries like Japan, Korea, Bangladesh, India, Malaysia, Pakistan, Vietnam and Philippines providing 35 - 75% of the calories consumed by more than 3 billion Asians (Khush, 2005). A rice crop requires 1,000 - 3,000 mm of water from a combination of irrigation and rain (Rice Knowledge Bank, 2009). In developing countries, 85% of their available water is used for irrigation in lowland rice cultivation (Amin et al., 2011). However, nearly half the global population are already experiencing water scarcity for at least a month per year brought about by the combination of increase in competition for water demand in agriculture and industries, and climate change. About 73% of the affected people live in Asia (Burek et al., 2016) where more than half of rice world supply is produced. This water shortage is responsible for the greatest crop losses globally and are expected to worsen as water scarcity looms.

Modern agriculture is a big contributor to global warming that alters normal climate patterns. During Green Revolution in the 1960, yield improvements in staple crops such as wheat, corn, and rice helped saved hundreds of millions of lives from hunger through luxurious use of inorganic fertilizers and pesticides (Pingali, 2012). As a consequence, loss of soil fertility, soil erosion, soil toxicity, diminishing water resources, pollution and salinity of underground water, increased incidence of human, and livestock diseases have been experienced as the negative impacts of over adoption of modern agricultural technologies (Rahman, 2015).

In an effort to address the need of the ever-growing human population without compromising the environment, it is necessary to practice sustainable agriculture by producing safe and healthy food, conserving natural resources and protecting biodiversity, ensuring animal welfare, and improving quality of life in farming areas (European Commission, 2012).

Reclaiming and improving the physical, biological, and chemical properties of the soil can be done through application of organic materials to sustain the overall soil health status. Vermicompost is an excellent organic fertilizer as it provides good aeration and water-holding capacity, good drainage and improved status of mineral nutrients, and biological properties of the soil (Atiyeh et al., 2002; Pant et al., 2011; Joshi et al., 2015). It contains humic acids that bind to water and nutrients helping plants to absorb them efficiently by increasing the number and length of root (Meléndrez, 2017) under nutrient and water-limited environments.

The effect of water deficit on rice has negative impact on plant growth and development, especially during flowering, grain-filling, and maturity stage (Zhang et al., 2018). The plant root system should be fully developed before early season drought affects early growth stages of the rice crop, particularly in direct seeding. This can be associated with seedling vigor and root to shoot ratio (Mackill and Redona, 1997) and increased rooting depth, root density, root pulling force, and penetration ability through hardpans (Uyprasert et al., 2004).

It is essential that mechanisms employed by single or integrated use of inorganic and organic fertilizers to rice in relation to shoot and root system development must first be thoroughly understood to improve tolerance and plant performance under drought conditions. An increased understanding of the underlying mechanisms will lay the foundation for future efforts on rice breeding and agronomic management. In this study, it was hypothesized that the use of organic fertilizer as partial substitute for inorganic fertilizer requirements would maintain root growth and development during vegetative drought and hence maintain yield even under drought. Hence, it aimed to quantify the effect of single and combined use of inorganic and organic fertilizers on root system development, water use, growth, and yield of rice under drought.

Materials and Methods

Experimental Conditions, Design, and Treatments

The study was conducted in the greenhouse at the Philippine Rice Research Institute-Central Experiment Station (PhilRice-CES), Muñoz, Nueva Ecija, Philippines (15°40'N, 120°53'E, 57.6 MASL) from October 2019 to January 2020. During the growing period, the average minimum and maximum temperature was 28.2°C and 42.9°C, respectively. The average daily solar radiation, minimum and maximum temperatures, and relative humidity were 15.5 MJ m^{-2}, 23.1°C, 32.5 and 75.6%, respectively.

The experiment was laid out in a split-plot design arranged in randomized complete block design (RCBD) with six replications. The water treatments were assigned as main plot while the fertilizer treatments as sub plot. The water treatments were continuously waterlogged (CWL, control) and progressive drought (DRT) conditions. On the other hand, fertilizer treatments include no fertilizer applied (NF, control), inorganic fertilizer (IF), organic fertilizer (OF), and combined IF and OF (IF/OF).

Cultural Management and Imposition of Treatments

NSIC Rc 226 (Tubigan 20), a high yielding irrigated lowland rice variety under inorganic and organic system under both dry and wet seasons (Manigbas et al., 2018) was used in this study. This variety is moderately resistant to brown plant hopper and green plant hopper but susceptible to blast, tungro virus and bacterial leaf blight (Pinoy Rice Knowledge, 2019).

The seeds were soaked overnight for 12 h in tap water, drained and incubated at 28°C for 24 h prior to sowing. Three pre-germinated seeds were sown in each 10 L-capacity plastic pot (22.5 x 25 x 20 cm top and bottom diameter) filled with 8 kg of dry sieved loamy soil. The detailed chemical analysis is shown in Table 1. The seedlings were thinned to one seedling per pail 3 days after sowing (DAS).

Water Treatments

The plants were subjected to either CWL or DRT conditions. SMC was maintained at 43% which approximately has water level of 5 cm above soil surface from 3 DAS to a week before harvesting. In DRT, the SMC was also maintained at 43% to allow optimal growth of seedlings from 3 to 14 DAS. Thereafter, watering was withheld to impose progressive soil drying. The soil was allowed to dry down to 12% SMC and maintained in that level until 40 DAS. The target SMC of each treatment was maintained by adding the equivalent amount of water lost through evapotranspiration daily by gravimetric method following the formula:

$$\text{SMC} = \frac{(\text{FWsoil (kg)-DWsoil(8 kg)})}{(\text{DWsoil (8 kg)})}$$

where:

SMC- Soil Moisture Content (%)

FW- Fresh Weight

DW- Dry Weight

At maximum tillering (40 DAS), three replications from each treatment combination were sampled for plant growth measurements while the remaining three replications were continued to grow until maturity under waterlogged conditions.

Fertilizer Treatments

Plants were either subjected to no fertilizer application (NF) or fertilized using pure inorganic fertilizer (IF), pure organic fertilizer (OF) or combined IF and OF (IF/OF). The sources of inorganic fertilizer used were urea (46-0-0, NPK), complete fertilizer (14-14-14, NPK) while that of organic fertilizer was vermicompost. The chemical analysis of vermicompost are shown in Table 1.

Table 1. Chemical analysis of the soil medium and vermicompost used in this study. Analyses were done at the Central Analytical Services Laboratory (CASL), Philippine Root Crops Research and Training Center, Visayas State University, Visca, Baybay City, Leyte.

Parameters		Garden Soil	Vermicompost
pH (1:2:5)		7.94	8.14
OM (%)		0.87	17.38
Total N (%)		0.08	0.79
Available P (mg kg^{-1})		24.13	0.13
Exchangeable (mg kg^{-1})	K	189.01	789.13
	Na	148.53	291.10
	Ca	2948.69	58.70
	Mg	663.18	1170.93
Extractable (mg kg^{-1})	Fe	31.81	2090.95
	Mn	22.00	448.78
	Cu	2.78	32.95
	Zn	2.02	80.70
CEC		5.33	-
Texture Class		loamy sand	-

For IF treatment, basal fertilizer was applied at the rate of 120-60-60 kg ha^{-1} N, P_2O_5, K_2O (1.46 g 14-14-14 and 0.44 g Urea pot^{-1}). In OF treatment, pots were only fertilized with vermicompost at the rate of 10 t ha^{-1} (2.27 kg pot^{-1}). In IF/OF treatment, ½ of those in the IF treatment (60-30-30 kg ha^{-1} N, P_2O_5, K_2O) plus ½ of those in the OF application and combined with ½ of and organic fertilizer rates (5 t ha^{-1} or 1.13 kg pot^{-1}). The vermicompost was mixed thoroughly into the soil medium to prevent damage on the seeds.

Shoot and Root Growth, and Yield Measurements

At 40 DAS (maximum tillering) plants from three replications of each treatment combination were sampled for various measurements. The number of tillers per plant was counted and recorded. The leaves were separated from the stems and their area was measured using leaf area meter (Li-300, LiCOR Inc., Lincoln, Nebraska, USA). Thereafter, the leaves and stems were oven-dried together at 70°C for 3 days before weighing. The root samples were carefully extracted and washed with water to remove dirt. The roots were stored in 95% ethyl alcohol prior to measurements. The total number of nodal roots were counted. The cleaned individual root systems were scattered uniformly in 5 mm water in a 30 x 20 cm clear glass tray. The total root length (TRL) was analyzed using scanned images of the roots at 600 dpi (EpsonNv700 Perfection) through the WhinRhizo v. 2007d (Régent Instruments, Québec, Canada) software. A pixel threshold value of 230 was set for the analysis. The lateral root length (LRL) was computed as the difference between the TRL and total nodal root length (TNRL). After scanning, the roots were oven-dried at 70°C for 3 days and weighed.

At maturity, the remaining three replications from each treatment combination were harvested. The number of tillers and panicles were counted. The stem and leaves were oven-dried shoots at 70°C for 3 days to record the shoot dry weight. The number of filled and unfilled spikelets were separated and counted. The 1000 grain weight was weighed and adjusted to 14% moisture content. The total grain weight was also weighed and values were adjusted to 14% moisture content.

Statistical Analysis

The data were subjected to two-way ANOVA using Statistical Tool for Agricultural Research (STAR, version 2.0.1. January 2014). The means were compared by the least significant difference (LSD) at 0.05 probability.

Results and Discussion

Yield and Yield Components Responses of NSIC Rc 226 to Drought and Fertilizer Treatments

The ANOVA showed no significant interaction between water and fertilizer treatments on grain weight, number of tillers, number of panicles, total number of spikelets, number of filled spikelets, and unfilled spikelets per panicle (Table 2). Grain weight was significantly affected by fertilizer treatments only. Regardless of water treatments, application of fertilizers generally had significantly higher grain weight than the NF control. Both IF and IF/OF treatments had comparable grain weight. However, IF had significantly higher grain weight than OF while IF/OF had comparable yield. Elhabet (2018) also showed comparable yield between those applied with 4.76 t ha^{-1} composted rice straw + 110 kg N ha^{-1} and applied with recommended dose of inorganic N at 165 kg ha^{-1}. However, those applied with 4.76 t ha^{-1} composted rice straw + 110 kg N ha^{-1} had resulted in savings of one third of inorganic N. Furthermore, Adhikari et al. (2018) showed comparable grain yield between recommended dose of inorganic fertilizers (i.e 250, 126, 120, 100 and 10 kg ha^{-1} N, P, K, -S, and Zn, respectively) and recommended dose of inorganic fertilizer + 5 t ha^{-1} vermicompost. The recommended fertilizer rate is important to ensure maximum yield under drought-prone rainfed lowland rice fields (Linquist and Sengxua, 2001; Inthavong et al., 2014).

The number of tillers per plant was significantly affected by fertilizer treatment although it was relatively lower in DRT than in CWL though not statistically significant (Table 2). Under both water treatments, the number of tillers at maturity was significantly higher in fertilized treatments than NF control. Among fertilized treatments, no significant differences were observed. The number of panicles in the fertilized treatments were also significantly higher than in the NF control, regardless of water treatments. However, IF/OF treatments showed significantly higher number of panicles than either IF or OF alone. Maximizing the tillering potential is key to maintaining higher yield in rice via increase in number in panicle bearing tillers and other yield components (Badshah et al., 2014). Under vegetative stage drought, maximizing the number of tillers could be done by improving root system developmental responses to the condition through genetic improvement and/or agronomic management (Suralta et al., 2016). Sumon et al. (2018) showed that panicle bearing tillers per hill can be maximized by applying either with 80% recommended dose of N, P, K, S, and Zn + 3.5 t ha^{-1} green manure or with full recommended doses of N, P, K, S, and Zn or using System of Rice Intensification (SRI). The SRI is a system, which aims to improve soil conditions through the enrichment with organic matter (Stoop et al., 2002).

Furthermore, the average number of spikelets, number of filled and unfilled spikelets per panicle was not significantly affected by either water or fertilizer treatments (Table 2). Heading stage drought can markedly reduce the number of spikelets per panicle (Yang et al., 2019) and filled grains (Pandey et al., 2014; Zhang et al., 2018; Yang et al., 2019), resulting in a significant decrease in grain yield (Zhang et al., 2018; Yang et al., 2019). The reduction in filled grain under post flowering drought is related to the reduction in assimilate partitioning and activities of sucrose and starch enzymes (Anjum et al., 2011) as a consequence of a decrease in photosynthesis (Xu et al., 2020). In this study, however, drought was imposed at pre-heading stage and subjected to continuous waterlogging thereafter; hence, spikelet fertility under DRT was generally not affected. The increase in grain yield brought about by fertilizer applications under DRT was clearly due to the differences in number of tillers and panicle bearing tillers per plant (Table 2) which was generally higher in fertilized treatments than in NF control.

Shoot Growth Responses of NSIC Rc 226 to Drought and Fertilizer Treatments at Maximum tillering

There was a significant interaction between water and fertilizer treatment on shoot dry weight, leaf area, and stomatal conductance while it was not significant on the number of tillers (Table 3). Under CWL, shoot

Table 2. Yield and selected yield components of NSIC Rc 226 grown under various water and fertilizer treatments.

Water (W) / Fertilizer (F)	Grain Yield (g plant^{-1})	Tiller (no. plant^{-1})	Panicle (no. plant^{-1})	Spikelets (no. panicle-^{1})	Filled Spikelets (no. panicle^{-1})	Unfilled Spikelets (no. panicle^{-1})
Continuously waterlogged (CWL)						
NF	4.02^{bc}	5^{b}	5.1^{cd}	99.5^{ab}	36.7^{a}	62.7^{ab}
IF	10.16^{a}	9^{a}	10.0^{abc}	117.9^{a}	43.3^{a}	74.5^{a}
OF	6.89^{abc}	10^{a}	7.7^{bcd}	90.7^{ab}	39.4^{a}	51.3^{abc}
IF/OF	8.26^{ab}	11^{a}	13.3^{a}	72.5^{b}	26.9^{a}	45.6^{bc}
Progressive drought (DRT)						
NF	2.59^{c}	3^{c}	2.8^{d}	72.0^{b}	37.7^{a}	34.3^{c}
IF	7.22^{abc}	7^{a}	8.0^{abc}	93.0^{ab}	37.8^{a}	55.2^{abc}
OF	5.79^{abc}	7^{a}	8.3^{abc}	89.9^{ab}	31.6^{a}	58.2^{abc}
IF/OF	7.19^{abc}	9^{a}	11.3^{ab}	84.3^{b}	29.6^{a}	54.6^{abc}
W	ns	ns	ns	ns	ns	ns
F	**	**	**	ns	ns	ns
W x F	ns	ns	ns	ns	ns	ns

NF – no fertilizer applied, IF – inorganic fertilizer alone, OF – organic fertilizer alone, IF/OF- combined inorganic/organic fertilizer. In a column, means followed by the same letter(s) are not significantly different at 5% level by LSD; * and **, significant at 5 and 1% level, respectively; ns, not significant.

dry weight was highest in IF while under DRT, it was significantly highest in IF/OF treatment (Table 3).

Kobata et al. (1996) and Suralta et al. (2010) showed that dry matter production is a function of water use (WU), especially under water-limited conditions. The shoot dry weight in IF/OF fertilizer under DRT was significantly highest than other treatments. The total dry matter production in field applied with inorganically fertilizer with readily available NPK is normally higher than that applied with organic fertilizer (Chhogyel et al., 2015; Iqbal et al., 2019).

The number of tillers per plant was significantly affected by both water and fertilizer treatments (Table 3). Vegetative stage drought significantly reduced tillering ability in rice (Mostajeran and Rahimi-Eichi, 2009) due to reduced stomatal conductance, transpiration, and photosynthesis (Xu et al., 2020) that limits assimilate production for growth and development. Regardless of water treatments, IF and IF/OF had comparable tillering ability, which was significantly higher than other fertilizer treatments (Table 3).

The leaf area was significantly affected by both water and fertilizer treatments (Table 3). Drought significantly decreased the leaf area. Farooq et al. (2010) observed a reduced leaf area in IR64 and four of its near-isogenic lines unique for leaf size traits (long, broad, short, and narrow leaves) under progressive soil drying. Reduction in leaf area, either through size reduction, shedding or death of leaves, is one of the morphological changes to a plant under drought which could be due to impaired cell division and plant water relations, and limited photosynthesis as a result of declined Rubisco activity (Bota et al., 2004). Under CWL, IF had the significantly highest leaf area while under DRT, IF/OF had the significantly highest leaf number. This might be due to inorganic fertilizer that readily provided nutrients unlike the organic fertilizer, which needs to undergo decomposition process before a slow and steady release of nutrients occur (Chhogyel et al., 2015; Elhabet, 2018; Roba, 2018).

Dynamics in Daily Water Use and Cumulative Water Use of NSIC Rc 226 to Drought and Fertilizer Treatments at Maximum Tillering and Maturity

Under CWL, daily water use was generally higher in fertilized treatments than in NF (Figure 1). Similarly under DRT, fertilized treatments had generally higher daily water use and thus, reached 12% SMC one day earlier than NF. This result indicates that fertilized plants had greater ability to take up water from the drying soil, which is directly related to the size of their root system as will be discussed in the succeeding section.

The total water use or the whole plant transpiration during vegetative stage was calculated from 14 to 40 DAS (maximum tillering) when plants were grown either under continuous waterlogging or progressive

Table 3. Shoot growth and stomatal conductance and water use at maximum tillering (40 DAS) of NSIC Rc 226 as affected by various water and fertilizer treatments.

Water (W) / Fertilizer (F)	Shoot Dry Weight (g plant^{-1})	Tiller (no. plant^{-1})	Leaf Area (cm^2 plant^{-1})
Continuously waterlogged (CWL)			
NF	2.10^{de}	6.0^{bcd}	318.36^{c}
IF	5.00^{a}	10.0^{a}	568.67^{a}
OF	2.72^{c}	7.0^{abc}	345.28^{c}
IF/OF	3.43^{b}	9.0^{ab}	415.56^{b}
Progressive drought (DRT)			
NF	0.89^{g}	3.0^{d}	52.29^{e}
IF	2.05^{ef}	7.0^{abc}	223.84^{c}
OF	1.47^{fg}	5.0^{cd}	184.93^{d}
IF/OF	2.66^{cd}	8.0^{abc}	347.36^{c}
W	**	*	**
N	**	**	**
W x F	**	ns	**

NF – no fertilizer applied, IF – inorganic fertilizer alone, OF – organic fertilizer alone, IF/OF- combined inorganic/organic fertilizer. In a column, means followed by the same letter(s) are not significantly different at 5% level by LSD; * and **, significant at 5 and 1% level, respectively; ns, not significant.

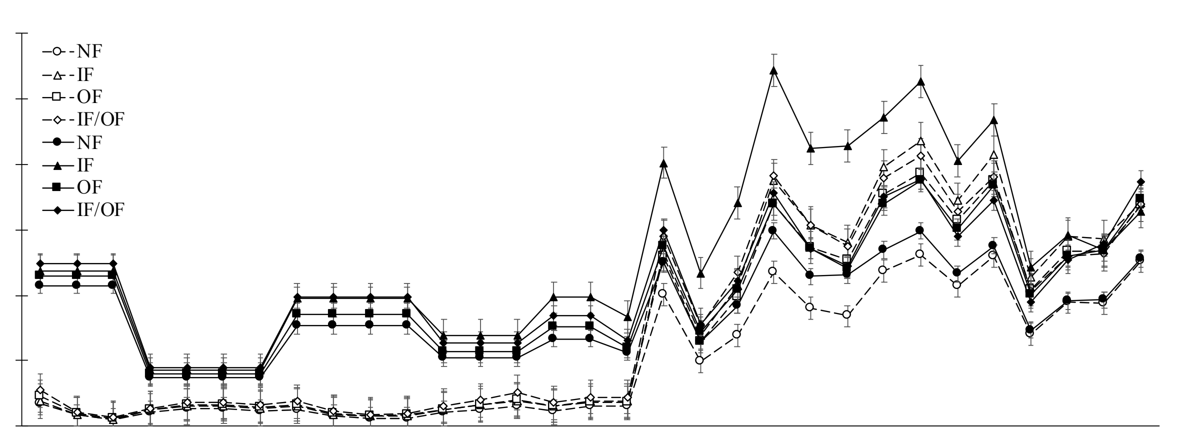

Figure 1. Daily water use of NSIC Rc 226 grown under different water and fertilizer treatments. ○, NF – no fertilizer applied; Δ, IF – inorganic fertilizer alone; ○, OF – organic fertilizer alone; ◊, IF/OF – combined inorganic and organic fertilizer. Continuously waterlogged (CWL, shaded) and progressive drought (DRT, non-shaded).

drought conditions (Table 3). The ANOVA showed no significant interactions on the cumulative water use at maximum tillering (40 DAS) and between maximum tillering to maturity and the overall total water use (Table 3). The water use in both stages as well as the total water used were significantly affected by fertilizer treatments (Table 3).

Regardless of water regimes, water use at vegetative stage was similar for all fertilizer treatments except IF under CWL (Table 3). Furthermore, water use from maximum tillering (40 DAS) to maturity as well as total water use at maturity were significantly highest in IF under CWL while the fertilized treatments had generally higher water use than NF under DRT (Table 3). The grain yield between IF and IF/OF fertilizer treatments with 1.9 and 0.03 g difference under CWL and DRT conditions, respectively, were not significantly different (Table 1). Hence, this study indicates that substituting a portion of inorganic fertilizer with organic fertilizer similar to that in IF/OF treatment could reduce water requirements by 5.71 (30.1%) and 0.08 (0.54%) in CWL and DRT conditions, respectively, without yield penalty.

Root System Developmental Responses of NSIC Rc 226 to Drought and Fertilizer Treatments at Maximum Tillering

The ANOVA showed no significant interaction between water and fertilizer treatments on total nodal root length (TNRL), total lateral root length (TLRL), and total root length while it was significant on nodal root production (Table 4). Drought generally reduced nodal root production relative to CWL regardless of fertilizer treatments (Table 4). The number of nodal roots were significantly highest in IF under CWL while it was highest in IF/OF under DRT indicating a positive influence of vermicompost on vegetative growth, stimulating shoot growth and root development when combined with inorganic fertilizer (Edwards et al 2004).

The TNRL, TLRL and TRL were generally reduced by DRT relative to CWL condition and regardless of fertilizer treatments (Table 4). Under CWL, TNRL, TLRL and TRL were significantly highest in IF while under DRT, they were higher in IF and IF/OF than in either NF and OF. These results suggest that nodal root development was more susceptible to drought than the root traits. Furthermore, vegetative stage drought can be markedly affected root system development in rice genotypes with poor root plasticity (Suralta et al., 2008). Root plasticity responses are key during vegetative-stage water stress and a major determinant for overall plant performance and yield (Suralta et al., 2016). Root system developmental responses to vegetative stage drought can be enhanced with N application; thereby, improving the maintenance of higher dry matter production under the condition (Tran et al., 2014) and in this study.

Relationship Among Above- and Below-Ground Parameters of NSIC Rc 226 as influenced by Drought and Fertilizer Treatments

The reduction in the dry matter can be related to the reduced leaf area causing slower photosynthetic rate that resulted in limited assimilates under drought (Mostajeran and Rahimi-Eichi, 2009). These differences in leaf area under CWL and DRT conditions among fertilizer treatments showed a strong positive linear relationship with shoot dry weight at maximum tillering (r= 0.77 and 0.79, respectively at P>0.01) as well as with grain weight (r= 0.89 and 0.72, respectively at P>0.01). Dry matter production under drought would contribute significantly to the final grain yield (Kobata et al., 1996; Saikumar et al., 2016). In this study, the relationship between shoot dry weight at maximum tillering and maturity stage with grain weight was strong and positive under both water treatment (r=99 and 0.77, respectively at P>0.01. Saikumar et al. (2016) also observed a reduction of grain yield due to reduction in shoot dry weight ranging from moderate to very severe drought.

Dry matter production is a function of water use, especially under water-limited conditions (Kobata el al 1996; Suralta et al 2010). The relationships between water use at maximum tillering with number of tillers per plant (CWL, r= 0.86, P>0.01; DRT, r=0.87, P>0.01) and shoot dry weight at maximum tillering (CWL, 0.79, P>0.1; DRT, r= 0.93, P>0.01) were significant. These results suggest that IF/OF treatment enabled plants to use water more efficiently under drought. The response of lateral root development to moisture stress determines plant growth and responsible for the amount of water and nutrient absorbed by the whole root system (Bañoc et al., 2000; Suralta et al., 2008); thus, the increase in TLRL as a function of fertilizer application enhanced TRL under CWL and DRT (r=0.99, P>0.001) treatments. Plants under drought

Table 3. Cumulative water use at maximum tillering (40 DAS), from maximum tillering to maturity and total water use of NSIC Rc 226 grown under continuous waterlogging (CWL) and progressive drought (DRT) conditions and applied with different sources of fertilizers.

Water (W) / Fertilizer (F)	Water Use at Maximum tillering (40 DAS) (kg plant^{-1})	Water Use from Maximum tillering to Maturity (kg plant^{-1})	Total Water Use (kg plant^{-1})
Continuously waterlogged (CWL)			
NF	0.44^{b}	8.7^{cd}	9.2^{cd}
IF	0.67^{a}	18.3^{a}	19.0^{a}
OF	0.48^{b}	12.0^{bcd}	12.5^{bcd}
IF/OF	0.52^{ab}	12.7^{bcd}	13.2^{bcd}
Progressive drought (DRT)			
NF	0.15^{c}	8.1^{d}	8.2^{d}
IF	0.20^{c}	14.7^{ab}	14.9^{ab}
OF	0.21^{c}	13.2^{bc}	13.4^{bc}
IF/OF	0.26^{c}	14.6^{ab}	14.8^{ab}
W	ns	ns	ns
N	*	**	**
W x F	ns	ns	ns

NF – no fertilizer applied, IF – inorganic fertilizer alone, OF – organic fertilizer alone, IF/OF- combined inorganic/organic fertilizer. In a column, means followed by the same letter(s) are not significantly different at 5% level by LSD; * and **, significant at 5 and 1% level, respectively; ns, not significant.

Table 4. Root system developmental responses of NSIC Rc 226 to different water and fertilizer treatments at maximum tillering (40 DAS).

Water (W)/ Fertilizer (F)	Nodal Roots (no. plant^{-1})	Total NRL (cm plant^{-1})	Total LRL (cm plant^{-1})	TRL (cm plant^{-1})
Continuously waterlogged (CWL)				
NF	83.9^{d}	4685.44^{bc}	17577.40^{bc}	22262.84^{bc}
IF	167.2^{a}	7188.15^{ab}	29197.74^{ab}	36385.88^{ab}
OF	123.0^{c}	5079.43^{abc}	16615.54^{c}	21694.97^{c}
IF/OF	157.2^{b}	8714.42^{a}	30479.53^{a}	39193.95^{a}
Progressive Drought (DRT)				
NF	25.3^{f}	2419.00^{c}	6665.65^{d}	9084.65^{d}
IF	37.2^{f}	4874.50^{abc}	11033.48^{c}	15907.98^{c}
OF	48.3^{f}	2089.16^{c}	5611.03^{d}	7700.20^{d}
IF/OF	58.3^{e}	4234.90^{bc}	11543.88^{c}	15778.78^{c}
W	**	*	**	**
F	**	**	**	**
W x F	**	ns	ns	ns

NF – no fertilizer applied, IF – inorganic fertilizer alone, OF – organic fertilizer alone, IF/OF- combined inorganic/organic fertilizer. NR – nodal root, NRL – nodal root length, LRL – lateral root length, TRL – total root length. In a column, means followed by the same letter(s) are not significantly different at 5% level by LSD, * and **, significant at 5 and 1% level, respectively; ns, not significant.

with limited water use would often result in reduced leaf area; thus, limiting interception of solar radiation, leading to reduced dry matter production (Table 6) and ultimately, low productivity (Sinclair and Muchow, 2001).

The TRL had positive and significant relationship with shoot dry weight at vegetative stage (CWL, r=0.60, P>0.05, DRT, r=0.74, P>0.01), while TRL at vegetative stage had a highly significant and moderate positive relationship with shoot dry weight at maturity (CWL, r=0.60, P>0.05, DRT, r=0.74, P>0.01) and grain weight (Table 5). These results indicate that increments in dry matter production and rice yield are directly and strongly dependent on root growth and development especially under water-limited conditions. Mohankumar et al. (2011) and Iqbal et al. (2019) also showed that root traits of rice at tillering stage *viz.* total root length significantly influenced the yield and total shoot dry weight. Plasticity in total root length, lateral root length and branching have been linked to improved shoot dry weight, water and nutrient uptake, and photosynthesis under drought (Suralta, 2010; Kano-Nakata et al., 2011; Tran et al., 2014).

The number of panicles per plant had significant and positive relationship with total number of filled grains under CWL (r=0.86, P>0.01) and DRT (r=0.98, P>0.001); and hence, significantly increased grain weight under CWL (r= 0.86, P>0.01) and DRT (r=0.96, P>0.001), respectively. Lanceras et al. (2004) also showed that the increase in number of panicles produced significantly increased grain yield only under well-watered and mild drought but not under severe drought. The increase in number of panicles was positively influenced by the number of tillers produced during vegetative stage drought (r=0.99, P>0.001) brought about by fertilizer applications.

Conclusion and Recommendations

The application of pure inorganic and the combination of both inorganic and organic fertilizer produced better root system development in terms of total root length, which in turn increased the water use of rice plants from the drying soil. Consequently, high water use contributed significantly to dry matter production and ultimately, grain yield through increased total number of filled grains per plant, panicle number per plant, tiller number per plant at vegetative growth stage, and number of productive tillers per plant at maturity. Depending on the inherent nutrient supply of the soil, combination of organic and inorganic sustains the optimum growth and yield performance of rice while keeping a healthy soil.

As the response of rice plant under IF and IF/OF were comparable, further study is needed with the same set of treatments under field conditions. Along term experiment may be conducted to determine whether the continuous use of organic fertilizer in IF/OF will have positive results on yield.

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FULL PAPER

BOOSTING THE ENTREPRENEURIAL SKILLS OF FARMERS FOR A SUSTAINABLE COMMUNITY ENTERPRISE

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Abstract

This research aimed to develop farmers become sustainable hyperlocal wealth-creators through community enterprise development (CED). The development of a rice-based enterprise started with a focus group discussion (FGD) among 15 farmer-leaders (60% male, 40% female) of a group in Macarse, Zaragoza, Nueva Ecija, Philippines. The participants discussed and generated their own product and marketing ideas considering their own local produce. The most favored product ideas were subjected to consumer sensory evaluation (n=30) and test marketing for products' acceptability. FGD results showed that coffee-like beverages using rice and mungbean were the most preferred ideas. Rice and mungbean brews were developed with acceptability index (AI) of 80%-84.6% ($\bar{x}$=36.9 y/o) and 64.6-69.4%, respectively. Both products obtained high willingness-to-buy percentages (53.9 and 92.3%). The women-farmers sold their products at 0.41 USD (Php 20.0)each (three tea bags of brew/pack) during the test marketing. These activities stimulated their creativity and entrepreneurial skills by doing on their own the preparation, packaging, and actual marketing of the product, as a start of their chosen enterprise. They have, thus far, bought a grinder for the beverage production and successfully launched their products. This shows that CED intervention should capitalize on women- farmers' entrepreneurial instinct and capabilities to ensure sustainability and positive economic outcomes. Thus, making them own and take responsibility in managing the production and selling of their product.

Keywords: *Enterprise Development, Community, Rice-Based, Sustainability, Value-Adding.*

Introduction

Poverty, which leads to hunger and malnutrition, is a chronic problem in rural agricultural communities. Two-thirds of the Philippine rural poor are agricultural workers (WFP, 2017) with farmers (34.3%) having the highest poverty incidence (PSA, 2014. Enhancing agriculture production is closely associated with solving poverty in rural communities; however, it is not always enough to alleviate poverty and improve the economic status through employment generation, increase income, and enhanced food accessibility. Building social enterprises (Smith and Stevens, 2010), on top of known interventions, may well be among the strategies that can further improve the economic situation in local communities. Adding value to agricultural products or agro-processing promises sustainability and increased rural incomes of community-based enterprises.

Women-farmers are known to be less paid and less productive in agriculture due to their traditional responsibilities in the household (FAO, 2011). However, women-farmers tend to contribute to agricultural productivity and rural economies by having various livelihood activities such as processing and marketing (SOFA Team and Doss, 2011). Women tend to dominate food and marketing of vegetable crops (Lu, 2007). Thus, there is wisdom in empowering women-farmers through value-adding and entrepreneurship as a way to push rural economy forward.

The lead agency of the Philippines for rice research and development is Philippine Rice Research Institute (PhilRice). Its Rice Business Innovations System (RiceBIS) Program aims to build sustainable communities by developing rice and rice-based enterprises to address farmers' need and support to them towards increasing farm productivity and profitability through value addition. Value-adding opens opportunities for farmers to create new sources of income and generate employment aside from rice production. Value-addition in rice is not new. It is a strategy that has long been practiced to promote nutrition and health (Shih and Diagle, 2002). Some of the known rice-based food products are puffed rice, fermented sweet rice, and alcoholic beverages

(Arendt and Zannini, 2013). With RiceBIS, the focus is not only on increasing production but also on making farm households become entrepreneurs through value-adding of their raw products.

This study focused on the development of value-added products utilizing rice and other crops produced in the rice-based farming communities as an initial step to create rural-agro enterprises to provide nutritional and economic benefits to farming families. This study used a participatory, community-based, and customer-oriented approaches allowing the RiceBIS farmers of Macarse, Zaragosa to conceptualize products of their preference and collect feedbacks from the target consumers and market.

Methodology

Research Site

This work was conducted at the RiceBIS community in Barangay Macarse, Zaragoza, Nueva Ecija, Philippines. Sites were selected based on the following criteria: yield - should be less than 4 tha^{-1}, 100 ha rice area, presence of a farmer organization, and active Local Government Unit (LGU).

RiceBIS-Macarse is located approximately 41 km south of PhilRice Central Experiment Station in Science City of Munoz, Nueva Ecija. The community is composed of 57 farmer-members with rice production as their main source of income. Rice yield is low in the community because the use of high quality seeds is not practiced. The community was organized to increase their yield, lower their production cost, and link them to market and business development service providers.

Engagement of Stakeholders

This work used action research (Markley et al., 2015) and community development principles (Quimbo et al., 2018) in engaging the stakeholders. In action research, farmers and product developers, market and community development specialists at PhilRice worked together to identify and reflect on the circumstances that led them to developing a rice-based enterprise for the community. Community enterprise development employed a multifaceted approach, i.e., participatory, community-based, entrepreneurial and consumer-driven. The study capitalized on the willingness of farmers, especially women-farmers to work together toward the same goal of solving rural poverty by using their community's resources and on their potential as entrepreneurs. To ensure active participation of women-farmers, they handled control and responsibility over agroenterpriese plan and implementation, costing, purchase of materials, market outlet identification, and income for them to have a sense of ownership, appreciation, and responsibility of outcomes.

Idea Generation

Idea generation was facilitated through an FGD with 15 women-leaders of RiceBIS-Macarse. The first FGD was conducted among the farmers and the second was with women who were organized by the officers to take care of the product development. In the FGD with the women, each participant was asked about the agricultural commodities, i.e., aside from rice, indigenous crops, and food products/traditional food products that are usually produced in their village. Their responses were used as take off points in generating rice-based products. In addition, they were also asked to rate the value-adding potential of each produce.

Product Development, Training, and Entrepreneurial Stimulation

Drawing on from the key ideas generated from FGD, food products were pre-developed. The RiceBIS-Macarse women-farmers underwent a training on food products preparation. Some of these food products were rice ball stuffed with mungbean and meat, shanghai stuffed with rice, mungbean and other vegetables, and coffee-like beverages from rice and mungbean. They were also trained on the basics of maintaining food quality and safety, proper packaging, costing, and marketing strategies. The pre-developed products underwent final selection by the women based on their preference and self-perceived entrepreneurial abilities. The women were asked to choose products, which they are willing to produce and market as their community-based enterprise.

Coffee-like beverages from rice and mungbean emerged as their top preferences. Hence, these two products were further developed. One kilogram of milled rice was roasted evenly in a pan under medium to high heat for approximately 1½ - 2 h until the external color of the roasted rice was blackish and the internal color was dark brown. Likewise, the mungbean was roasted in the same way. The roasted rice and mungbean were allowed to cool down and ground using a commercial grinder purchased by the cooperative as an initial investment for the rice-based enterprise.

Consumer sensory evaluation followed. Fifteen randomly-selected PhilRice staff members and 15 members of the RiceBis-Macarse were recruited to assess the products' acceptability in terms of color, aroma, taste, price, packaging, and overall liking using the 5-point unipolar Likert-type scale (1= I do not like it at all; 5= I like it extremely). The samples were prepared by adding 7.5 tbsp of rice or mungbean powder to 1 L of hot water, and added with 5 tsp sugar. Sensory panelists were given with 30 mL coded hot brew of each treatment and a glass of water as palate cleanser. Commercial coffee powder was used as the control. The data collected was encoded in MS Excel 2013 and analyzed using descriptive statistics and Acceptability Index (AI). Acceptability Index (AI) (de Lima et al, 2017) was used to characterize the acceptability of the products developed. ANOVA test was performed on the sensory scores and mean comparisons were analyzed using Tukey's B test using SPSS for Windows version 20 statistical software package (IBM SPSS Statistics). The level of significance used was p=0.05.

Product Packaging, Market Testing, and Branding

The Macarse women participated in the Farmers' Day Fair in their town to test the marketability of their roasted and powdered rice and mungbean products. They produced and packed the beverages based on their learnings from the training. They also participated in improving their product. PhilRice and the Department of Trade and Industry (DTI) assisted the farmers in packaging the products. After the successful test marketing and soft launching, the RiceBIS-Macarse women were asked to discuss among themselves about product branding and plan for their venture's next steps of their venture as facilitated by DTI and PhilRice representatives.

Results

RiceBIS-Macarse Farmers' Produce and Product Ideas

The focus group discussion was participated in by farmer-leaders (female, 60%; male, 40%) of RiceBIS-Macarse. Table 1 shows the profile of the participants.

Table 1. Socio demographic characteristics of the FGD participants (n=15).

	Mean	Percentage (%)
Sex		
Male		40
Female		60
Age (y)	52.8	
Marital Status		
Married		93
Singe		7
Education		
Elementary Graduate		9
High School Graduate		45
College Level		18
College Graduate		27

Aside from rice, FGD participants most commonly produced crops were string bean, mungbean, eggplant, lady's fingers, jute, squash, mango, bitter gourd, and chili. Some of them planted these crops in their backyard, while some allotted land in their farm or the vegetables such as chili. The fresh vegetables were sold in their town's public market or in their neighborhood. Mungbean was sold as dried beans to pick-up buyers, locally known as *byaheros*. Eventually, these dried mungbeans were sold in vegetable markets in the province or in Tarlac, their neighbouring province. Farmers informed that they usually have a surplus of mungbean. These vegetables are rich sources of nutrients. The availability of these vegetables and their nutritional values were considered in developing value-added products. Additionally, the mungbean shared a number of rice-based food products that they have already tasted such as *espasol*, *biko*, *duro-duro*, and *sumang malagkit*. These food products were not usually sold but were prepared for special occasions.

For rice-based product ideas, the top three preferred products were rice ball or rice *suman* stuffed with local vegetable crop, particularly mungbean; coffee-like rice-based beverage; and rice spring roll wrapper stuffed with mungbean and other vegetables. Other product ideas were rice wine, rice noodles, rice juice, *arroz caldo*-like product, rice doughnut, and *chao-fan*-like product. After naming these rice-based food products, FGD participants identified their possible markets. The markets identified were nearby community schools, *sari-sari* (variety) stores and the public market in Zaragoza. The participants expressed their desire to sell their products in the biggest city nearby (i.e., Cabanatuan City) where many potential outlets are located, should they be assisted in improving the packaging of their products.

The Chosen Products: Rice Brew and Mungbean Brew

The women enthusiastically chose a coffee-like product, which they specifically called as "rice coffee". They associated this product with their past experiences on how they used to roast rice and make it into a coffee. They shared that rice coffee provides many health benefits and is associated with long life. With this shared enthusiasm among them, coffee-like beverages made from rice and mungbean were further developed. In the process, the RiceBIS women thru several trials on site discovered that using firewood could lessen the roasting time to about an hour without jeopardizing the desired quality of the roasted product. One kilogram of rice yielded 851.6 g of roasted and ground rice with particle size of 10 mesh 1.7 mm^{-1}. On the other hand, 1 kg of mungbean obtained 921.4 g of roasted and ground mungbean with particle size of 9 mesh/2.0 mm. This particle sizes reveals their differences as mungbean is finer than the rice; thus higher residue percentage from the 1.3 mm hole of teabag.

Initially, the target market were mainly ≥40 years old, the age group which most of the consumers fall under. The reason for this was they thought that people belonging to this age group would most likely have the same experience (such as taste and health benefits) with them as regards "rice coffee". After some consultations made with the DTI provincial office, these coffee-like beverages were then labeled as rice brew and mungbean brew, respectively.

Acceptability of Rice Brew and Mungbean Brew

The basic profile of the 30 participants of the sensory evaluation of the brew products is shown in Table 2. Fifty-seven percent were women and 43% were men. There was an equal number of panelists from PhilRice and Macarse.

Table 2. Participants of the consumer sensory evaluation for rice and mungbean brews (n=30).

	Mean	Percentage (%)
Sex		
Female		57
Male		43
Panelists' Location		
PhilRice		50
Macarse		50
Age (y)		
PhilRice panelists	28.6	
Macarse panelists	46.3	

Rice brew obtained the highest overall acceptability scores among PhilRice (3.67) and RiceBIS-Macarse (4.53) consumer panelists. The panelists liked it very much (Table 1). It also obtained the highest liking for taste among RiceBIS-Macarse panelists (4.47) and PhilRice panelists (3.60). Mungbean brew came second to overall acceptability score and was liked moderately among the panelists from PhilRice (2.60) and RiceBIS-Macarse (3.20).

According to de Lima (2017), AI of ≥70% indicates that the product is acceptable in terms of its sensory attributes. Figure 1 shows that rice brew placed first in terms of acceptability index among panelists from both groups. According to the panelists from RiceBIS-Macarse, only rice (90.67%) and mungbean (76%) brews were acceptable in terms of sensory attributes. On the other hand, rice brew (73.33%) was the only acceptable sample among participants from PhilRice but had a lower acceptability index than that of RiceBIS-Macarse participants. Table 3, however, shows that there is no significant difference in the overall acceptability scores of rice brew between the respondents from RiceBIS-Macarse and PhilRice. The average age of participants from RiceBIS-Macarse was 46.31 years old. This explains why their acceptability indices were higher compared to those of PhilRice consumer panelists who were younger than them (mean age=28.67). All participants knew that roasted mungbean and rice were among the ingredients, but they did not know which specific samples contained the ingredients. Roasted ingredients were preferred more by the older panelists from Rice-BIS-Macarse. According to them, roasted ingredients brought in old memories from their childhood. On the other hand, younger participants found rice brew acceptable in terms of sensory attributes (≥ 70% AI).

Rice brew had the highest acceptability index for both male (80%) and female (84.62%) panelists among other brews (including the control - commercial instant coffee). As rated by male panelists, mungbean brew (69.41%), rice-mungbean brew (63.75%), and control (61.25%) had acceptability indices below 70% (Figure 2). Likewise, female panelists' acceptability indices for the commercial instant coffee and other brews were below 70%. In this case, the rice brew was the only acceptable product (female and male consumers).

Table 3. Sensory attribute scores of rice brew, mungbean brew, rice-mungbean brew, and commercial instant coffee powder between PhilRice and RiceBIS-Macarse consumer panelists.

Sensory Attributes*	Control (Commercial Coffee Powder)		Rice Brew		Mungbean Brew		Rice-Mungbean Brew	
Aroma	3.8^{a}	3.1^{a}	2.9^{a}	3.7^{a}	2.5^{a}	2.8^{a}	2.5^{a}	3.1^{a}
Color	4.1^{a}	3.7^{a}	3.7^{a}	4.0^{a}	2.7^{a}	2.7^{a}	2.7^{a}	3.0^{a}
Taste	2.5^{a}	2.6^{a}	3.6^{a}	4.5^{a}	2.6^{b}	3.2^{a}	2.8^{a}	3.3^{a}
Overall Acceptability	2.7^{a}	3.3^{a}	3.7^{a}	4.5^{a}	2.9^{ab}	3.8^{a}	2.5^{a}	3.5^{a}

n=15 PhilRice panelists; *n*=15 RiceBIS-Macarse panelists
*Scale: 1- I do not like it at all; 2-like it slightly; 3-like it moderately; 4- like it very much; 5- like it extremely
Mean scores with the same letter within a row are not significantly different from each other at $p<0.05$

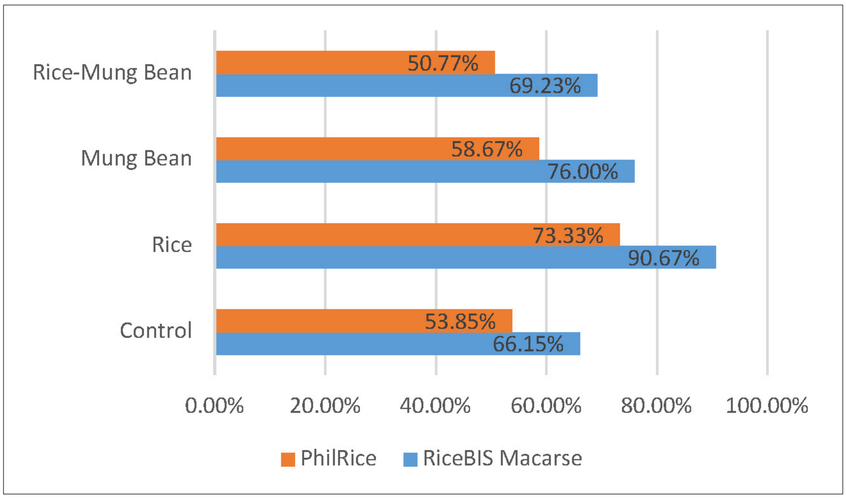

Figure 1. Acceptability index based on the overall acceptability of the different brews between panelists from PhilRice (n=15) and RiceBIS-Macarse (n=15).

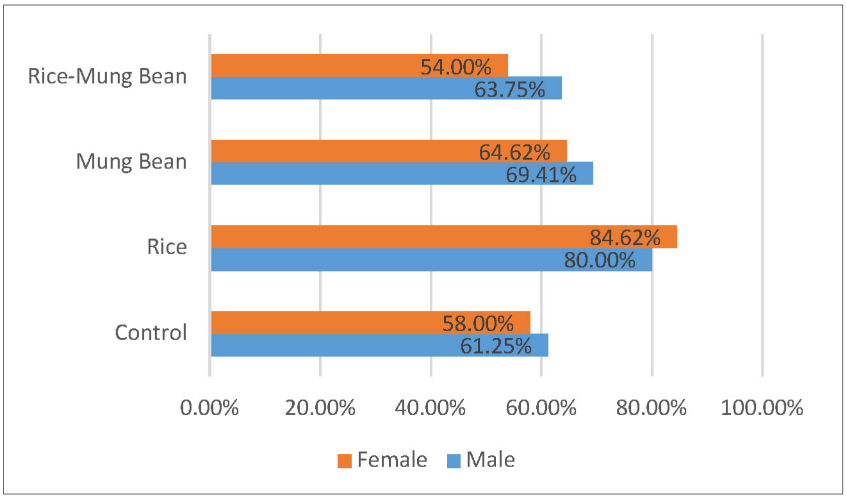

Figure 2. Acceptability index based on the overall acceptability of the different brews between female (n=13) and male panelists (n=17).

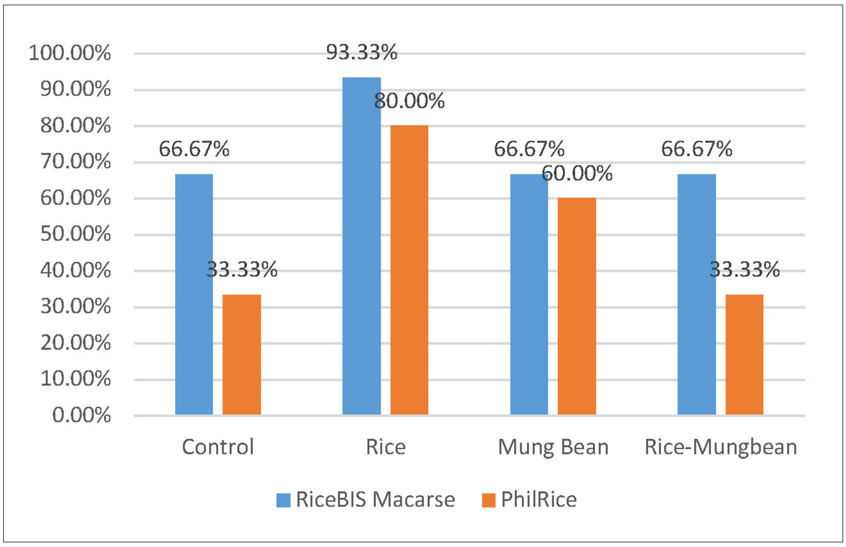

Figure 3. Willingness-to-buy (Yes) rice, mungbean, and rice-mungbean brews and the control (commercial instant coffee) among male and female participants from PhilRice (n=15) and RiceBIS-Macarse (n=15).

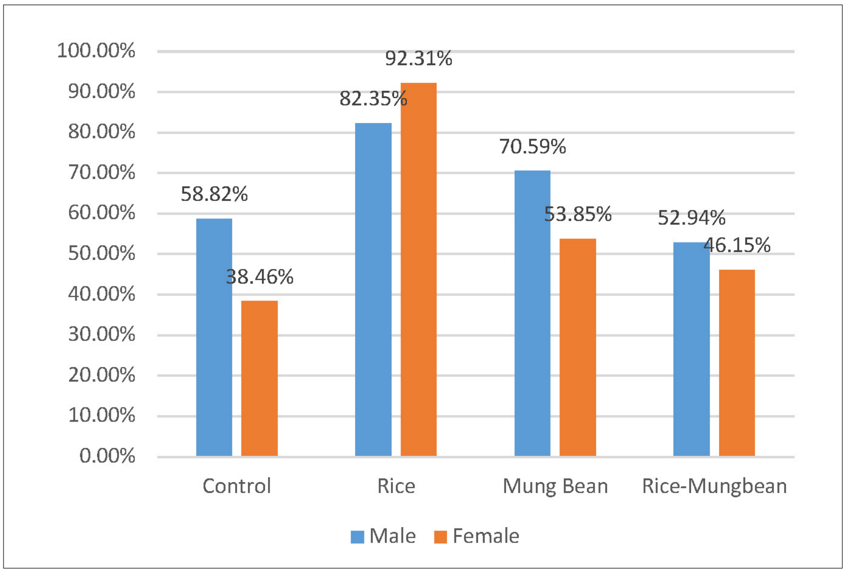

Figure 4. Willingness-to-buy (Yes) rice, mungbean, and rice-mungbean brews and the control (commercial instant coffee) among male (n=17)and female (n=13) participants.

Willingness to Buy for Rice Brew and Mungbean Brew

Most participants from RiceBIS-Macarse (93.33%) and PhilRice (80%) were willing to buy rice brew if it were available in the market, and majority of them (60-66.7%) were also willing to buy mungbean brew. The figure also indicates that only a few panelists from PhilRice were willing to buy rice-mungbean brew (33.33%) and the control (33.33%). On the contrary, majority of the RiceBIS-Macarse were also willing to buy rice-mungbean (66.67%) and the control (66.67%) (Figure 3). This may be due to older participants preference for hot drinks like coffee.

Figure 4 shows that most panelists were willing to buy rice brew — male, 82.4% and female, 92.3%. A slightly lower pecentages regarding willingness to buy were noted for mungbean brew — male, 70.6% and female, 53.9%. Lastly, less than half of the female panelists were willing to buy rice-mungbean (46.15%) and commercial coffee (38.46%).

The Birth of Macarse Brews

During the test marketing of the rice and mungbean brews, all the packed products were sold out (Figure 5). Each pack was priced at USD 0.41 (PhP 20.0) [1] although the computed cost was at USD 0.25 (PhP12.0). Each pack contained three tea bags of roasted and ground rice and mungbean. Figure 6 shows the samples of rice and mungbean brews. Women farmers agreed that this was a wholesale price, and they would increase the price in their future ventures. The RiceBIS women participated actively during the preparation and test marketing, and many of them were delighted about the outcome of the initial launching of their products as mentioned by those who participated in the event.

As the product progressed, RiceBIS-Macarse women finally agreed to brand the products as *Macarse* Rice Brew and Mungbean Brew. They were excited and wanted their village to be known as a producer of rice and mungbean beverages. Based on the community members' feedback, beverages from rice and mungbean were the products that encouraged participation and enterprise engagement among them. They were able to launch their products in an event attended by fellow farmers, researchers and development workers of PhilRice, and other stakeholders in another RiceBIS community (Figure 7).

Sustainability Plan for Rice and Mungbean Brew

Macarse brews are best for coffee lovers and health conscious people. The product is packed in a box containing 10 packs (each pack contains 3 teabags, 3 g per teabag) sold at USD 3.10 (PhP 150.0) per box and USD 0.41 (PhP 20.0) per pack. Rice is the major commodity of the community and based on initial cost analysis, rice brew is more feasible than mungbean brew owing to better marketability. On the other hand, mungbean will still serve as an alternative raw material as it is among the crops planted in the community. Thus, production of rice brew will be made more frequently than mungbean by 5 production staff members 5 days per week for 8 h per day. This is based on the estimated human resource of Php 3,000-Php 5,000 monthly compensation. Total production target would be 11,000 packs of rice brew per month to be distributed in nearby towns. Initial distribution outlets identified were local restaurants and souvenir shops. Other target outlets are nearby public and private offices. Given that rice drink is not a new product, competition is expected to be stiff. To compete well and sustain productivity, quality control

1 Exchange rate as of October 10, 2020.

and packaging, strong market linkages, promotional strategies, and critical factors (climatic risk factors, accessibility, and market risk factors) and its contingency plans must be ensured. Despite all this, a 5-year business plan for rice brew enterprise shows that it is feasible and profitable in a commercial scale as a community enterprise.

Discussion

This work optimized the use of the bottom-up approach as evidenced by the members of the community actively participating in putting up the enterprise. Originally, the ideas were generated by both men and women farmers. However, along the way, only the women-farmers pursued the development of the enterprise eventhough engagement of men farmers was actively sought but they were busy in rice production. This case shows the strong interest of the Macarse women-farmers in this entrepreneurial endeavor. Entrepreneurs are seen to be innovators and the engine for economic development as they try to maneuver opportunities in a way that they can offer new products and services with the use of their own ideas, capabilities, and creativity, which eventually lead to economic development (Mamun and Ekpe, 2016). This was shown by the women-farmer participants when they initiated the establishment of

Figure 5. The RiceBIS-Macarse women during the Farmers' Day Fair in Zagaroza, Nueva Ecija.

Figure 6. Rice brew (left) and mungbean brew (right) samples sold by RiceBIS-Macarse women during the Farmers' Day Fair in Zagaroza, Nueva Ecija.

Figure 7. Packaging (sachet) and launching of *Macarse* Brews - Rice brew and bungbean brew participated in by the RiceBIS-Macarse women, PhilRice researchers and development workers, and other stakeholders.

rice brew as their start up food business. The past few decades saw how women entrepreneurship served as a major force driving economic growth at the global level. Ali (2018) noted that female entrepreneurship generated positive economic impact such as employment creation, economic growth, innovation, and entrepreneurial diversity. Ní Fhlatharta and Farrell (2017) found that female innovators had contributed economic, social, and cultural impact promoting rural development. During the course of idea generation, farmers mentioned their local resources such as rice and vegetables. In addition, they shared that they usually experience a surplus of mungbean. These locally available commodities were strongly considered in coming up with an enterprise and product ideas, such as rice ball or rice *suman* stuffed with local vegetable crops and coffee-like rice-based beverage. They identified possible markets for their product ideas, such as schools and small stores in the area, the town public market and the nearby city. All of these, imply a trajectory of enhancing local economic transactions, such as sourcing of raw materials from local producers and selling products to local markets. This could eventually lead to local economic growth for Macarse community.

As this enterprise development proceeded, a hands-on cooking and food product development training was conducted. Only women-farmers who participated in the FGD were in attendance. While they were also invited, men farmers did not show interest to participate. To understand this difference in project reception, a gender dimension proves useful. Similar to the findings of Arakawa (2013), domestic chores such as cooking and food preparation are in general, not for men's activity. Women are likely to take on entrepreneurial activities that relate well to their inherent or gender-assigned skills but today women tend to transform these skills into economically productive labor that allows them to generate additional income (Anthopoulou, 2010). This was the same observation among the Macarse farmers.

When women-farmers decided on the final product for their community enterprise, they chose a coffee-like beverage either from rice and mungbean due to their close affinity to this kind of product. This product, according to them, enlivened memories from the past saying "we better prefer to develop a coffee like beverages because of the good experiences we had when drinking rice coffee in the past" — those from their younger selves, their ancestors, and their traditions. They added that people of their age would likely be their market owing to share views and experiences. This view was validated during the sensory evaluation. This finding is shared by Anthopoulou (2010) who found that "nostalgic trip back to the realms of the food granny used to cook" page would likely take its consumers in a nostalgic experience on how grandparents used to cook and prepare food, which further entails a distinct niche market given that the target consumers are in pursuit of traditional or authentic tastes of the past.

Moreover, regarding the marketability of the coffee-like beverages, the consumer acceptability test shows high potential. Apart from its acceptable sensory attributes, rice brew has been appealing to consumers, especially to the elderly, because it does not contain caffeine and artificial ingredients. Aside from that, Filipinos used to believe that rice coffee has positive impact on health, specifically in treating stomach pain due to gas, ulcer, and liver problems. On the other hand, mungbean brew had also received a positive impression from the consumer panelists. Majority were willing to buy it eventhough it had received a low acceptability index relating to its sensory attributes (<70%). The findings suggest that producing and marketing of rice brew is feasible to become a value-adding activity and be a source of added income to the RiceBIS community. While mungbean had a low sensory acceptability, it remained appealing to consumers. Likewise, it should be noted that mungbean is packed with other beneficial properties such as being a good source of nutrients and minerals (e.g., protein, folate, manganese, vitamin B1, B2, B5, B6, and iron) (Raman, 2018). Thus, mungbean brew needs to be further developed to improve its sensory attributes and maximize its potential as an enterprise for the community. Marketing these coffee-like beverages can still be challenging eventhough Filipinos are known to be avid drinkers of coffee beverages. Coffee mixes (e.g., 3-in-1 coffees) take hold of the lion's share of the market in both urban and rural households (Manila Bulletin, 2015). While this is the case, coffee-like rice and mungbean beverages may operate on a niche market targeting consumers who prefer traditionally prepared beverages with added health and nutritional value.

Throughout the course of this project, women-farmers tried to improve the products on their own. The *"Macarse Brews"* branding reflects their vision for their community to be known as a producer of traditional beverages like the "rice coffee." The strong cultural connection of this enterprise among Macarse women increases the chance for their success in a niche market. In a study of rural women in Greece engaging in agro-food production business, it was observed that their success rests solely on the marketability of their products. Their success did not help them achieve their aspirations relating to social emancipation and social recognition. The reason for this is because women are tied up to their stereotypical roles such as "nurturers and guardians of traditional values rooted in motherhood" (Anthopoulou, 2010). In the case of Macarse women, they might have not explicitly expressed these aspirations as they concentrate on how to augment their family's income. However, their enthusiasm to pursue this community enterprise and the joy brought by the product launching success are manifestations of their latent need for self-achievement and identity recognition in the society. Sharma et al. (2012) observed that among the drivers of success of women in entrepreneurship are social identity, achievement of excellence, and confidence. These suggest that the first step towards improving women's entrepreneurial skills and ensure the success of their community enterprise, is to help them achieve their personal aspirations (i.e., to be known for the food business and to earn more income outside farming) and economic motivations.

Additionally, to ensure success for this community enterprise, it is important to identify other entrepreneurial traits that may need to be developed among Macarse women. A study in Malaysia on women-led micro-enterprises showed that the most important trait that positively affects the performances of the enterprises was entrepreneurial alertness or the ability to spot and tap opportunities in the environment (Mamun and Ekpe, 2016). These opportunities may differ among entrepreneurs depending on various factors, such as accessibility of information, acquisition of resources (e.g., microcredit, training, and social capital), and their ability to make use of the resources around them. However, it should be noted that Macarse women could face barriers in accessing needed resources given that they are in a rural area. Leahy et al. (2017) reported that female entrepreneurs from Cambodia, Indonesia, and the Lao People's Democratic Republic experienced challenges in establishing their businesses. These challenges include inherent biases in socio-cultural norms (work versus family duties), poor education, poor access to finances, and improperly tailored support services. While the Philippines may already have plenty of gender equality policies in place, the challenges that its neigbours experience may also be experienced by Filipino women entrepreneurs albeit on a slightly different level (Asian Development Bank, 2013).

Macarse women are organized, which is their advantage. Hence, it is wise to assume that they will work collectively to make their community enterprise successful. Jones et al. (2012) observed that as a group, women are able to improve their production and trade practices by learning from each other, pooling capital and other resources, and improving their skills and knowledge by attending training programs.

Therefore, an enabling environment for women-entrepreneurs should be strongly supported. Government interventions should capitalize on the use of bottom-up approaches to ensure that women's unique needs are addressed (Alarcón and Sato, 2019). Capital is one of the constraints of women to engage in entrepreneurial activities. Support from local banking or microfinance institutions by providing microcredit or financial products suitable to women's needs and situation (as creditors) is warranted. If these are already available, they should reach to rural communities and make these financial products and services known and accessible. It is also paramount to understand the dual role of women, i.e., doing family duties and aspiring to achieve social and professional recognition. Thus, family and community support and encouragement have become crucial to achieve success for these women entrepreneurs (Alarcón and Sato, 2019; Movahedi et al., 2016). Additionally, involved women may actively inform, encourage, and engage other women in the community for opportunities offered by institutions (e.g. skills training, development projects). Such actions lead to a more cohesive community development. Finally, institutions and interventions should seriously consider these aspects to ensure success and sustainability for projects that are aiming for both women empowerment and rural economic growth.

Conclusions and Recommendations

The community's suggestions and experiences, inspired by their enthusiasm, had encouraged them in their voluntary engagement in the enterprise. This work highlights the importance of taking into account the community's preferences, abilities and available resources in product development as well as consumer feedback, in creating a successful community-based enterprise. Being participatory, community-based, and consumer-driven stimulated the women's creativity and entrepreneurial skills, which empowered them in creating their own income-source. The agro-enterprise development approach of the RiceBIS not just focused on the farmers' increased productivity, but also involved other members of the family as income generators, particularly the women who are often neglected and disadvantaged.

The need to conduct post-intervention activities and evaluation to ensure sustainability and impact to hyperlocal economies must be made. This undertaking must also be duplicated in other communities.

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FULL PAPER

ROOT AND SHOOT GROWTH OF RICE IS ENHANCED BY NITROGEN APPLICATION DURING VEGETATIVE STAGE SOIL MOISTURE FLUCTUATIONS

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Abstract

Soil moisture fluctuation (SMF) requires a specific combination of root adaptations, which is uniquely different from either continuously waterlogged (CWL) or progressive drought. Under real field conditions, SMF is caused by erratic rainfall pattern brought about by climate change. Root plasticity is one of the key traits for plant's adaptation under abiotic stresses including SMF. This study evaluated the effect of nitrogen (N) fertilizer on root plasticity expression and shoot development of INL60, an introgression line from a cross between IR64 and CSSL74, under SMF. SMF generally reduced shoot dry weight (SDW) relative to CWL. During initial drought at 10% soil moisture content (SMC) in SMF, SDW was significantly higher in 120 and 150N than in 0N. Under subsequent 10-day waterlogging after initial drought in SMF, SDW was significantly higher with 120 and 150N fertilizer relative to 0N. The increase in SDW under SMF brought about by higher N application was due to the increase in water use attributed by the increase in root system development based on total root length. Results showed that INL60 expressed root plasticity in response to SMF, which can be further enhanced with higher N application which consequently enhanced water use and maintenance in greater shoot dry matter production. This line can be a good material in breeding program targeting yield improvement in rice growing areas prone to SMF.

Keywords: *Root Plasticity Expression, Soil Moisture Fluctuation, Continuously Waterlogged, Nitrogen Application.*

Introduction

Rice is a major food crop for most of the world's population. It is widely grown in Asia including rainfed areas, which is heavily affected by drought (Manickavelu et al., 2006). In the Philippines, irrigated rice ecosystem constitutes 60% of the production area while the remaining areas are under upland and rainfed lowland condition (PSA, 2018). One of the major constraints in upland and rainfed ecosystems for better crop productivity is the availability of water, which is aggravated by the impact of climate change that looms water scarcity.

More than 50% of the rainfed areas are drought-prone; hence, the amount and timing of water supply available for rice production are basically constraining the crop productivity (Wade et al., 1999). Under real field conditions, the erratic rainfall pattern and irrigation systems of water-saving technology practicing intermittent irrigation (Suralta et al., 2010) exposes rice to a continuous and recurring cycles of soil moisture fluctuation from waterlogging to drought and vice versa (Niones et al., 2012) and water deficit (Owusu-Nketia et al., 2018). These changes in the soil moistures have severe marked effects on the soil condition, availability of nutrients and water, root development and functions (Suralta et al., 2008).

Under abiotic stress condition, roots play an important role for rice adaptation especially in water and nutrient acquisition and for maintaining growth and yield especially under various water stresses. The promotion of root development under water stress conditions is called root plasticity (Kano et al., 2011), which is defined as the ability of an individual genotype to modify or alter its phenotype in response to the changes in physical environment (Bradshaw, 1965).

Under rainfed and upland rice growing conditions, root plasticity is one of the key traits for plant's growth adaptation to various intensities of drought stress in rice (Kano et al., 2011; Owusu-Nketia et al., 2018; Suralta et al., 2016; Wang and Yamauchi, 2006. Niones et al. (2015) showed that L-type lateral roots (LR) plasticity of the root system development is the key trait that plays a significant role in the plant's broader adaptation under soil moisture fluctuation condition. Other important root plasticity trait found to be useful under rainfed lowland ecosystem are plasticity in lateral root development at the shallow soil layer under transient drought-to-waterlogged condition with hardpan (Kano-Nakata et al., 2013), plasticity in L-type LR at deeper soil layer under transient waterlogged-to-drought condition without hardpan (Suralta et al., 2010), plasticity in deep rooting under the absence of hardpan inhibiting root growth during drought periods (Henry et al., 2011), and ability to induce root cortical aerenchyma in previously drought stressed roots under transient drought-to-waterlogged condition (Suralta et al., 2008; Suralta et al., 2010). Owusu-Nketia et al. (2018) showed an improved adaptation to soil moisture fluctuating stress through promoted production of nodal roots and development of lateral roots using backcross inbred lines of rice.

With the erratic rainfall pattern and the increasing pressure on crop production, rainfed lowland and upland ecosystems are being sought as additional areas for increasing crop productivity under varying soil moisture conditions. Breeding rice with root plasticity trait for these target rice environments is needed and its corresponding optimum agronomic management that may enhance its expression and function must be given full attention (Suralta et al., 2016).

Understanding the contribution of different levels of N fertilization for improving nutrient management under soil moisture fluctuation is needed to enhance the expression of root plasticity trait for maximized water and nutrient acquisition from the soil. Status of nutrients in the soil is critical to root developmental process (Lopez-Bucio et al., 2003). In the 21st century, increased in crop yields had been due to the increased application of chemical fertilizer, particularly N fertilizer (Tilman et al., 2011; Fan et al., 2011). Nitrogen is a necessary nutritional element in crop production, and also one of the main limiting factors for crop yield (Ferguson et al 2002; Samonte et al., 2006). The application of N can increase root dry weight, total root length, root volume, and root surface area (Fan et al., 2010; Wang and Yamauchi, 2006). Tran et al. (2014) showed that the application of N under mild drought conditions enhanced plasticity in lateral root development for lowland rice. Moreover, the regulation of N application rates under moderate drought conditions can manipulate root morphology and can improve productivity. However, genotypic variation and different field conditions may influence efficacy (Menge et al., 2018).

An introgression line (INL60) identified from a cross between IR64 and CSSL47 showed good root system development and shoot growth under SMF (Niones et al., unpublished data). This INL can be used as a material for improving varieties suitable for rainfed lowland conditions. However, its root system developmental and shoot growth in response to N application under SMF has yet to be quantified. In this study, it is hypothesized that application of N will enhance the root system developmental responses of this INL to SMF; thus, maintains higher dry matter production. This study determined the effect of N fertilization on enhancing the root system developmental responses of INL60 during SMF and maintaining greater dry matter production by quantifying root and shoot developmental responses and soil water uptake.

Materials and Methods

Time and Place of the Study

This study was conducted under glasshouse conditions of the Agronomy, Soils, and Plant Physiology Division (ASPPD) from February to May 2020 at the Philippine Rice Research Institute-Central Experiment Station (PhilRice-CES), Science City of Muñoz, Nueva Ecija, Philippines (15°40' N, 120°53' E, 57.6 masl). The average daily solar radiation, minimum and maximum temperatures, and relative humidity during the conduct of the experiments were 21.45 MJ m^{-2} d^{-1}, 22.7°C, 34.3°C, and 76.5%, respectively.

Fifty-four black plastic pots without drain holes measuring 26 cm in height, 24 cm in bottom diameter, and 30.5 cm in top diameter were used as growing containers.

Genotype Used

An introgression line (BC_1F_3 QTL-1-64-B-1-1-1) derived as cross between IR64 x CSSL47 (chromosome segment substituted line number 47 of Nipponbare/Kasalath) at PhilRice-CES was used and herein referred as 'INL60'. The INL60 was selected based on the plasticity index and shoot dry matter production from the F_7 population of the IR64*2/CSSL47 cross. The INL60 has high plasticity index (0.90) and shoot dry weight under SMF at 1.31 g $plant^{-1}$ based from the previous root box experiment until vegetative stage at PhilRice (Niones et al., unpublished data).

Cultural Management

Seeds of INL60 were soaked overnight in 5% sodium hypochlorite, drained, and incubated at 28°C for 48 h prior to sowing. The pre-germinated seed were sown at 3 seeds per plastic pot (26.5 cm in height, 24 cm in bottom diameter, and 30.5 cm in top diameter) filled with 8 kg of dried and sieved garden soil. Thinning was done to 1 seedling at 5 days after sowing (DAS). Six pots with soil but without plants were added to the experiment with three pots representing each of the CWL and SMF were used to determine the water lost via evaporation alone.

Experimental Design and Treatments

The study was laid out in a split-plot arranged in randomized complete block design with six replications. Soil moisture treatments were assigned as main plot and levels of N applications as subplots. The three replications were sampled at the end of initial drought while the other three replications were collected after the subsequent waterlogging treatment under SMF. The timing in plant samplings in CWL were also based on SMF samplings.

Nitrogen Treatments

Four N treatments based on the rate of N applications was imposed such as 0 N (0N, control), low N rate (60N, 60 $kgha^{-1}$), recommended rate (120N, 120 $kgha^{-1}$), and high N rate (150N, 150 $kgha^{-1}$). The source of N fertilizer was urea (46-0-0) and were applied at three splits (7, 21 and 46 DAS) at 30:35:35 ratio of the N rate. Phosphorus (0-18-0, Solophos) and Potassium (0-0-60, Muriate of Potash) were applied to pots at basal at the rate of 60 $kgha^{-1}$.

Soil Moisture Treatments

The plants were exposed to two soil moisture treatments: continuously waterlogged (CWL) and soil moisture fluctuation (SMF) regimes. In the CWL condition, water level at 5 cm above the soil surface (50% SMC) was maintained throughout the study. For SMF, plants were exposed to episodes of waterlogged-drought-waterlogged conditions. The water level in SMF was kept at 5 cm depth from sowing up to 20 DAS. Thereafter, imposition of drought was done by withholding water at 21 DAS and allowed to dry down to 10% SMC. SMC was maintained for 7 days (45 DAS) before bringing the soil moisture back to the level of CWL for another 10 days (56 DAS). The SMC in each pot was calculated as the proportion of water weight (difference between the wet weights of the soil excluding the pail on a given day) to the dry weight of the soil (Suralta et al., 2015). Rewatering was done to replace the amount of water lost through evaporation. The amount of water added was computed as the difference between the current weight and target weight at 10% SMC.

Physiological Measurements

The photosynthesis, stomatal conductance, and transpiration rate were measured on the second youngest fully expanded leaf using LI-6800 Portable Photosynthesis System Version 1.3 (Li-COR Inc., USA) between 10 am to 2 pm timed at the end of transient drought (45 DAS) and subsequent waterlogging (56 DAS) in SMF.

The water use was estimated as the difference in the amount of water lost through evapotranspiration (pots with plants) and evaporation (pots without plants). The cumulative water use is the sum of daily water use from each pot.

Shoot and Root Growth Measurements

Two plant samplings (three replications per sampling) were done; one each at the end of initial drought stress (45 DAS) and end of subsequent waterlogging (56 DAS) in SMF. Prior to cutting of shoots at the ground level, the number of tillers was manually counted. The leaves were separated from each stem and the leaf area was determined using LI-3100 leaf area meter (LI-COR, Inc. Lincoln, Nebraska USA). The leaves were then combined with the stem in a designated paper bags and oven dried at $70^{o}C$ for 72 h prior to weighing of shoot dry weight (SDW).

The roots from each sample were carefully extracted, washed gently with water using a hose sprayer to remove soil, and was stored in 95% alcohol for further measurements. Alcohol was later drained and roots were stained in 0.25% Coomassie Brilliant Blue R 250 aqueous solution for 12 h. Roots were stained to take high-resolution photographs of the roots. After staining, roots were washed with water to remove excess stain. The number of nodal roots at the base of each plant were manually counted. Thereafter, the roots were cut into small pieces and placed in a clear glass tray with 5 mm water and scanned using Epson perfection v700 scanner at 600dpi. Digital images were saved using tiff format. Root length analyses were done using WinRhizo v. 2007d (Régent Instruments, Québec, Canada) with the pixel threshold value of 215. Root lengths were classified based on their diameters, <0.1 mm, 0.1–0.2 mm and >0.2 mm. Roots with a diameter less than 0.2 mm were considered as lateral roots (Sandhu et al., 2016), and roots with 2.0 mm and greater were considered as nodal root. The total nodal root length (NRL) was estimated as the difference between the total root length (TRL) and the total lateral root length (LRL).

Plasticity index was determined as the difference between the expressed root traits in SMF and CWL treatments in each N rate relative to 0N.

Statistical Analysis

The ANOVA was conducted using the Statistical Tool for Agricultural Research (STAR; version 2.0.1, January 2014). Means were compared using Least Significant Difference (LSD) test at 5% level of significance.

Results

Shoot Growth

Under SMF, transient drought (20-45 DAS) and waterlogging (46-56 DAS) were imposed, in which timing of plant samplings and measurements were also done simultaneously with CWL. Initial drought in SMF (45 DAS) significantly reduced SDW, leaf area, and relative growth rate of INL60 regardless of N treatments (Table 1). Under initial drought in SMF (45 DAS), SDW and relative growth rate (RGR) were generally higher with N application relative to 0N particularly in 120N and 150N. The number of tillers on the other hand was not significantly affected by SMF although tillering ability tended to be lower in SMF relative to CWL, regardless of N treatments (Table 1). Under initial drought in SMF (45 DAS), tillering was significantly higher with N application relative to 0N. At 56 DAS, the imposition of waterlogging after transient drought in SMF have resulted in the recovery of SDW, leaf area, tillering, and RGR back to the level of their CWL counterpart, regardless of N treatments (Table 1). Under SMF, the SDW, leaf area, tillering, and RGR were generally higher with N application especially in 120N and 150N.

Leaf Photosynthesis, Stomatal Conductance, Transpiration, and Water Use

At 45 DAS, initial drought in SMF significantly reduced the leaf transpiration (Tr), stomatal conductance (SC), and water use (WU) but not photosynthesis (Ps) of INL60 relative to CWL (Table 2). The Ps, Tr, SC, and WU under SMF were generally higher with N application relative to 0N, especially in 120N and 150N. At 56 DAS, the imposition of waterlogging after transient drought in SMF have resulted in the abrupt increase in Tr, SC, Ps, and WU back to the level of their CWL counterparts (Table 2) regardless of N treatments. Under SMF, the application of N significantly increased WU only in 120N and 150N relative to 0N.

Root System Development

Except for the total nodal root length (TNRL), initial drought in SMF (46 DAS) significantly reduced the number of nodal roots (NNR), total root length (TRL), and total lateral root length (TLRL) of INL60 relative to CWL (Table 3). During this period in SMF, N application did not enhance the root number and length of INL60. At 56 DAS, the imposition of waterlogging after transient drought in SMF significantly increased the NNR and TNRL similar to the CWL level across N treatments. Meanwhile, the TRL and TLRL were still significantly lower under SMF than under CWL across N treatments. However, those applied with N generally had increased root lengths under especially TRL at 120 and 150N, and TLRL and TNRL at 150N, relative to 0N. The estimated change in TLRL and TRL during the period of drought to waterlogged conditions was greatest in 150N, which even surpassed the change in root length of its N counterpart under CWL (Table 3).

Table 1. Shoot growth and relative growth rates at 45 and 56 days after sowing (DAS) of INL60 grown under continuously waterlogged (CWL) and soil moisture fluctuation (SMF) conditions and applied with different rates of N fertilizers. End of initial drought (45 DAS) and end of waterlogging after transient drought (56 DAS) in SMF.

Water (W)/ Nitrogen (N)	Shoot Dry Weight (SDW) (g plant^{-1})		Leaf Area (cm^2 plant^{-1})		Tiller (no. plant^{-1})		Relative Growth Rate (g SDW plant^{-1} day^{-1})	
	DAS		DAS		DAS		DAS	
	45	56	45	56	45	56	45	56
CWL								
0N	3.9^{b}	5.9^{c}	411.6^{b}	421.1^{c}	6.7^{b}	4.3^{b}	0.09^{b}	0.18^{b}
60N	5.5^{ab}	10.9^{b}	556.2^{ab}	1229.4^{ab}	10.3^{a}	8.0^{a}	0.12^{ab}	0.49^{b}
120N	4.6^{ab}	14.3^{a}	545.1^{ab}	1418.4^{a}	10.7^{a}	10.3^{a}	0.11^{ab}	0.88^{a}
150N	5.8^{a}	15.4^{a}	734.7^{a}	1604.8^{a}	10.7^{a}	11.3^{a}	0.13^{a}	0.87^{a}
SMF								
0N	3.4^{b}	5.3^{c}	291.5^{a}	389.9^{c}	5.7^{b}	5.0^{c}	0.08^{b}	0.17^{b}
60N	3.7^{ab}	7.4^{b}	385.0^{a}	718.7^{ab}	8.0^{ab}	9.0^{ab}	0.08^{b}	0.34^{ab}
120N	4.7^{a}	10.4^{a}	441.6^{a}	1090.5^{a}	7.7^{ab}	10.3^{a}	0.10^{a}	0.52^{a}
150N	4.2^{a}	10.8^{a}	425.5^{a}	1201.3^{a}	9.0^{a}	12.7^{a}	0.10^{a}	0.59^{a}
W	*	ns	*	ns	ns	ns	*	ns
N	*	***	**	***	***	***	*	*
W x N	ns	ns	ns	ns	ns	ns	ns	ns

*, **, *** indicates significance at P<0.05, P<0.01, and P<0.001, respectively. ns, not significant. Values are means from three replications. Means followed by the same letters are not significantly different within each water treatment at 5% LSD.

Table 2. Leaf photosynthesis, stomatal conductance, transpiration, and water use of INL60 at 45 and 56 days after sowing (DAS) under continuously waterlogged (CWL) and soil moisture fluctuation (SMF) conditions and subjected to different rates of N fertilizer. End of initial drought (45 DAS) and end of waterlogging after transient drought (56 DAS) in SMF.

Water (W)/ Nitrogen (N)	Photosynthesis ($\mu mol\ m^{-2}\ s^{-1}$)		Stomatal Conductance ($mol\ m^{-2} s^{-1}$)		Transpiration ($mol\ m^{-2}\ s^{-1}$)		Water Use ($kg\ plant^{-1}$)	
	DAS		DAS		DAS		DAS	
	45	56	45	56	45	56	45	56
CWL								
0N	12.6^{b}	13.3^{b}	0.29^{b}	0.34^{a}	0.012^{ab}	0.016^{a}	1.5^{b}	2.0^{c}
60N	12.7^{b}	15.2^{ab}	0.24^{b}	0.38^{a}	0.009^{b}	0.016^{a}	1.6^{b}	4.3^{ab}
120N	17.0^{a}	22.5^{a}	0.34^{a}	0.58^{a}	0.012^{ab}	0.024^{a}	1.9^{a}	5.3^{ab}
150N	18.8^{a}	20.2^{ab}	0.37^{a}	0.51^{a}	0.015^{a}	0.022^{a}	1.9^{a}	6.4^{a}
SMF								
0N	9.9^{b}	13.2^{a}	0.11^{b}	0.30^{a}	0.005^{b}	0.016^{a}	1.2^{b}	2.1^{b}
60N	13.5^{a}	18.6^{a}	0.16^{ab}	0.46^{a}	0.008^{ab}	0.019^{a}	1.4^{ab}	3.6^{ab}
120N	14.9^{a}	18.1^{a}	0.17^{ab}	0.55^{a}	0.010^{a}	0.021^{a}	1.5^{a}	4.9^{a}
150N	15.5^{a}	19.1^{a}	0.21^{a}	0.54^{a}	0.011^{a}	0.022^{a}	1.6^{a}	5.7^{a}
W	ns	ns	*	ns	*	ns	*	ns
N	*	**	*	ns	*	*	*	*
W x N	ns	ns	ns	ns	ns	ns	ns	ns

*, ** indicates significance at P<0.05 and P<0.01, respectively. ns, not significant. Values are means from three replications. Means followed by the same letters are not significantly different within each water treatment at 5% LSD.

Table 3. Number of nodal roots and component root lengths of INL60 at 45 and 56 days after sowing (DAS) under continuously waterlogged (CWL) and soil moisture fluctuation (SMF) conditions and subjected to different rates of N fertilizer. NNR, number of nodal roots per plant (NNR); total root length (TRL); total lateral root length (TLRL); and total nodal root length (TNRL).

Water (W)/ Nitrogen (N)	NNR ($no.\ plant^{-1}$)		TRL ($m\ plant^{-1}$)			TLRL ($m\ plant^{-1}$)			TNRL ($m\ plant^{-1}$)	
	45	56	45	56	Δ	45	56	Δ	45	56
CWL										
0N	135.3 b	157.7 b	723.5 a	715.4 b	60.6 b	596.9 ab	564.6 b	35.9 b	126.5 a	150.7 b
60N	205.3 a	241.3 ab	1140.9 a	1189.2 a	48.2 b	971.3 a	926.1 b	-45.2 b	169.6 a	263.0 ab
120N	182.7 ab	308.7 a	773.8 a	1358.3 a	584.5 a	665.2 ab	1079.5 a	414.2 a	108.6 a	278.8 a
150N	220.0 a	325.3 a	839.0 a	1280.4 a	441.3 a	723.4 a	1010.5 a	287.1 ab	115.7 a	269.9 a
SMF										
0N	48.0 c	167.0 a	431.9 a	678.0 b	246.1 b	330.0 a	509.1 b	179.0 b	101.8 a	168.9 b
60N	60.7 c	201.7 a	396.9 a	721.9 b	324.9 ab	286.7 a	541.2 b	254.5 b	110.2 a	180.7 ab
120N	71.3 c	226.3 a	436.3 a	856.3 a	420.0 a	288.8 a	652.2 a	363.4 ab	147.5 a	204.1 ab
150N	64.3 c	234.7 a	385.5 a	926.5 a	541.0 a	274.7 a	708.5 a	433.8 a	110.8 a	218.0 a
W	**	ns	*	*	ns	*	*	ns	ns	ns
N	**	***	ns	**	*	ns	**	**	ns	*
W x N	*	ns	ns	ns	ns	ns	ns	ns	ns	ns

Δ, increase in root lengths from 45 DAS (initial waterlogged in CWL or initial drought in SMF) to 56 DAS (continuous waterlogging in CWL or transient waterlogged conditions in SMF). *, **, *** indicate significance at P<0.05, P<0.01, and P<0.001, respectively. ns, not significant. Values are means from three replications. Except for NNR at 45 DAS, means followed by the same letters are not significantly different within each water treatment at 5% LSD.

Root Plasticity Index

The root plasticity index (RPI) was observed in TRL and TLRL but not in TNRL at the end of initial drought in SMF (45 DAS) (Table 4). During this condition, RPI of both root number and lengths was not significantly different among N treatments under SMF. At 56 DAS, RPI of all component root length was significantly lower under SMF than in CWL across N treatments. However, the application of N significantly enhanced the plasticity in TRL and TLRL but not in TNRL after the imposition of transient waterlogging especially in 120N and 150N (Table 4).

Relationship among Root and Shoot Traits and Water Use Under SMF as Influenced by Rates of N Application

The relationship between water use (WU) and shoot dry weight and between total root length and WU under SMF was strongly positive and significant at 45 and 56 DAS (Figure 1). Similarly, the relationships between total lateral root length, total nodal root length, and number nodal roots with total root length were also strongly positive and significant at 45 and 56 DAS except for the relationship between number of nodal roots and total root length at 45 DAS, which was not significant (Figure 2).

Discussion

INL60 was used in this study, an introgression line (BC_1F_3 QTL-1-64-B-1-1-1) derived from a cross between IR64 x CSSL47 (chromosome segment substituted line number 47). The CSSL47 was previously identified from a series of experiments using the chromosome segment substitution lines (CSSLs) from a cross between Nipponbare (*japonica*) and Kasalath (*indica*) subjected to soil moisture fluctuations (SMF) as simulated using hydroponics (Suralta et al., 2008), root box experiments (Suralta et al., 2010), and under field conditions with cycles of alternating waterlogged and drought conditions (Niones et al., 2012). This line generally had similar shoot and root growth with the recurrent parent Nipponbare under non-stressed conditions such as continuously waterlogged conditions (Suralta et al., 2008; 2010; Niones et al., 2012). However, under SMF, CSSL47 exhibited greater root plasticity than Nipponbare and consequently contributed to greater water use, dry matter production, and yield (Suralta et al., 2008; 2010; Niones et al., 2012). Fluctuating soil moistures is a unique stress, which requires set of root adaptations different from simple continuous waterlogging and progressive drought conditions because of the nature of soil moisture stress fluctuating between waterlogged and aerobic (mild drought)

Table 4. The root plasticity index of the different root lengths under continuously waterlogged (CWL) and soil moisture fluctuation (SMF) conditions and various rates of N application. End of initial drought (45 DAS) and end of waterlogging after transient drought (56 DAS) in SMF.

Water (W)/ Nitrogen (N)	Plasticity Index					
	TRL (cm plant^{-1})		TLRL (cm plant^{-1})		TNRL (cm plant^{-1})	
	45	56	45	56	45	56
CWL						
0N	-	-	-	-	-	-
60N	0.58^{a}	0.66^{a}	0.63^{a}	0.64^{a}	0.34^{a}	0.74^{b}
120N	0.07^{ab}	0.90^{a}	0.11^{a}	0.91^{a}	-0.14^{a}	0.85^{a}
150N	0.16^{ab}	0.79^{a}	0.21^{a}	0.79^{a}	-0.09^{a}	0.79^{b}
SMF						
0N	-0.40^{a}	-0.05^{b}	-0.45^{a}	-0.10^{b}	-0.20^{a}	0.12^{a}
60N	-0.45^{a}	0.01^{b}	-0.52^{a}	-0.04^{b}	-0.13^{a}	0.20^{a}
120N	-0.40^{a}	0.20^{a}	-0.52^{a}	0.16^{a}	0.17^{a}	0.35^{a}
150N	-0.47^{a}	0.30^{a}	-0.54^{a}	0.25^{a}	-0.12^{a}	0.45^{a}
W	**	*	**	*	ns	ns
N	ns	**	ns	**	ns	*
W x N	ns	Ns	ns	ns	ns	ns

*, **, ** indicates significance at P<0.05 and P<0.01, respectively. ns, not significant. Values are means from three replications. Means followed by the same letters are not significantly different within each water treatment at LSD 0.05.

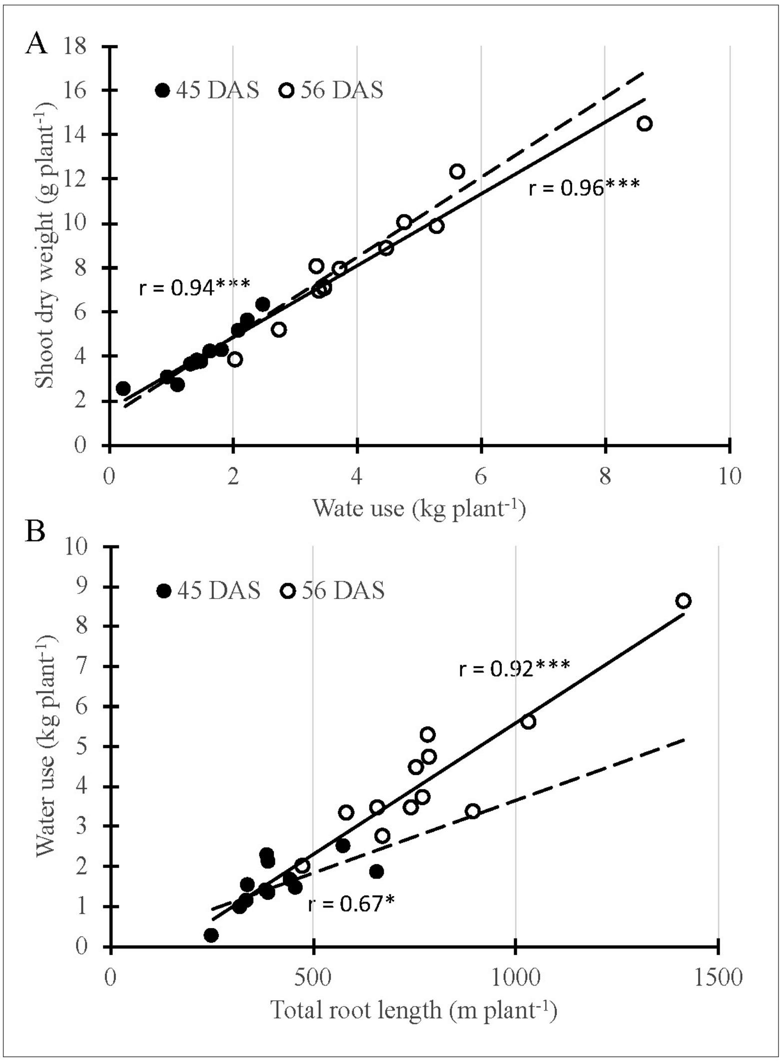

Figure 1. The relationship between water use and shoot dry weight (A) and total root length and water use (B) at the end initial drought until 45 DAS and at the end transient waterlogging at 56 DAS in soil moisture fluctuation condition. * and ***, significant at P>0.05 and 0.001, respectively.

conditions (Boling et al., 2004, 2008). Thus, CSSL47 possessed a unique root plasticity trait more adapted to SMF (Suralta et al., 2008, 2010; Kano et al., 2011; Niones et al., 2012).

Results showed that the shoot dry weight of INL60 was significantly reduced by SMF particularly after initial drought (45 DAS). However, such reduction in shoot dry weight was mitigated by application of high N via maintenance of high tillering production and higher RGR that compensated well despite reductions in leaf area at 45 DAS (Table 1). At 56 DAS, during which the 10 days of waterlogging had elapsed, INL60 quickly recovered from the effect of initial drought, in which the increase in shoot dry matter production was higher with higher rate of N application such as 120N and 150N (Table 1) via higher formation of additional tillers and leaf area development and thus RGR (Table 1). This above ground observations in INL60 was well supported by its leaf physiology under SMF. Although SC, Tr, and WU were generally reduced by SMF especially after initial drought (46 DAS), its leaf Ps was unaffected (Table 2). During the measurements at 45 DAS, the Ps was already low under CWL (Table 2), possibly due either to the time of last N application (25 days back) and the size of shoot at 45 DAS or both, which created a temporary N stress as the next application was done at 46 DAS simultaneously with the imposition of waterlogging in the SMF.

The cumulative whole plant transpiration through the measurement of WU was significantly higher with higher N application. At 56 DAS, these leaf physiologies especially SC, Tr, and WU had quickly recovered back to the levels similar to those in CWL conditions (Table 2). In particular, WU was enhanced by higher N application (Table 2) that resulted in the maintenance of higher dry matter production under SMF (Figure 1) via higher growth recovery (Table 1). Thus, application of fertilizer particularly N mitigate

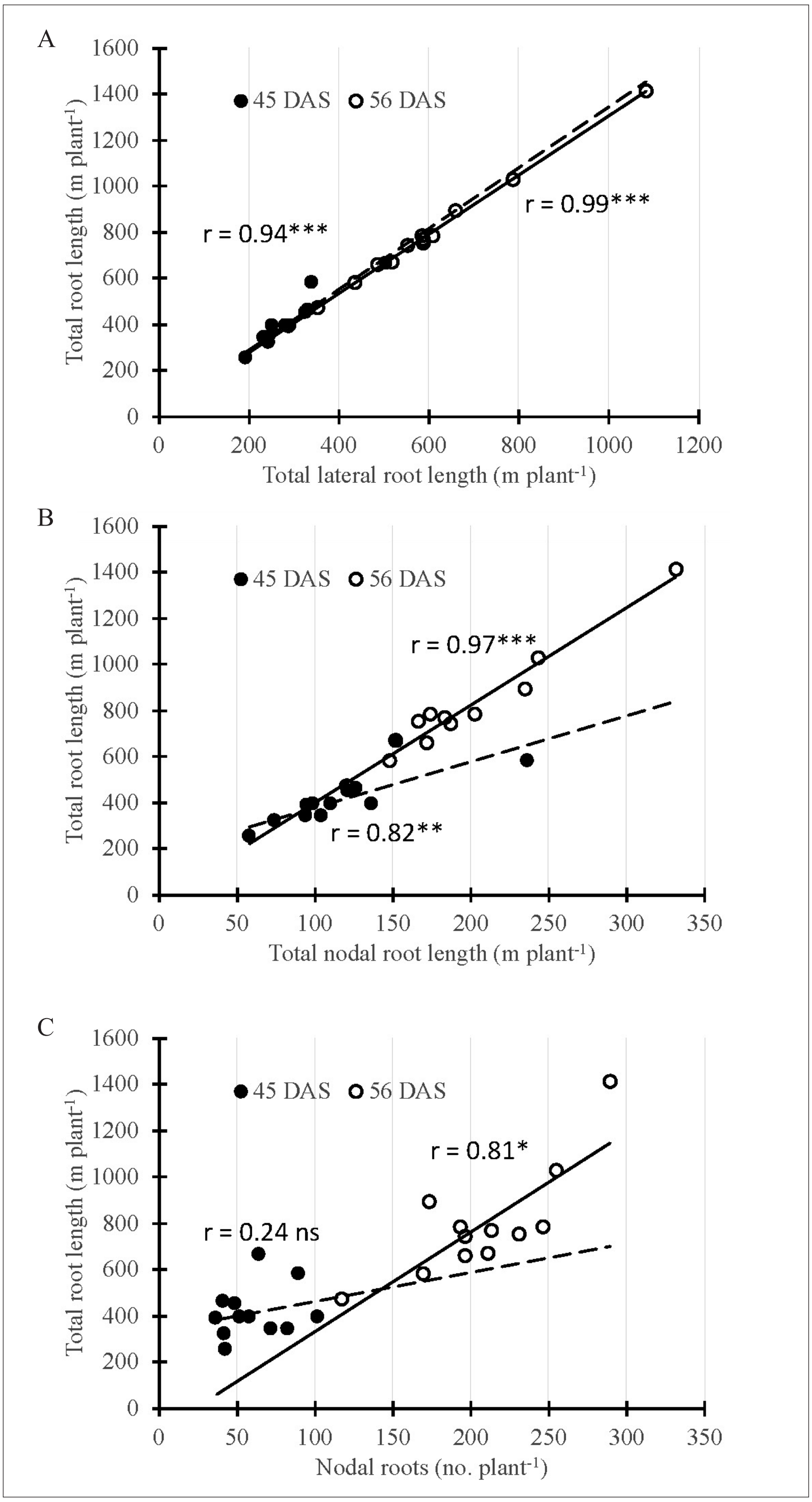

Figure 2. The relationship of total lateral root length (A), total nodal root length (B) and nodal root production (C) with total root length at the end initial drought until 45 DAS and at the end of transient waterlogging at 56 DAS in soil moisture fluctuation condition. ns, not significant; *, ** and ***, significant at P>0.05, P>0.01 and P>0.001, respectively.

the negative impacts of SMF through improved nutrient concentrations in the leaves, shoot growth and enhanced or maintained physiological processes (Percival et al., 2003; Alsafar and Al Hassan, 2009), and higher growth recovery during periods of transient waterlogging.

Plants have the ability to survive water deficit condition through drought (dehydration) avoidance mechanism by maintaining water (Serraj et al., 2009; Gowda et al., 2011) and nutrient uptake from the drying soil (Suralta, 2010). Tran et al. (2015) previously showed that root plasticity expression, especially under mild drought can be enhanced by N application regardless of N form indicating that root plasticity in response to water stress under field conditions is dependent on soil fertility status such as N level. In this study, however, root number and component root lengths were generally reduced by initial drought of SMF due to its lower expression of root plasticity at 45 DAS (Tables 3 and 4). However, the TRL quickly increased upon re-introduction of waterlogging (56 DAS) to almost similar lengths with those under CWL conditions particularly in SMF treatments with higher N applications. This increase in total root length was accomplished via the rapid increase in the total lateral root length and total nodal root length (Figure 2), which contributed to the maintenance in high water use under SMF (Figure 1).

The maintenance of leaf transpiration and thus WU under SMF particularly during drought-waterlogged condition is attributed to the rapid recovery in stomatal conductance and higher growth rate during rewatering (Suralta et al., 2010). The immediate recovery in transpiration, stomatal conductance, and photosynthesis after re-watering was dependent on pre-drought intensity and duration (Xu et al., 2010) and rates of N applied (Tran et al., 2014). This resulted in greater shoot dry matter production (Table 1) as reflected in higher WU (Table 2) through high expression of root system development (Figure 2) and root plasticity (Table 4).

Previous studies using the Nipponbare/Kasalath CSSLs showed that QTLs associated with lateral root plasticity under SMF are found in chromosome 12 regions namely the *qTLRN-12* at seedling stage and *qLLRN-12* at vegetative stage (Niones et al., 2015). These QTLs are present in INL60, which may be responsible to its high root plasticity index especially during the episodes of drought to waterlogging condition of SMF with higher N application (Table 4). Some QTLs associated to root plasticity response to limited N have been mapped (Manangkil et al., 2019). Thus, QTLs that are linked to root plasticity expression in response to high N application under SMF also need to be identified in future studies.

In this study, the plants were not grown until maturity when yield response could be measured but in exchange for the higher precision of root plasticity quantifications. Although root plasticity expression under SMF can greatly contribute to yield (Niones et al., 2012; Niones et al., 2015), additional study is recommended under field conditions to validate yield response.

Conclusions

Results showed that INL60 possessed root plasticity in response to SMF conditions and this can be further enhanced with higher N application, which consequently enhanced water use and maintenance in shoot dry matter production via high response of nodal and lateral root elongation during drought to waterlogged conditions. INL60 can be used for improving rice adaptation and productivity in rice growing environment prone to fluctuating soil moisture stress conditions either as a candidate line or source of root plasticity traits in varietal improvement program.

Acknowledgment

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FULL PAPER

SOCIOECONOMIC IMPACT OF ADOPTING RICE COMBINE HARVESTER IN THE PHILIPPINES

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Abstract

Adoption of rice combine harvesters (RCH) is low at the national level. In 2012, harvesting machine usage concentrated only in some Luzon provinces. This study assessed farmers' perception and level of awareness on RCH in five major rice-producing provinces. Treatment effects-two steps model was used to determine factors of RCH adoption and its effect on farmers' income and labor producitivity. Costs and returns and partial budget analyses (PBA) identified the adoption impact on profitability. Social welfare and economic effects of RCH adoption were also determined. PBA showed positive effects from combine harvester. Estimated change in net income between usage and non-usage of combine harvester amounted to PhP 5,000. This implies that RCH has more benefits than reaping paddy rice manually. Labor use and costs on harvesting and threshing of RCH adopters were significantly lower by 91 and 86%, respectively. RCH was perceived negatively due to its adverse impact on manual laborers during harvesting and transplanting. Treatment effect estimations showed that education, larger farm size, and landownership are significant and positively influence the likelihood of adopting RCH. Second step estimation under Model 1 showed that RCH adoption, yield, farming experience, and landownership have direct and positive effect on farmer's net income. Model 2 showed that adoption of this technology significantly lowers labor use. These results are potentially relevant when designing policies to increase RCH adoption and reduce production costs; thereby, increase net income of farmers, as well as boost their labor productivity. Agricultural skills training programs can also be provided to farm laborers with the increased use of farm machineries.

Keywords: *Combine Harvester, Adoption, Treatment Effects, Costs and Returns, Partial Budget.*

Introduction

Rice farming is generally labor and capital intensive. Among the labor activities in domestic rice production, harvesting and threshing entailed the bulk of work days with 32% of the total labor requirements (Bordey et al., 2017). Currently, Filipino rice farmers practice four methods of harvesting and threshing: manual harvesting and manual threshing, manual harvesting and mechanical thresher, mechanical reaper and mechanical thresher, and use of combine harvester.

Manual harvesting is the cutting of rice panicles using hand tools, usually operated by either family members or hired laborers. In contrast, mechanical reaper is a process of cutting rice using a machine, which requires less labor. Another method of mechanical harvesting is the use of combine harvester, a machine developed to cut, thresh, and pre-clean paddy grains in one operation (IRRI, 2016). Recently, other combine harvesters included bagging in its design (Praweenwongwuthi, et al., 2010). Japan began using combine harvesters in the 1970s (Pingali, 2007), which were introduced in the Philippines with modifications (De Padua et al., 2004). According to de Padua et al. (2004), cleaning and bagging were not part of the original designs of the machine but were later included because these were important requirements for Filipino farmers wanting to dispose of their harvest right after threshing and bagging. Manual threshing is done by hand beating the rice paddy against an object or through foot trampling in order to detach the grain from the straw. This is in contrast with mechanical threshing, in which rice paddy are fed into a machine (IRRI, 2016).

In 2011-2012, rice combine harvester (RCH) can harvest and thresh in an average of 3 mdha^{-1} (Arida et al., 2016). This was significantly lower than labor work days of other harvesting and threshing methods: manual harvesting and manual threshing (12 md ha^{-1}), manual harvesting and mechanical thresher (20 md ha^{-1}), and mechanical reaper and mechanical thresher (11 md ha^{-1}).

During the same period, harvesting and threshing accounted to 44% of the total labor and power costs (Arida et al., 2016). Overall, the percent share of labor and power cost was about 44% of the total rice production costs (Bordey et al., 2017). This is comparatively higher than in other neighboring countries like Vietnam (33%) and Thailand (33%) (Moya et al., 2016).

High percent share of harvesting and threshing in the total labor costs could be attributed to farm practices of our local farmers such as hiring 10-20 farm laborers during peak harvests. Moya and Dawe (2006) recommended the adoption of combine harvester to help reduce rice production costs. Unfortunately, Rice-Based Farm Household Survey (RBFHS) conducted in 33 major rice producing provinces in the Philippines showed that RCH had low adoption rate with only less than 1% of the interviewed farmers in 2011-2012. The predominantly used method was manual harvesting and mechanical thresher with about 88%, followed by manual harvesting and manual threshing at 11%, and mechanical reaper and mechanical thresher at 1%. Five years later, adoption rate of combine harvester increased to about 33% only (PhilRice-SED, 2019). Manual harvesting and mechanical thresher was still popularly used at 55%.

Even with significantly lower work days for combine harvester, labor costs of adopting this machine during the same period was found to be not significantly different with other harvesting and threshing methods. Hence, identifying the change in adoption rate and its effects on work days and costs can provide insights on the economic importance of agricultural machineries, particularly the combine harvester, to the Filipino rice farmers.

Presently, researches on technology adoption were conducted, however, few studies focused on rice combine harvester. In fact, study on factors affecting farmers' decision to adopt RCH is not yet available in the country. Addressing this research gap, this study aimed to provide insights on the socioeconomic impact of adopting rice combine harvester in the Philippines. Specifically, farmers' level of awareness, perceptions, and RCH adoption were assessed. Determinants of RCH adoption, as well as its effect on farmers' income and labor producitivity were also identified. In addition, social welfare and economic effects of RCH adoption were determined. This study generated policy implications that can help improve provision and support on the RCH use in the Philippines.

Overview of Combine Harvester in the Philippines

The need for mechanized agriculture started during the latter years of Green Revolution era in the 1960s, when rice farmers used modern varieties and intensified their cropping systems. In 1978, Regional Network for Agricultural Machinery (RNAM) was established in the University of the Philippines Los Baños, which boosted the development of mechanical harvesters in the country (Tado and Panagsagan, 2015). Trials of using foreign-designed mechanical harvesters started in 1980s, which included mechanical reapers, stripper-harvesters, and combine harvesters. Western-type designs were not applicable in Asia due to different field conditions in the region. This prompted several countries to develop rice combines suitable in Asian farms. In the Philippines, Japan head-feed rice combines were rejected by farmers due to its low harvesting capacity and high shattering losses while Thailand design was too big and heavy for the local rice fields (Tado and Panagsagan, 2015). In 1998, the Crop Tiger*,* a multi-purpose combine harvester designed by CLAAS firm, captured the interest of progressive farmers and local government officials. This model has been widely used and offered for custom harvesting of rice and corn in Isabela (Tado and Panagsagan, 2015).

Modernization of Philippine agriculture was strengthened through continuous development of machinery. In 2002, the Philippine Rice Research Institute (PhilRice) in collaboration with Briggs and Stratton Corporation, modified the Chinese-designed small rice combine, which originally has no cleaning and bagging functions. The new design was called rice mini-combine, which can harvest, thresh, clean, and bag rice grains in one single pass. This machine was introduced in the Philippines and Vietnam in 2004 with higher adoption in Vietnam. In 2009, manufacturing of mini-combine was phased out due to arrival of new rice combines like Kubota. This mini-combine harvester was commonly adopted in large farm areas. Since then, awareness on RCH increased through field demonstrations and training under the mechanization programs of the Department of Agriculture (DA). In a span of two years (2014-2016), DA distributed 216 units of RCH among rice-producing regions in the Philippines, 50% of which were allocated in Mindanao while the rest were distributed in part of Luzon and Visayas. Currently, Kubota and Yanmar are the leading brands of RCH used by most farmer-adopters in the country.

Methods

Site Selection and Description

The study covered five major rice-producing provinces: Nueva Ecija, Pangasinan, Tarlac, Cagayan, and Isabela. In 2019, Nueva Ecija is the top rice-producing province with annual production of 1.89M mt, followed by Isabela (1.19M mt), Pangasinan (1.08M mt), and Cagayan (0.83M mt). Tarlac ranked 7th among the major rice-producing provinces with 0.57M mt rice production.

Four of these provinces were among the five provinces that used machine in harvesting based from the 2011-2012 (RBFHS) national survey (Figure 1). Machines used include rice reaper and combine harvester. This study focused on sampled respondents with irrigated ecosystem rice areas for better comparisons between users and non-users. The list of municipalities considered are enumerated in Table 1.

Data Collection Methods

The study applied three procedures in data collection: focus group discussion (FGD), key informant interview (KII), and survey. Three batches of FGDs were conducted on three groups of rice stakeholders such as agricultural extension workers, users and non-users of combine harvester, and landless laborers. Landless laborers are paid workers hired to work on a specific farm operation, and do not own or manage rice farms. Composed of at least eight respondents, the focus groups were interviewed separately to ensure that each group will be able to express their sentiments openly without the potential of hostility from the other groups particularly on sensitive matters. Information from the FGD were also used to improve the survey questionnaire.

Table 1. Municipalities covered in the surveys.

Province	Municipality
Nueva Ecija	Aliaga, Cabanatuan City, Cabiao, General Natividad, Jaen, Llanera, Rizal, San Jose City, Science City of Muñoz, Sta. Rosa, Talavera, and Zaragoza
Tarlac	Camiling, Concepcion, La Paz, Moncada, Tarlac City, and Victoria
Pangasinan	Alaminos, Binalonan, Manaoag, Mapandan, Rosales, San Nicolas, Sta. Barbara, Sual, and Urdaneta
Isabela	Alicia, Cauayan, Cordon, Echague, Luna, Naguilian, Ramon, Reina Mercedes, San Isidro, San Manuel, and Santiago
Cagayan	Amulung, Baggao, Gattaran, Lallo, Pamplona, Peñablanca, and Tuao

KIIs were conducted among rice millers, palay traders, *cabecillas*, and service providers of combine harvesters. The results from the KII were also useful in drafting the survey questionnaire.

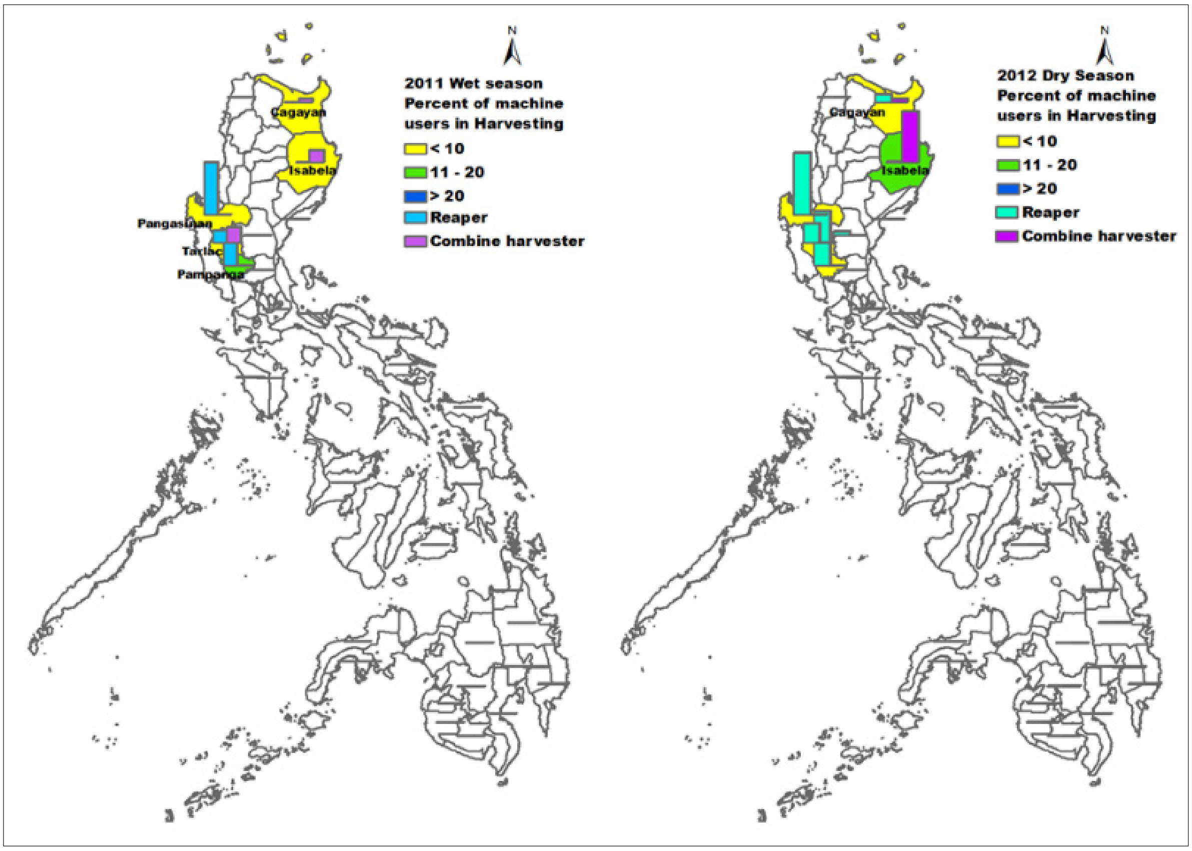

Figure 1. Percent of rice farmers who used machine in harvesting, 2011-2012 RBFHS.

Survey was conducted through personal interviews of rice farmers using a structured questionnaire. Farmers' socio-characteristic profiles, farming practices, production, marketing practice, and technology awareness were gathered from the survey.

Sampling Procedure

The survey covered dry season (January - June 2015) and wet season (July - December 2015) harvests. Sample respondents were from the master list of respondents from RBFHS Project who were selected using simple random sampling method. Sample respondents were segregated into two groups of farmers: users and non-users of combine harvesters. Users were farmers who adopted combine harvester during the reference period considered, while non-users were farmers who practiced traditional method of manually harvesting and mechanically threshing the rice paddy. Farmers totaling 450 per cropping season were interviewed using a structured questionnaire. During the first survey round, 90 respondents were interviewed per municipality, equally distributed to users and non-users.

The same set of farmers were interviewed during the two survey rounds, except for 35 replaced respondents in the latter survey. Replacements were due to the following: death, farmer was no longer the major decision-maker/operator of the farm, farmer migrated to other province that is no longer covered by the survey, and he/she stopped rice farming permanently.

Theoretical Framework

The analysis on farmer's decision to adopt a technology essentially employs dichotomous choice methods. As such, the choice between adopters and non-adopters are assumed to be influenced by some set of characteristics. The process of farmer's choice, whether to use or not to use a combine harvester, was determined using the expected utility framework (Valencia et al., 2009; Singerman et al., 2010; Kolady and Lesser, 2006). Autor (2004) explained that under expected utility theory, consumer choices may be selected among risky bundles (e.g., preferences over lotteries).

This follows that the utility function of the farmers is assumed Von Neuman-Morgenstern (NVM) expected utility function. This means that the *i*th farmer seek to maximize their expected utility over wealth (W) such that if expected utility with adoption $EU_{Ai}(W)$ is greater or equal with the expected utility with non-adoption $EU_{Ni}(W)$, then the *i*th farmer would prefer $EU_{Ai}(W)$ to $EU_{Ni}(W)$ or that they would be indifferent.

As the expected utility of farmers cannot be directly observed and measured, observable measures can be defined as factors influencing the distribution. It can be used as a vector of explanatory variables (farmers' characteristics and fixed inputs), X, of a choice made by an individual farmer *i,* and a random disturbance term (Thou et al., 2010; and Coble et al., 1996).

Following the discussion of Coble et al. (1996) on discrete choice analysis, in order to limit the non-linearity in the likelihood function, it is assumed that $EU_{Ai}(W)$ and $EU_{Ni}(W)$ can be expressed mathematically as follows:

$$EU_{Ni}(W) = \beta'_N X_i + \varepsilon_{Ni} \qquad (1)$$

$$EU_{Ai}(W) = \beta'_A X_i + \varepsilon_{Ai} \qquad (2)$$

In addition, the difference in expected utility from equations (1) and (2) can be written as:

$$\begin{aligned} EU_{Ai}(W) - EU_{Ni}(W) &= (\beta'_A X_i + \varepsilon_{Ai}) - (\beta'_N X_i + \varepsilon_{Ni}) \\ &= (\beta'_A - \beta'_N)X_i + (\varepsilon_{Ai} - \varepsilon_{Ni}) \\ EU_{Ji}(W) &= \beta' X_i + \delta_i \end{aligned} \qquad (3)$$

where $EU_{Ji}(W) = EU_{Ai}(W) - EU_{Ni}(W)$,

$\beta' = (\beta'_A - \beta'_N)$, and

$\delta_i = (\varepsilon_{Ai} - \varepsilon_{Ni})$.

Farmers will decide to adopt rice combine harvester if $EU_{Ai}(W) - EU_{Ni}(W) > 0$, and farmer will choose not to adopt the technology if $EU_{Ai}(W) - EU_{Ni}(W) < 0$.

Analytical Procedure

The study used the two types of treatment effects models (two-step) on the impact of technology adoption. Partial budget analysis, costs and returns, labor requirement and productivity, descriptive, and inferential statistics were also utilized.

Technology adoption models with binary dependent variables of values 0 and 1 usually employ Logit and Probit models (Ibrahim et al., 2013). However, in comparing the profitability of rice farmers with and without adoption of combine harvester, taking the difference of net income between the two groups may not be attributed to the technology adoption alone. There could be other factors contributing to the difference in net income that are unobservable characteristics of farmers like management skills (Varadan and Kumar, 2012). Similarly, direct comparison of labor productivity among users and non-users of technology may not consider other contributory factors leading to their differences.

Following the models used by Varadan and Kumar (2012) and Asfaw et al., (2011), this study estimated standard treatment effects model with two step model estimators. Model 1 focused on the effect of RCH adoption on net income of farmers while Model 2 aimed to determine RCH adoption's impact on labor productivity. These models eliminate biased estimation that could be encountered if a simple comparison of the average net income or labor productivity is used. The models consider the effect of an endogenously chosen binary treatment on another endogenous continuous variable, conditional on two sets of independent variables. Each model can be mathematically expressed as:

$$Y_i^* = \beta_0 + \beta_j X_i + \mu_i \quad (4)$$

$$R_i = \alpha + \gamma Z_i + \delta Y_i + \varepsilon_i \quad (5)$$

$$Y_i = \begin{cases} 1 \; if \; Y_i^* > 0 \\ 0 \; otherwise \end{cases} \quad (6)$$

where Y_i^* is the unobservable or latent variable for technology adoption, X_i are the set of characteristics that determines adoption, Y_i is the observable counterpart, a dummy variable taking the value of 1 if a farmer adopts combine harvester and 0 otherwise, R_i is a vector of net income/labor use of the i^{th} farmer, Z_i are vectors of exogenous variables believed to affect the net income/labor use, and μ_i and ε_i are random disturbance terms associated with the adoption of combine harvester and net income/ labor use models, respectively. β_j, γ and δ are the parameters to be estimated. The main focus is the δ parameter estimation, which represents the impact of adopting rice combine harvester to the net income and

Table 2. Definition of the variables considered in the adoption model.

Variables	Description
Dependent variables (Model 1)	
RCH	1 if farmer used/rented combine harvester; 0 otherwise
INC	Net income per hectare from rice farming (largest parcel)
Dependent variables (Model 2)	
RCH	1 if farmer used/rented combine harvester; 0 otherwise
LABORTLMDHA	Total labor man-days per hectare on all activities
Independent variables	
Adoption	
RCH	1 if farmer used/rented combine harvester; 0 otherwise
Farmer characteristics	
FARMEX	Length of farming experience in years
Human capital	
HHSIZE	Number of family household members working in the farm
EDUC	1 if farmer reached college level in education; 0 otherwise
Production	
YIELDDRY	Normal yield produced by farmer in kilograms/hectare (at 14% moisture content)
SEASON	1 if wet season reference period; 0 otherwise
Extension	
ATTSEM	1 if farmer attended rice related seminars; 0 otherwise
MEMBER	1 if farmer is a member of rice-based organization; 0 otherwise
Resource Constraint	
BORROWER	1 if farmer has access to credit; 0 otherwise
TENURIALSTAT	1 if farmer owns the land; 0 otherwise
FARMSIZE	Farm size of area planted in hectares
Shocks/External Effect	
LODGE	1 if farmer experienced lodging of rice; 0 otherwise
DRAIN	1 if farmer practices fully drained field before harvesting; 0 otherwise
EFFECT1	1 if RCH affected manual harvesters in the area; 0 otherwise
Location dummy	
PROVTAR	1 if farmer is from Tarlac province; 0 otherwise
PROVPAN	1 if farmer is from Pangasinan province; 0 otherwise
PROVISA	1 if farmer is from Isabela province; 0 otherwise
PROVCAG	1 if farmer is from Cagayan province; 0 otherwise

labor productivity. Table 2 summarizes the variables and descriptions used in the models.

Partial budget estimated the changes in costs and income, between the use of combine harvester and manually harvested-mechanically threshed paddy. Profitability was compared using costs and benefits between the two harvesting methods, with and without combine harvester adoption. Similarly, costs and returns and labor requirement and productivity were analyzed and compared. Descriptive and inferential statistics were also employed through means and frequency distribution. T-test was used to discern whether the differences in yield, inputs, costs, and labor use between users and non-users were statistically significant.

Limitation of the Study

The scope of the study is limited only to five provinces where machines are used in harvesting based in the 2011-2012 RBFHS. These provinces are pioneers in adopting the combine harvester in the country. Results from this study could only be true to these provinces and could not be used to represent the national data.

Results and Discussion

Farmer's and Farm Profile

Table 3 shows the profile of respondents. They were typically 55 years old, male with an average of 10 years of schooling (equivalent to secondary education graduate), 19 years of farming experience, and land owners with a household size of five members. About 75% of the total annual household's income came from rice farming. Other sources of income were from non-rice farming, self-employment (e.g., owning *sari-sari* store or *carinderia*, tricycle driving carpentry, sewing, and welding), and honoraria.

Table 3. Sociodemographic profile of farmers, 2015.

Item	User (n=299)	All (n=485)
Age (yr)	56	55
Sex (% male)	88	88
Education (yr)	10	10
Farming experience (yr)	25	19
Household size (no.)	4	5
Organization (% member)	62	58
Training (% trained)	57	56
Capital (% borrower)	51	52
Rice income share (%)	79	75

Comparing the sociodemographic profile among RCH users and non-users, Table 4 showed apparent differences between the two groups. Users were older, have more farming experience, higher educational attainment, and relatively smaller household size. RCH users were also members of organization, used their own capital in producing rice, and derived larger share of household income from rice farming. However, the users and non-users appeared seemingly the same in sex composition and training participation.

Generally, the characterization between users and non-users conformed with earlier studies that early adopters of technologies were more educated, engaged in higher social participation (indicated by organizational membership), and have greater degree of upward social mobility (Rogers, 1983). Rogers identified such sociodemographic characteristics as typical among progressive farmers. Further information on the landholdings between each farmer group showed that users were more well-off than non-users (Table 4).

Table 4. Rice area profile of farmers, 2015.

Items	User (n=299)	Non-user (n=186)	All (n=485)
Ave. no. of rice parcels	2.00	1.69	1.88
Parcel details (%)			
1 parcel only	47	63	53
2-3 parcels	42	30	38
More than 3 parcels	11	7	9
Total area cultivated (ha)	3.03	1.80	2.56
Ave. size of largest parcel (ha)	1.82	1.29	1.62
Distribution of farmers by farm size (%)			
0.10 to 0.50 ha	9	14	23
0.51 to 1.00 ha	16	19	35
1.01 to 1.50 ha	9	6	15
1.51 to 2.00 ha	6	5	11
More than 2.00	11	5	15
Tenure status (%)			
Owner	52	47	50
Non-owner*	28	44	34
Combination	20	9	16

*Non-owner includes farmers who are either tenants, lessees, renters, and/or tills the land free of charge.

On average, farmers cultivated more than one parcel, with 2.56 ha allocated for rice and the average size of the largest parcel planted with rice was 1.62 ha per cropping season. Two-thirds (66%) of the farmers own land that they cultivated in which 16% of them also rented land to further expand their rice production area. The rest (34%) do not own rice lands.

RCH users have more rice parcels cultivated than non-users. More than half (53%) of users farmed more than one parcel compared with 37% among non-users. Users also cultivated larger rice area and their largest rice parcel were bigger than non-users. About three-fourth (72%) of users owned the land as opposed to 56% among non-users. This conforms to earlier observations that farm size and land ownership, being wealth indicators, influenced the use of new technologies as it correlates with risk-aversion (Feder and Umali, 1993; Doss and Morris, 2001).

Most (70%) irrigated farmers rely on National Irrigation Authority (NIA) and Communal Irrigation System (CIS), whether conveyed by gravity or with the use of pumps (Figure 2). About 30% depend on other sources such as shallow tube wells (STW), small farm reservoirs, rivers or streams, deep well pumps, and electric pumps.

On average, farm distance to the nearest market was 5.8 km and the main road structure was concrete (85%). Farmers primarily used tricycles (67%) as their means of transportation, while some used hand tractors or *kuliglig* (16%) and jeepney or truck (11%). Thus, market access was not a problem for these farmers.

Period of Awareness

In 2009, PhilRice in collaboration with Briggs and Stratton Corporation, designed a rice mini-combine to help farmers reduce labor cost and drudgery during harvesting (Hermida, 2009). However, before 2011, the policy of the Philippine government was selective mechanization, which meant mechanizing should not lead to net labor displacement or must be combined with livelihood-generating projects (Ashburner and Lantin, 2013). The issue on labor displacement is often cited as one of the major stumbling blocks in implementing mechanization in agriculture (Dela Cruz and Malanon, 2017; and Hegazy et al. 2016). Thus, mechanization took a long time to push through as governments stayed neutral by neither rejecting nor pushing initiatives for agricultural mechanization (Lantin, 2016). This concern had empirical basis as observed in earlier studies (Medrano et al. 2016) and also supported in this study.

In 2011, the Department of Agriculture (DA), through the Philippine Center for Postharvest Development and Mechanization (PHilMech), implemented the Rice Mechanization and Postharvest Program (RMPP), which distributed machinery under the 85:15 scheme (Lantin, 2016). Under this arrangement the government shouldered 85% of the machinery cost while the remaining 15% were paid by the farmer-beneficiaries. The push for mechanization was further strengthened with the passage of the Agricultural and Fishery Mechanization Law (AFMech Law) in 2013.

The 85:15 scheme may have contributed to the use of more advanced and efficient RCH imported models as the machine started gaining popularity in 2012. RCH awareness was low before RMPP implementation. Among the respondents, only 3% were cognizant of RCH pre-2010 (1981 - 2009) with an additional 9% increase in awareness from 2010 - 2011 (Figure 3). However, within 2012 - 2015, 89% became aware of the technology. The three-year period coincided with

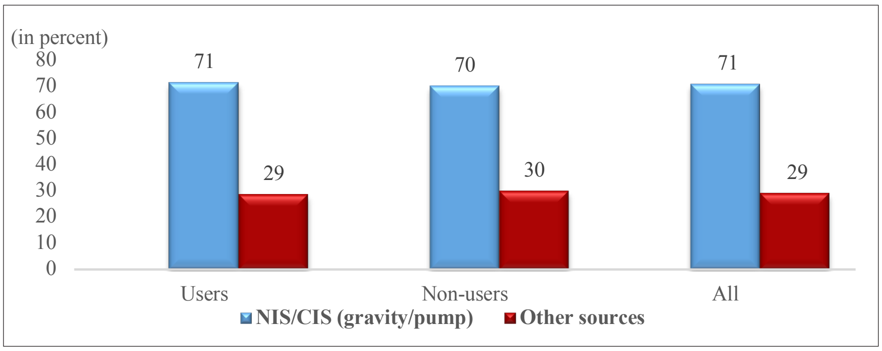

Figure 2. Irrigated farmers' source of irrigation for largest parcel, 2015.

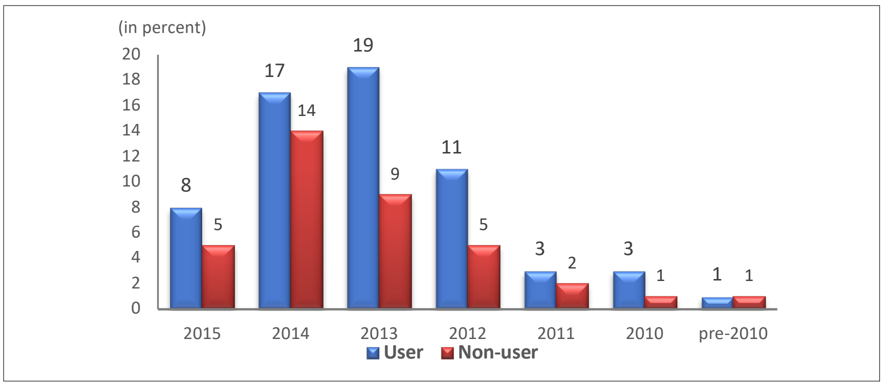

Figure 3. Period of awareness.

RMPP implementation. Further, there appeared to be an anticipation of the passage of the AFMech Law as the estimated number of RCH sold in 2013 was about 1,500 units (Lantin 2016).

Figure 4 showed that farmers obtained information about RCH through co-farmers (64%) and service providers (21%). Co-farmers being the main source of farmer information conforms to the 2011-2012 RBFHS result (PhilRice, 2014). Other respondents became aware through farm demonstrations (9%) conducted by government agencies and private entities. A few also became aware of the machine through agents of RCH service providers or by discovering the technology by themselves (4% each). Other RCH sources of information include mass communication, social media, and hired laborers.

Farmers' Reason for RCH Use or Non-Use

According to Berger and Luckmann (1966), people tend to construct their reality and their actions are guided by how they shape and view the world. Social construction approach in technology states that the emergence of particular technologies, choices between competing technologies, and the way these technologies are actually used owe a great deal to socially grounded forces like political power, social class, gender, and organizational dynamics (Volti, 2014).

Figure 5 presents the reasons cited by RCH users for using the machine. The top reasons cited were fast and convenient (48%), reduced cost (31%), unavailability of manual harvesters (19%), and reduced risk during rainy season (15%), which can be the relative advantage of RCH from other harvesting technologies.

The FGD supported claims that using RCH was fast and convenient. Farmers stated that it takes three to four hours to harvest, thresh, and bag a hectare of produced *palay* with machine than with manual harvesting, which takes three days or more. Farmers also reported that the average number of hours to harvest a one-hectare field was about one to two hours. Moreover, paddy rice is directly bagged so they can just sit and watch while the machine does its job.

Both FGD and survey results confirmed that the use of RCH can reduce cost because farmers no longer spent on sacks, twine, and food for the workers. Farmers also saved on hauling because some RCH service providers offered free hauling as part of the machine rental package. In a study among Ilocano farmers, Baradi and Kang (2018), also concluded that

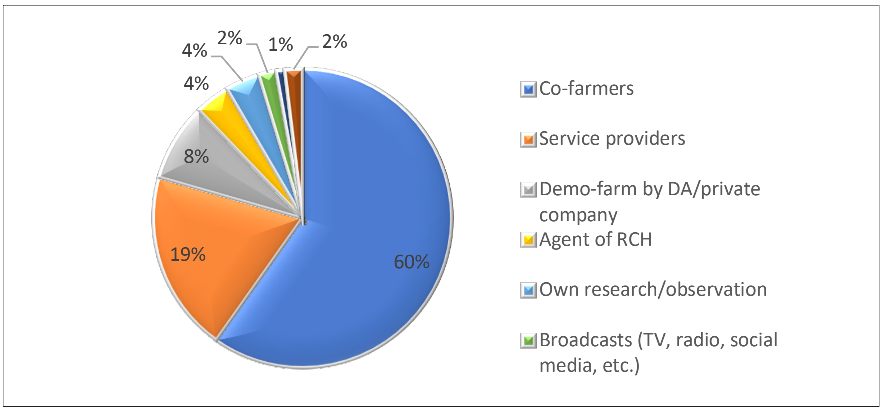

Figure 4. Sources of information on RCH.

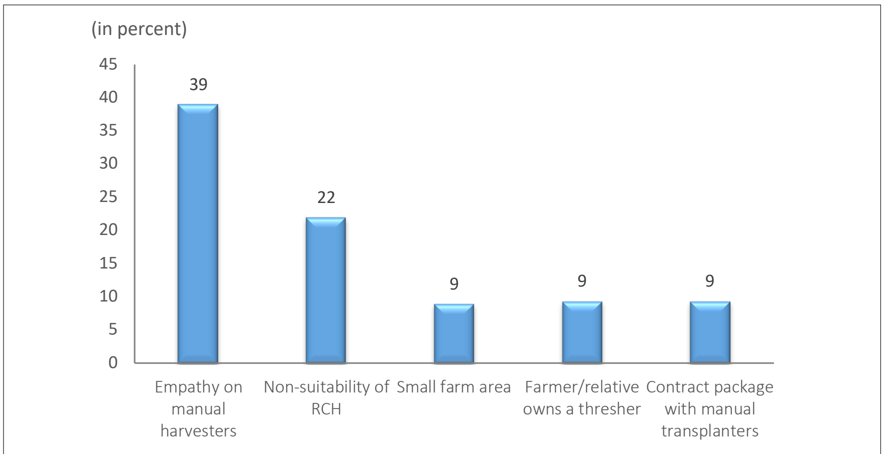

Figure 5. Main reasons for not using RCH, non-users.

cost was reduced with RCH. Lantin (2016) reported that the estimated cost of traditional method of manual harvesting and mechanical threshing shared 19% of the gross harvest while only 10% for RCH, consequently increasing farmers' income by reducing their cost spent in rice farming.

Further, RCH use addressed concerns about farm labor shortage during peak of harvesting season and reduced potential damage due to erratic weather condition, especially in the wet season. Decrease in labor availability in agriculture was already observed as early as 2010; thus, the need to mechanize (Amongo et al., 2011). The quick harvest and disposal is also a bonus for farmers as the risk exposure of rice crop to changing weather conditions is reduced (Lantin, 2016).

Douthwaite et al. (2001) stated that the relative advantage is the degree to which an innovation is perceived as being better than the technique it supersedes. Relative advantage is probably the most important in explaining adoption rate. Compatibility can also explain RCH adoption. Compatibility is the degree to which an innovation is perceived as consistent with the existing values, past experiences, and needs of potential adopters (Rogers, 1983). In short, RCH adopters value a form of rationalization in production activities characterized by efficiency, predictability, calculability, and control over their production (Ritzer, 2002), which are addressed by RCH.

Meanwhile, reasons for non-adoption of the technology include issue of non-compatibility. This issue is linked either in the social meaning construed with the technology or with the inherent flaw in its design.

The main reasons of non-users for not using the machine were empathy to manual harvesters (39%), non-suitability of RCH in the rice paddy (22%), small farm area (9%), the farmer or their relative owns a mechanical thresher (9%), and contract package with manual harvesters (9%) (Figure 5). Non-compatibility binds these reasons, which are tied to the constructed meanings associated with RCH.

The concept of *kapwa* addresses the farmer's decision to continue the conventional method as they do not want to disrupt the contractual *"pakikipagkapwa"* relationship they established in the community. They also want to maintain good relations with relatives owning mechanical threshers. According to Nadal (2011), it is the value of *kapwa* that ensures a Filipino to be generous to others, especially to relatives who are viewed as part of the family.

The issue of non-compatibility is also reflected in the RCH design as well as its applicability to the size of farmer's fields. Douthwaite et al. (2001) refers to this as fitness of the technology. Non-users stated that the RCH design was not suited to the characteristic of the paddy field which were muddy, water-logged, steeped and unleveled, have no access road, or have multiple plots. Others also stated that the size of their farms were small making it difficult for the RCH to maneuver. This shows that the social construction of the farmers with the machine is not only subjective in character but also has objective basis.

Farmers' Perception on Combine Harvester

Figure 6 showed that among users, 16% appropriately addressed RCH as combine harvester. However, more than half (55%) call it as reaper while others call it *halimaw* (36%). The percentages among the non-users in terms of how they call the RCH approximate those of the users (Figure 6). These also corresponded with the results of the FGD. For some of the farm laborers who participated in the FGD, they associated and called RCH as *gutom,* which translates to hunger or starvation.

Halimaw roughly translates to beast or monster, which has a positive or negative connotation in everyday communication. The positive association with word *halimaw* refers to the RCH's efficiency in harvesting, threshing, and automatically bagging the harvested rice. The negative association refers to the

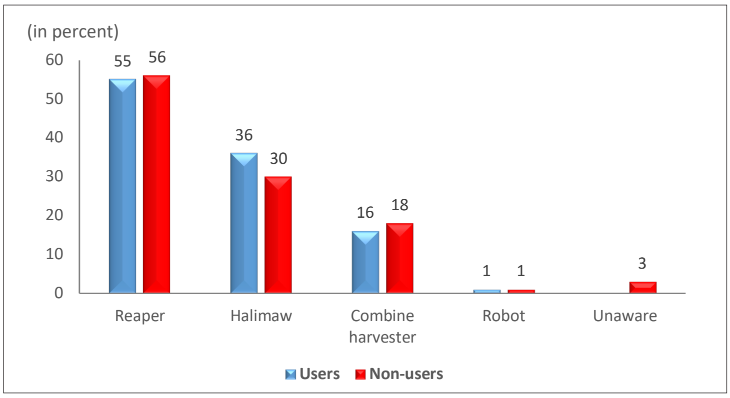

Figure 6. Local term for RCH.

perception that the machine lead to labor displacement in communities where it was introduced. Among users and non-users who called the RCH *halimaw*, 47% and 46%, respectively, used the term in a positive sense and the rest used it in the negative sense.

Positive perception. Among users, the top advantages cited for using the machine were: fast and convenient; thus, saves time and effort (82%); and 2) reduced cost due to freebies such as twine, sacks, and in some areas, hauling (57%) (Figure 7*)*. These reasons were consistent with their motives for using RCH, which is to rationalize the harvesting process. Even among non-users (Figure 7), they also acknowledged fast and convenient (72%) and reduces labor cost (52%) as RCH advantages.

This suggests that both farmer groups recognize the relative advantage of RCH against the conventional harvesting and threshing method. However, compatibility between the values of users and non-users factors significantly in the adoption of RCH. The users value efficiency, predictability, and control, which were their reasons for shifting to RCH. Meanwhile, non-users value the sense of community through the notion of *kapwa*.

Negative perception. Among users, 32% perceived that RCH use have no adverse impact in their community. Other users however, believed that some of the disadvantages of RCH use were: 1) it negatively impacts manual laborers and makes labor for transplanting unavailable (28%), 2) adversely affects the soil making land preparation difficult (23%), and 3) it leads to higher grain losses (13%) (Figure 8). Among non-users, 15% perceived that RCH use has no negative impact in their community (Figure 8).

Non-users stated RCH has adverse impact among manual laborers and disrupts labor availability during transplanting (37%). The FGD results indicated that this happens particularly in areas where farm laborers available for transplanting and harvesting were the same persons. Thus, labor displacement occurs with mechanization, which conforms to the observations of Medrano et al. (2016). They also observed that the sense of *bayanihan* (spirit of communal unity and cooperation) is degraded in the community. This sense of *bayanihan* is also connected with the Filipino value of *kapwa* (Nadal, 2011).

Other non-users also perceived that RCH use also adversely affects the soil. For the using, the machine makes land preparation difficult (15%). The same number (15%) also perceived that RCH use leads to higher grain losses. The perception of the users and non-users of RCH in relation to the perceived impacts of a technology supports at how farmers construct their reality influences their adoption of a technology or otherwise (Volti, 2014).

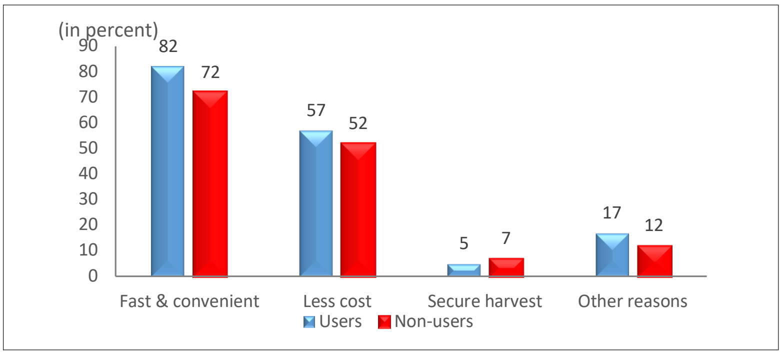

Figure 7. RCH positive perception.

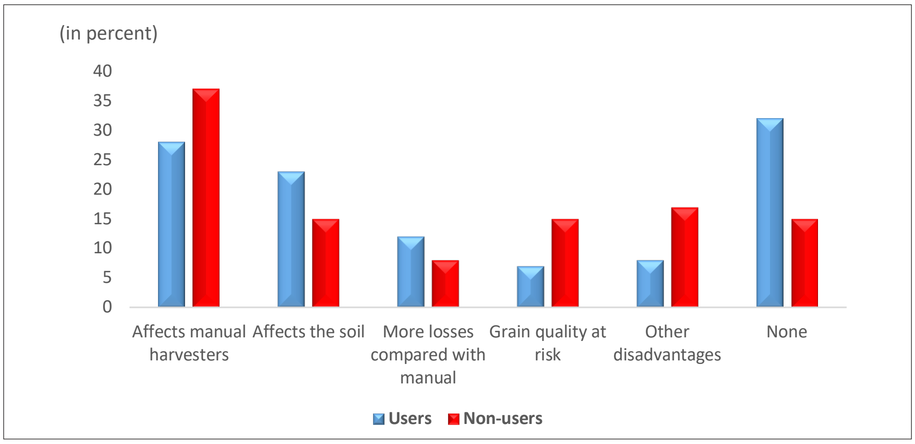

Figure 8. RCH negative perception.

Perceived effects on manual farm laborers. All of the users believe that laborers engaged in harvesting would be affected by the advent of RCH (Figure 9). Most (81%) perceived that manual harvesting laborers would be significantly affected. Almost half (45%) perceived that manual harvesters would be extremely affected and 36% sensed that these labor group would be very affected. For non-users, 83% perceived that manual harvesting laborers would be significantly affected by the RCH (Figure 9). More than half (51%) perceived that this labor group would be extremely affected while 36% believed that they would be very affected. This issue on labor displacement is often cited as one of the major stumbling blocks in implementing mechanization in agriculture (Dela Cruz and Malanon 2017; and Hegazy et al., 2016).

Perceived effects on rice farming activities. Besides harvesting and threshing, farmers perceived that RCH also affected land preparation and transplanting costs (Figure 10 and Figure 11). However, it can be observed that number of farmers who perceived such changes in land preparation cost increased after one cropping season (Figure 10). On one hand, the number farmers who perceived increase in transplanting costs remained almost the same (Figure 11).

The possible reason for the perceived increase in transplanting cost was due to labor displacement in harvesting. Hence, the farm laborers opted to offset their income losses by imposing additional fee during transplanting. Assuming that there is a substantial increase in transplanting cost as demanded by the farm laborers, this might force farmers to shift either to direct seeding to cut cost or to mechanical transplanting if machine use proves to be cost-saving and has no negative impact on rice yield.

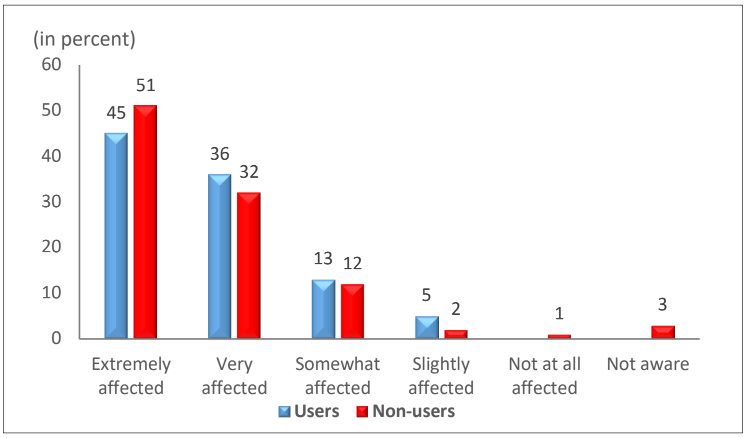

Figure 9. Perceived effects on manual harvesting laborers.

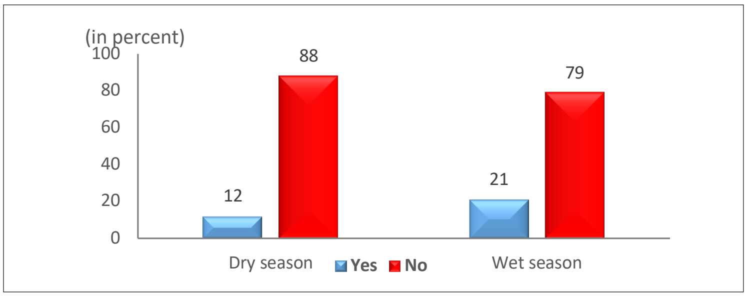

Figure 10. Perceived increase in land preparation cost by season, 2015.

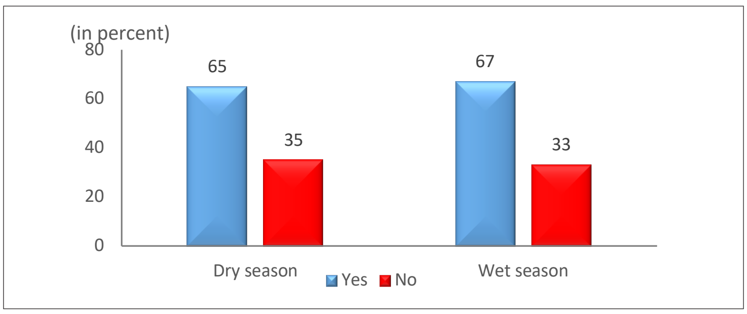

Figure 11. Perceived increase in transplanting cost by season, 2015.

Farmers' Intensity of Adoption

This section looks at the intensity of farmers' use of RCH. Within 2011-2015, 1.7% used RCH each season (Figure 12). Many (41%) used RCH twice followed by those who used it once (19.1%). The figures coincided with the observation of an agricultural products entrepreneur. In a personal interview with Tilah (a private company in Munoz, Nueva Ecija), owner said that that the use of the machines among rice farmers rapidly increased, particularly in Central Luzon and Northern Luzon, within 2013-2014.

Area harvested using RCH increased from 2011 to 2015 (Figure 13). For the dry season (DS), the increase was almost 14 times since 2011 and a 10-fold increase in the wet season (WS). This change happened within a short period, which can be compared to the time when modern varieties were introduced in the 1960s. Generally, the RCH area harvested was higher in WS than DS. This could be attributed to the higher risk of damage that rice can incur. This conforms with the statements of the farmers as well as the studies by Lantin (2016) and Feder and Umali (1993) that if there is an opportunity, farmers will try to avoid the risks although the risk-tolerance and risk-adversity is different for each farmer given that they are heterogeneous.

Cost and Returns Analysis

Table 5 summarizes the comparison of costs and returns between users and non-users of combine harvester by cropping season for the covered provinces. Rice production in dry paddy ranged 4.7-5.0 kg ha^{-1} (WS) and 5.1-5.7 kg ha^{-1} (DS). Results showed a 5% (WS) and 7% (DS) yield difference between users and non-users. These can be attributed to production losses incurred during manual harvesting and mechanical threshing. Correspondingly, total production costs of users were significantly lower than non-users with PhP 3,400 - 4,100 difference. This is primarily due to lower labor costs incurred by RCH users. Differences can also be attributed to other factors like costs of sacks and twine, food, and land rental. Overall, users have significantly higher net returns than non-users. This income advantage of RCH users was consistently observed for both cropping seasons.

Factors Affecting Adoption, Income, and Labor

Rice combine harvester arrived in Southeast Asian countries as early as 1970s. However, studies showed that adoption rate was low in 1980s due to small farm sizes and low harvest wages (Pingali et al., 1997).

Technology adoption analysis commonly use logit and probit models as its probabilities are bounded

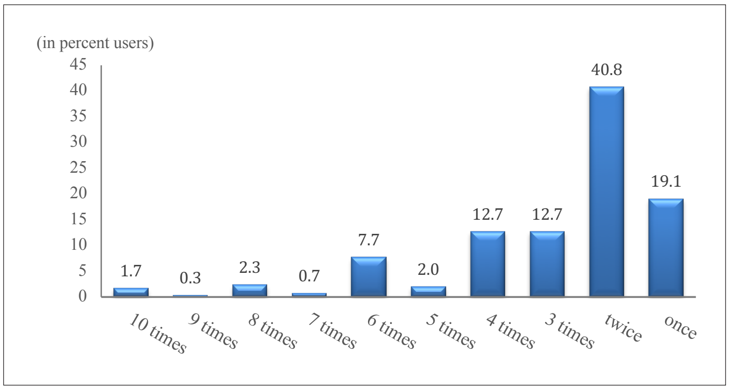

Figure 12. Frequency of usage by RCH adopters, 2011-2015.

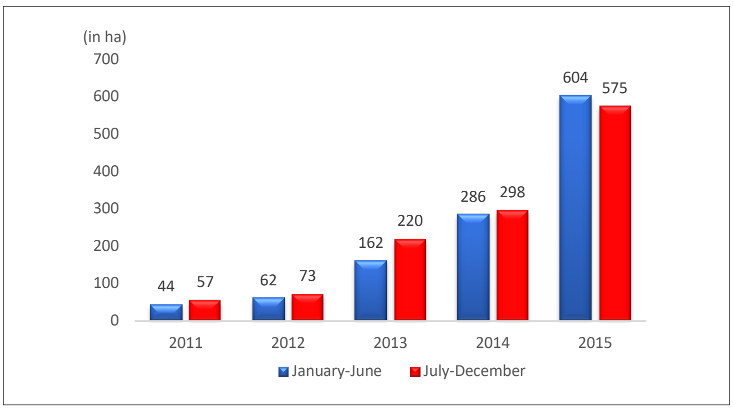

Figure 13. Area harvested (ha) by RCH through the years, 2011-2015.

Table 5. Costs and returns of paddy production in selected rice producing provinces in the Philippines, 2015 DS and WS.

Item	Wet Season		Dry Season	
	User	Non-User	User	Non-User
Returns				
Yield (kg ha^{-1}, fresh paddy)	5,463.86	5,206.30	6,205.27	5,578.06
Yield (kg ha^{-1}, dry paddy)	5,019.10	4,782.51	5,700.16	5,124.01
Dry paddy price (PhP kg^{-1})	15.67	15.62	16.97	16.87
Gross Revenue (PhP kg^{-1})	78,768.76	74,671.55	96,862.51	86,739.46
Costs				
Seed	3,054.61	2,969.32	3,883.85	3,741.68
Fertilizer	8,310.75	8,308.45	8,774.50	8,792.15
Pesticide	2,383.17	2,344.65	2,437.34	2,381.59
Hired labor	12,046.67	15,181.40	12,795.85	17,996.23
Operator, family, and exchange labor (OFE)	2,234.93	3,066.58	2,997.77	4,278.26
Irrigation	1,855.44	1,603.42	2,978.08	2,915.54
Food	1,381.92	1,898.58	1,399.05	1,601.60
Transportation	129.61	135.14	138.74	226.24
Animal and machine rental	7,020.76	4,805.61	9,488.12	5,415.66
Fuel and oil	785.43	873.52	936.82	921.23
Sacks and twine	41.8	897.51	97.82	1,116.21
Land tax	200.83	179.75	314.18	368.24
Land rent	12,188.22	13,345.21	12,574.70	12,819.03
Repair and maintenance	1,744.48	1,115.69	1,037.72	1,065.17
Other costs	383.44	294.91	213.22	170.09
Interest on capital	1,225.57	1,451.06	1,121.97	1,516.05
Total production costs	54,987.62	58,454.31	61,189.72	65,324.95
Cost (PhP kg^{-1})	11.4	12.61	11.27	13.61
Net income from rice farming (P ha^{-1})	23,781.14	15,802.40	35,672.79	21,414.51
Net returns from rice farming + own labor, land, and capital (P ha^{-1})	39,429.87	33,665.24	52,367.22	40,027.85

between 0 and 1. However, this study employed two types of treatment effects-two step models: Model 1 is treatment effect on combine harvester adoption and net income, Model 2 focused on treatment effect on combine harvester adoption and labor use. These are another methods to estimate the causal impact of technology adoption at the same time provide unbiased estimation of adoption impact on net revenue and labor use. These methods consider the unobservable characteristics of farmers that may contribute to the difference in net income (Varadan, et al. 2012; Asfaw, et al. 2011) and required labor among users and non-users of combine harvester.

Tables 6 and 7 summarize the results of the treatment effects – two step models. Model 1 focused on treatment effects on combine harvester adoption and net income while Model 2 focused on treatment effects on combine harvester adoption and labor use. Both models were statistically significant at one percent level as shown in the Wald Chi-square test. The significance of lambda in each model implied that the selectivity biased estimation from effects of unobservable factors such as characteristics of farmers has been corrected. The results also implied that separate comparison of net income or labor use between users and non-users of combine harvester may result to biased estimation.

Determinants of Rice Combine Harvester Adoption

The first step in this model used probit estimation to determine the factors affecting farmer's decision to adopt RCH. Results showed that three factors significantly and positively influenced farmers' adoption decision (Table 6). The probability of adoption was higher among farmers who reached college level, with larger farm size, and area landowners. Result on significance of education is consistent with the findings of Varadan et al. (2012) and Bruce et al. (2014) who found that educated farmers can better understand the information and importance of the technology. Hence, formal schooling plays a vital role in adoption of combine harvester.

Higher educational attainment can provide greater allocative ability than worker ability (Feder, Just, and Zilberman, 1985). There are two types of human capital: allocative ability and worker ability. Allocative ability is the ability to deal with new situation and learn new techniques, closely related to intelligence and formal education. They have the ability deal with disequilibrium, assess problems, make rational choices, and adjust to change. Worker ability has the capacity to perform hard manual tasks.

Results showed that farmers with larger farm size were more likely to adopt combine harvester. This also conforms to the results of Ayob (1979) and Hassena (2000) on their combine harvester adoption studies. Large farm areas can be associated with greater wealth, more stable control of land, greater access to credit and information related to farming. Thus, increase in farm areas has direct and positive effect on adoption. In addition, Figure 5 shows that 9% of farmers were hesitant to use combine harvester due to smaller farm size, opting them to hire manual harvesters instead. It was also indicated that about 14% of non-user has farm area ranging from 0.10 to 0.50 ha, a significantly low area compared with average farm of 2 ha (Table 4).

Land ownership showed a positive relationship to combine harvester adoption. This factor was also found as significant variable influencing rice farmers decision to adopt Urea Deep Placement (UPD) technology in Northern Region of Ghana (Azumah et al., 2016). Result showed that farmer-landowners may take the risks involved in any technology adoption. An example of the risk is the issue on how the combine harvester may damage the field. Farmers assumed that the impact of heavy farm machinery on the soil condition causes difficulty in preparing the land for next planting season; thus, increasing either the labor cost or requirement on land preparation. This is in contrast with non-landowners, who make possible restrictions from using any new technologies in the rice field.

Effects of RCH Adoption on Farmers' Income

The second step in Model 1 (Table 6) is the use of estimated predicted probabilities from the probit model and incorporating it as instrumental variable in the standard treatment effects model. The main focus in this model is the estimated coefficient of RCH adoption, which represents its impact to the net income of farmers, holding other factors constant.

Table 6 shows that there were four explanatory variables found to have direct and positive effect on net income that include the adoption of RCH, yield, years in farming experience, and land ownership. This implies that farmers who use RCH receives higher income than farmers who manually harvest their rice paddies. The expected positive sign of the combine harvester adoption's effect confirms the assumption that the use of machine reduces labor costs, in which bulk of the production costs is usually entailed; thus, farmers receive higher income.

Similarly, farmers who own the land have higher net income than non-landowners due to fixed land rents. Higher yield also results in higher income, holding others factors constant. Results indicated

Table 6. Estimated coefficients of the treatment effects-two step model on combine harvester adoption and its effect on income.

Variables	RCH Adoption			Net Income/ha		
	Parameter Estimates			Parameter Estimates		
	Coefficient	SE		Coefficient	SE	
RCH	-	-		3.7518	1.0302	***
YIELDDRY	-	-		0.7152	0.0589	***
FARMEX	0.0019	0.0036		0.0188	0.0079	**
HH SIZE	-0.0207	0.0224		-	-	
EDUC	0.0408	0.0202	**	-0.0359	0.0486	
ATTSEM	0.0867	0.0964		-	-	
FARMSIZE	0.1674	0.0417	***	-	-	
MEMBER	0.1431	0.1		-	-	
BORROWER	-0.077	0.0903		-	-	
TENURIALSTAT	0.205	0.0959	**	0.5683	0.2157	***
LODGE	-0.0248	0.0902		-	-	
DRAIN	-	-		-	-	
EFFECT1	0.9968	0.6225		-	-	
SEASON	-0.1967	0.2067		0.4476	0.4671	
PROVTAR	0.0074	0.1414		-	-	
PROVPAN	0.185	0.1519		-	-	
PROVISA	-0.0138	0.1383		-	-	
PROVCAG	0.0059	0.1407		-	-	
CONSTANT	-1.3946	0.6587	**	2.9869	0.5637	***
LAMBDA				-2.5214	0.9824	***
RHO				-0.7888		
Goodness-of-fit:						
Wald chi-square (9)	79.5	***				
Number of observations	861					

that farmers with longer farming experience receive higher net returns in rice production. These may be attributed to combined skills and learnings in rice farming through time that contributed significantly in attaining higher net income.

Effects of RCH Adoption on Labor

Table 7 presents the second step in Model 2 on the impact of estimated coefficient of RCH adoption to labor use per hectare, holding other factors constant. Results showed that RCH adoption has a direct and negative relation to labor use. This implies that farmers who use RCH during harvesting period incurred lower labor use in rice farming. Results conform to the studies that labor use in harvesting using RCH usually averages 3 md ha^{-1} while manual harvesting is finished within 20 md ha^{-1} (Arida et al., 2016). Other factors found significant were location dummy variables implying that farmers from Pangasinan and Cagayan have probably higher labor use in rice farming relative to Nueva Ecija. In addition, availability of farm laborers may also be higher in Pangasinan and Cagayan relative to Nueva Ecija as both provinces have relatively higher labor requirements on harvesting and threshing than the base province.

Potential Impact

Labor Requirement and Cost

Harvesting and threshing require high labor use in the Philippines especially when manually harvested. Based from 2011-2012 RBFHS results (Arida et al, 2016), harvesting and threshing constituted the biggest share with 32% of the total labor use, which contributed to the reduction in labor productivity. Results indicated that farmers who used RCH had less labor usage on harvesting and threshing than non-users (Table 8). On average, RCH users used only 1.7 md ha^{-1} than non-users with 18 md ha^{-1}, resulting in 91% reduction in labor use. Correspondingly, labor costs were PhP 1,000 and PhP 7,300 for users and non-users, respectively. About 86% cost difference between the two types of farmers. These costs were payments to machine operators and laborers. In some areas, manual harvesting is still practiced especially in areas where RCH cannot be operated/applicable.

This section also contains discussions on perception on change in land preparation and crop establishment due to RCH use. In terms of farmers' perception, about 84% of the total farmers disproved

Table 7. Estimated coefficients of the treatment effects-two step model on combine harvester adoption and its effect on labor use.

Variables	RCH Adoption			Labor Use/ha		
	Parameter Estimates			Parameter Estimates		
	Coefficient	SE		Coefficient	SE	
RCH	-	-		-1.2349	0.2095	***
YIELDDRY	-	-				
FARMEX	0.0019	0.0036		-0.00005	0.0016	
HH SIZE	-0.0207	0.0224		-	-	
EDUC	0.0408	0.0202	**	0.011	0.0097	
ATTSEM	0.0867	0.0964		-	-	
FARMSIZE	0.1674	0.0417	***	-	-	
MEMBER	0.1431	0.1		-	-	
BORROWER	-0.077	0.0903		-	-	
TENURIALSTAT	0.205	0.0959	**	0.0584	0.0462	
LODGE	-0.0248	0.0902		0.0066	0.0391	
DRAIN	-	-		-0.0203	0.0552	
EFFECT1	0.9968	0.6225		-	-	
SEASON	-0.1967	0.2067		-0.149	0.0927	
PROVTAR	0.0074	0.1414		-0.034	0.0618	
PROVPAN	0.185	0.1519		0.1417	0.0641	**
PROVISA	-0.0138	0.1383		-0.0387	0.0608	
PROVCAG	0.0059	0.1407		0.1987	0.0609	***
CONSTANT	-1.3946	0.6587	**	-0.002	0.0006	***
LAMBDA				0.5517	0.1303	***
RHO				0.9902		
Goodness-of-fit:						
Wald chi-square (9)	110.34	***				
Number of observations	861					

the issue on increased labor cost during land preparation operation (Table 8). This is in contrast with the issue on transplanting, in which 62% of respondents believed that the presence of combine harvesters resulted in increased labor costs of hiring manual transplanters.

Table 9 shows that, on the average, labor requirements and costs on land preparation for farmer users were low by 25% and 20%, respectively. On the other hand, Table 10 shows that labor costs on transplanting were only 1.42% higher than non-adopters. During peak transplanting, it is up to the manual laborers whether they would work for farmers who plan to hire combine harvester or demand higher payment for transplanting to compensate the expected income loss from manually harvesting the rice paddy.

Effect on Labor Productivity

Reduction in labor use directly influences labor productivity in rice farming. Labor productivity measures the rate of rice production (kg) per work day. RCH users have significantly higher labor productivity than non-users (Table 11). Across provinces, Isabela and Nueva Ecija attained the highest labor productivity with 167 kg work-day^{-1}. Cagayan has the lowest labor productivity but users of RCH were still 65% higher than non-user. This implies that the use of labor-saving technologies such as RCH can boost labor productivity.

Effect on Income

Partial budget analysis showed positive effects of using RCH on income due to cost reduction in labor that includes harvesting, threshing, and hauling (farm to road). Other costs considered were fuel and oil (for harvesting and threshing and hauling), machine custom fees (threshing and hauling), sacks and twine, as well as food costs during harvesting and threshing. Table 12 shows that the total reduced costs amounted to PhP 11,800 ha^{-1}. Adverse effects associated with RCH adoption is the rental fee for RCH amounting

Table 8. Labor requirements and costs on harvesting and threshing, 2015 DS and WS.

Province	Type of Farmer		Difference	% Change
	User	Non-User		
Labor requirements ha^{-1}		(in md ha^{-1})		
Cagayan	1.65	21.47	-19.82	-92.31
Isabela	1.89	18.14	-16.25	-89.57
Nueva Ecija	1.75	15.92	-14.17	-88.99
Pangasinan	1.62	17.24	-15.63	-90.63
Tarlac	1.56	16.83	-15.28	-90.75
Average	**1.7**	**18**	**-16.3**	**-90.57**
Labor costs ha^{-1}		(in PhP)		
Cagayan	1,013.84	7,103.87	-6,090.04	-85.73
Isabela	1,196.53	7,090.94	-5,894.41	-83.13
Nueva Ecija	1,057.34	8,970.97	-7,913.64	-88.21
Pangasinan	927.19	6,399.82	-5,472.64	-85.51
Tarlac	1,020.00	6,927.21	-5,907.21	-85.28
Average	**1,045.28**	**7,321.30**	**-6,276.02**	**-85.72**

Table 9. Labor requirements and costs on land preparation, 2015 DS and WS 2015.

Province	Type of Farmer		Difference	% Change
	User	Non-User		
Labor requirements ha^{-1}		(in md ha^{-1})		
Cagayan	6.52	8.04	-1.52	-18.9
Isabela	4.33	7.23	-2.9	-40.12
Nueva Ecija	5.02	5.24	-0.21	-4.06
Pangasinan	6.79	7.35	-0.56	-7.59
Tarlac	5.64	8.49	-2.84	-33.51
Average	**5.64**	**7.27**	**-1.63**	**-22.45**
Labor costs ha^{-1}		(in P)		
Cagayan	1,572.73	1,901.72	-328.99	-17.3
Isabela	1,289.96	1,941.11	-651.15	-33.55
Nueva Ecija	1,332.16	1,388.83	-56.67	-4.08
Pangasinan	1,991.44	2,199.25	-207.81	-9.45
Tarlac	1,571.76	2,243.84	-672.08	-29.95
Average	**1,545.40**	**1,925.36**	**-379.96**	**-19.73**

to PhP 6,800. Overall, income advantage of using RCH amounted to PhP 5,000 or about 20% of the net income. Table 13 summarizes the partial budget analysis among the covered provinces. Nueva Ecija has the highest income advantage from RCH adoption with PhP 6,000 followed by Cagayan and Pangasinan.

Social Effect

One of the effects of RCH use is that it is one more step in making rice production rationalized. Rationalization involves organizing belief and action so as to maximize the probability of achieving a defined end: attaining a rational belief system and methodical way of life or attaining an economic system oriented toward improving the standard of living and increasing the production of wealth" (Ritzer 2005:625). This suggests that the value of social life as means to an end is further dictated by rules and regulations (Scott, 2006). Rationalization is currently seen to reflect the paradigm of fast food industry, which are: efficiency, calculability, predictability, and

Table 10. Labor requirements and costs on transplanting method, 2015 DS and WS.

Province	Type of Farmer		Difference	% Change
	User	Non-User		
Labor requirements ha^{-1}		(in md ha^{-1})		
Cagayan	25.89	28.7	-2.81	-9.79
Isabela	20.48	21.34	-0.86	-4.04
Nueva Ecija	24.17	24.15	0.02	0.07
Pangasinan	23.88	23.39	0.49	2.09
Tarlac	23.35	22.99	0.36	1.56
Average	**23.6**	**24.42**	**-0.82**	**-3.37**
Labor costs ha^{-1}		(in P)		
Cagayan	6,644.43	6,647.93	-3.5	-0.05
Isabela	6,287.17	6,412.08	-124.91	-1.95
Nueva Ecija	6,171.23	6,053.00	118.23	1.95
Pangasinan	7,042.93	6,854.37	188.56	2.75
Tarlac	5,818.02	5,472.79	345.24	6.31
Average	**6,405.64**	**6,315.87**	**89.77**	**1.42**

Table 11. Labor productivity in select provinces, by type of farmer, 2015 DS and WS.

Province	Type of Farmer		Difference	% Change
	User	Non-User		
	(in kg man-day^{-1})			
Cagayan	114.78	69.46	45.32	65.24
Isabela	167.35	97.88	69.48	70.98
Nueva Ecija	163.98	110.21	53.77	48.78
Pangasinan	119.57	79.06	40.52	51.25
Tarlac	149.76	82.87	66.89	80.71
Average	**143.6**	**87.88**	**55.72**	**63.41**

Table 12. Partial budget analysis on use of combine harvester, all provinces, 2015 DS and WS.

Positive Effects	Value	Negative Effects	Value
Additional Income	0	**Reduced Income**	0
Total additional income	0	**Total reduced income**	0
Reduced costs		**Additional costs**	
Labor cost on harvesting and threshing	6,344.04	Rental fee (combine harvester)	6,797.14
Labor cost on hauling (farm to road)	96.14		
Fuel and oil (HT)	19.16		
Machine custom fee (thresher)	3,782.68		
Machine custom fee (hauling)	95.07		
Sacks and Twine	977.17		
Food cost for harvesting and threshing	490.89		
Total reduced costs	11,805.15	**Total additional costs**	6,797.14
A. Total additional income and reduced costs	11,805.15	**B. Total reduced income and additional costs**	6,797.14
C. Change in net income (A-B) = P5,008.01			

Table 13. Summary of partial budget results by province, 2015 DS and WS.

Province	Total Additional Income and Reduced Costs	Total Reduced Income and Additional Costs	Change in Net Income
Cagayan	11,725.34	6,394.49	5,330.85
Isabela	11,478.72	6,749.85	4,728.87
Nueva Ecija	13,568.32	7,528.10	6,040.22
Pangasinan	10,629.82	5,710.19	4,919.63
Tarlac	11,720.99	7,650.79	4,070.20

control through non-human technology (Plummer, 2010; Scott, 2006; Ritzer, 2002).

Applying this to rice farming, the reasons given by RCH users prioritize the values extolled by rationalization. This shows that these farmers were more inclined to regularize and standardize their lives, particularly in rice production. Moreover, this attitude to mechanize harvesting and threshing could be an indication or symptom that either farmers are more open to the idea of mechanizing major operations in rice production or that farmer characteristics are changing, in which mechanizing fulfills a need to modernize and rationalize.

This study showed apparent differences between the sociodemographic profile of users against non-users. Given this, the study expects that more progressive farmers will be engaged in farm mechanization. This is supported by an earlier study showing that progressive farmers are increasing (Malasa et al., 2008). Assuming that progressive rice farmers do not increase, the government and development workers must acknowledge that they are not homogeneous and there is a sizeable number of farmers who feel the need to mechanize farm operations.

At the onset, there is evidence that labor displacement occurs with the use of mechanization and this can be associated with social exclusion. The issue of social exclusion must be addressed to ensure that development is for all and not only for a particular segment of society. According to DFID (2005), social exclusion describes a process by which certain groups are systematically disadvantaged because they are discriminated against on the basis of their ethnicity, race, religion, sexual orientation, caste, descent, gender, age, disability, HIV status, migrant status or where they live. If the government turns a blind eye to the plight of the manual farm laborers, they will be excluded in development, which will again perpetuate the cycle of poverty and exclusion within this group.

There is also a need for the local government units (LGUs) and villages officials to mitigate the social disruptions that RCH might bring about. There is a need for pro-active community consultations among the stakeholders to ensure that community harmony is maintained. Other agencies and instrumentalities of the government should also be tapped by LGUs and village officials to foster more opportunity for the displaced laborer either by engaging them in other forms of employment or by introducing value-adding products.

Conclusion and Recommendations

Rice farming in the Philippines is generally labor and capital intensive compared with neighboring countries like Thailand and Vietnam. Majority of labor requirements and costs in domestic rice production can be attributed to harvesting and threshing. Thus, the use of combine harvester is highly recommended to help increase the land and labor efficiency of Filipino farmers. However, adoption of RCH in 2011-2012 was generally low with less than 1%, albeit its advantages on labor requirement. Similarly, low level of adoption can still be observed with 33% users based in the 2016-2017 RBFHS data. During this period, manual harvesting and mechanical threshing was predominantly used by 55% of the sample farmers. Costs of adopting RCH were not statistically significant with other methods of harvesting and threshing, which led to this study.

Results showed that there are factors significantly affecting farmers' decision to adopt RCH such as educational attainment, larger farm size, and tenurial status. Moreover, RCH adoption, yield, farming experience, and land ownership are significant and positive factors affecting the net income of rice farmers. Correspondingly, RCH users incurred significantly lower labor use per hectare than non-users.

Overall, results showed about PhP 5,000 change in the net income favoring farmers who used the technology. This implies that RCH use has more advantages than reaping the paddy rice manually.

One limiting factor that was not considered in this analysis was machine rental for land preparation. Adopters of combine harvesters reported the increase in the cost of machine rental during land preparation. This was due to the impact of the machine in the soil that led farmers to rent rotavators or four-wheel tractors. These machineries were required to better cultivate the land in preparation for the next planting season. Thus, the effect of this can be looked into in the future studies.

Similarly, studies can be done on introducing mechanization in a community to mitigate the possible negative impacts that might occur from its implementation. Moreover, a thorough study on cognitive dissonance can also be done to understand how farmers came up with the decision to adopt RCH. There is also a need to understand how the concept of *kapwa* can help in ensuring social harmony in the community even if farmers shift to farm mechanization. Further policy studies can also be conducted to evaluate the causes, issues, and challenges on labor displacement brought by high recommendation on RCH usage, and agricultural mechanization in general.

Findings from this study suggest the use of rice combine harvester can significantly reduce production costs particularly on labor costs on harvesting and threshing. Highly mechanized provinces such as Nueva Ecija and Isabela achieved higher labor productivity. Thus, adoption of labor-saving technology like combine harvester can boost the labor productivity of local rice farmers.

This study recommends a policy design in crafting programs that can intensify promotion and use of mechanization in rice production. Additional training programs on agricultural skills can also be provided to farm laborers with the advent of farm machineries from national agricultural programs.

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RESEARCH NOTE

CALAMANSI [*Citrofortunella microcarpa* (BUNGE) WIJNANDS]: A POTENTIAL INSECTICIDE AGAINST BROWN PLANTHOPPER (*Nilaparvata lugens* STÅL)

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Abstract

Brown planthopper or BPH (*Nilaparvata lugens* Stål) is an economically important rice pest in Asia. The use of synthetic-based insecticides was found to be effective against BPH. However, there are serious drawbacks in the use of chemical-based insecticides; thus, creating the need for safer insecticides from organic materials. This study determined the efficacy of calamansi, *Citrofortunella microcarpa*, against the third to fourth instar nymphs of BPH. Calamansi peels were dried, macerated for 48 h, and processed by rotary evaporation to obtain the crude peel extract. Varying solutions of 2, 4, and 6% calamansi peel crude extracts were formulated and applied to *N. lugens* in triplicates with 20 hoppers in each replicate. Percent mortality was recorded after 72 h of exposure to varying concentrations of calamansi peel extract. Increasing concentrations of calamansi peel extract was found to be directly proportional to the mortality of *N. lugens*. Furthermore, the LC50 and LC90 of calamansi peel extract was 8.37% and 54.57% respectively. Results suggest that calamansi peel extract is a potential insecticide against BPH and could be utilized as an organic plant-based insecticide.

Keywords: *Alternative Control, Botanicals, Bioinsecticide, D-Limonene, Probit Linear Regression.*

Introduction

Brown Planthopper or BPH (*Nilaparvata lugens* Stål) is one of Asia's significant rice pest that sucks plant sap and lays eggs in the tissues of plants (Cabauatan et al., 2009). This damages the rice plant and causes hopper burn, which are dry brown spots found on the leaves of the rice plant (Srivastava et al., 2009). The pest also transmits viral diseases on rice plants such as rice grassy stunt and rice ragged stunt (Suprihanto et al., 2015).

Brown planthopper was last reported to have caused significant damage in the Philippines on November 2017. In Samar alone, the damage was estimated at 4,000 ha of rice plants and costed about PhP18.75 million(Fernandez, 2017). Farmers in Pangasinan also appealed for help from the provincial government on February 2018 amid BPH attack on corn plants. According to the town's agricultural office, 750 ha of land are annually planted with corn and a percentage of this is already infested by the brown plant hopper (Pasion, 2018).

Farmers used chemicals as major control in managing brown planthopper. Pymetrozine, Glamore 80 WG (Ethiprole and Imidacloprid), Buprofezin 25 SC, Dinotefuram 20 SG, and Acephate 75 SP were proven to be effective BPH controls. These substances are all chemical-based (Seni and Naik., 2017). The most effective control for BPH is synthetic-based and can cause damage in rice plants due to residues.

However, due to the intensive use of insecticides to control this pest over years, resistance of BPH to most classes of chemical insecticides has been reported (Wu et al., 2018). BPH has been found to have significant resistance to insecticides such as Imidacloprid, Thiamethoxam, and Buprofezin (Wu et al., 2018). BPH management through Fipronil is not that effective in Albay, Philippines (Garcia, 2011). In addition, a 2010 data show an increase of resistance to Imidacloprid (Fabellar and Garcia, 2010).

Calamansi [*Citrofortunella microcarpa* (Bunge) Wijnands] is a citrus belonging to the family Ructaceae (Yee, 2014). The fruit is widely produced and used in beverages, flavor enhancement, and as a souring agent (Rodeo, 2016). Calamansi contains the compound D-Limonene, which is proven to have insecticidal activities (Yee, 2014). Limonene is a monocyclic monoterpene with a molecular formula of C10H16 and a constituent found in several Citrus peels (Yee, 2014).

Calamansi has flavonoids and alkanoids, which were found to have insecticidal effects (Hollingsworth, 2005). It was also found to be an effective larvicide against *Aedes aegypti* (Linn.) with determined lethal concentration 50 and lethal concentration 90 at 581 and 1009 mg l^{-1} respectively (De Villa et al., 2012).

This study determined the potential of calamansi crude peel extract as insecticide against BPH and determined the lethal concentration of calamansi crude peel extract required to kill 50% of the population (LC50) and 90% of the population (LC90). The findings of this study contributes to the knowledge of organic insecticides against BPH. It will help the local farmers to spend less on expensive pesticides and maintain safety to the rice plants and the environment.

Materials and Methods

Preparation of Calamansi Extract

Fresh calamansi fruits (15 kg) were obtained from a local market. The fruits were washed thoroughly, juiced, then manually peeled. The peels were wiped with clean cloth then all laid out on a surface without one overlapping with another. The peels were oven-dried in the school laboratory for 2 h at 79 °C. The dried calamansi peels were extracted at Department of Science and Technology Industrial Technology Development Institute-Chemical Energy Department (DOST ITDI-CED). The dried peels were pulverized using Wiley mill and macerated in 3 l of 95% ethanol for 48 h. The mixture was filtered, while the filtrate was concentrated using rotary evaporator at 60°C under vacuum for 2 h. The concentrated extract was further evaporated using water bath at 60°C to obtain a semi-solid extract.

Preparation of Brown Planthoppers

BPH were reared in the greenhouse of the University of the Philippines Los Baños-National Crop Protection Center (UPLB-NCPC). BPH nymphs instar totaling 300 were transferred, distributed, and caged in 15 mylar cylinders. Twenty hoppers were placed per cylinder, with rice seedlings as their source of food. The top of mylar cylinders were covered with organza fabric to prevent the insects from escaping while letting air ventilate through the cages.

Preparation and Application of Insecticides

The insecticidal solutions of 2, 4, and 6% crude extract solutions were formulated as experimental treatments. The synthetic insecticide Cymbush 5 EC (Cypermethrin) was utilized as the treatment for the positive control group and distilled water for the negative control group. Crude peel extract (2 g) was added to 98 mL of distilled water, 4 g to 96 mL of water, then 6 g to 94 mL of water; summing up to a 100 mL of solution. The solution was thoroughly stirred until the extract was dissolved completely and transferred to sprayers.

Hoppers in each cylinder were treated with 10 mL of the solutions. Insecticides were sprayed at the stem of the rice seedlings where BPHs were more abundant, at the middle through the slits on the mylar cylinders, and on top through the organza fabric. The experiment was arranged in completely randomized design (CRD) with three replications.

Data Collection and Analysis

Mortality was monitored after 24, 48, and 72 h after treatments were applied. The hoppers often stayed on the stem of the rice plant and fall instantly to the soil once dead. Dead hoppers were removed immediately every data collection.

Data were subjected to one-way Analysis of Variance to determine the significant difference between the mortality of BPH groups treated with varying concentrations of calamansi peel extract and the groups treated with control. All the treatment means were compared using the Tukey's HSD at 5% level of significance. The lethal concentration required to kill 50% (LC50) and 90% (LC90) of the hopper population was determined using Probit Analysis. All statistical tests were done through Data Analysis of Microsoft Excel.

Results and Discussion

Figure 1 presents the data collected on mortality after 72 h of exposure of *N. lugens* to different treatments and the comparison among treatments and controls with indicated Q-statistic values.

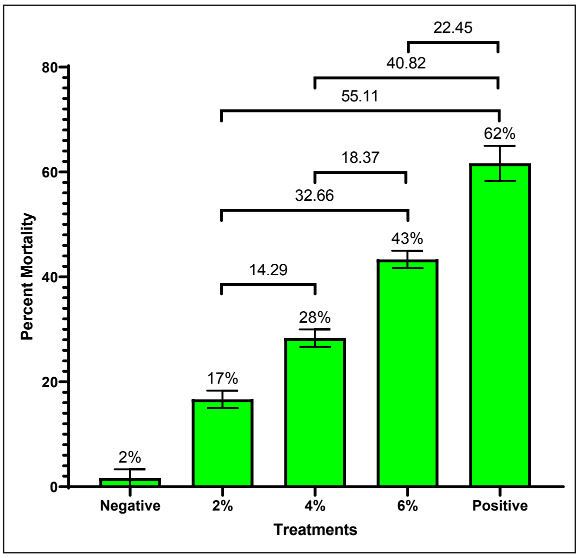

Figure 1. Percent mortality of BPH under different treatments and controls.

The highest percent mortality recorded among the concentrations of calamansi crude peel was 43% exhibited by the 6% calamansi extract (Figure 1). Calamansi extract at 2% concentration exhibited 17% mortality while 4% concentration resulted in 28% mortality. The negative and positive controls led to 2% and 62% mortality of BPH, respectively.

The mortality of BPH when exposed to the varying concentrations of calamansi may be attributed to the D-Limonene present in calamansi, which is proven to have insecticidal activities (Yee, 2014). The presence of phytochemicals such as flavonoids and alkaloids in calamansi, which was found to have insecticidal effects (Hollingsworth, 2005) may have caused the mortality of brown planthoppers.

One-way ANOVA showed that the variance of concentrations of *C. microcarpa* had an F-statistic value of 121.7, which is higher than the F crit value of 3.5 and a p-value of 1.95^{-08} indicating that there is a statistically significant difference among the treatments in relation to the mortality count of *N. lugens*. This means that one of the concentrations caused the change, proven to influence the mortality count of the insects.

Tukey's HSD test results of different concentrations of the *C. microcarpa* crude peel extract showed that the Q-statistic of all the concentrations is larger than their critical value, which means that all of concentrations have significant difference favoring the higher concentrations of the extract. This implies that Cypermethrin has a greater effect than the varying concentrations of *C. microcarpa* crude peel extract. Furthermore, the post-hoc test also implies that higher concentrations of *C. microcarpa* crude peel extract have a greater effect than the negative control and the lower concentrations.

Table 1 and Figure 2 present the results of regression analysis between the various concentrations of calamansi and percent mortality of BPH. It shows the regression line equation used to estimate the lethal concentration required to kill 50% (LC50) and 90% (LC90) of the population. The regression equation is $Y \fallingdotseq 1.5724 X + 3.5488$ with concentration of calamansi and percent mortality of BPH as X variable and Y variable, respectively. This indicates that with increase of one unit of concentration (X), the mortality of the BPH increases by a unit of 3.5. The extent to which concentration (X) predicts the mortality of the BPH (Y) was found at 97.05% coefficient of determination. Thus, insect mortality has a direct relationship with the concentration of solution, in which an increase in concentration will yield an increase in percent mortality.

The regression equation is used to predict the lethal concentration of calamansi peel extract required to kill 50% of the population (LC50) and of 90% of the population (LC50). Regression analysis showed that LC50 can be managed by 8.37% of calamansi peel extract while LC90 at 54.57% concentration. Thus, mortality is affected by the extract's increased concentration. This is supported by the study of De

Table 1. Calculated LC 50 and LC 90 of *C. microcarpa* peel extract against *N. lugens.*

LC 50 (%)	LC 90 (%)	Lower 95%	Upper 95%	Regression line equation	R
8.37%	54.57%	1.480473952	5.61711809	y= 1.5724 X + 3.5488	0.9705

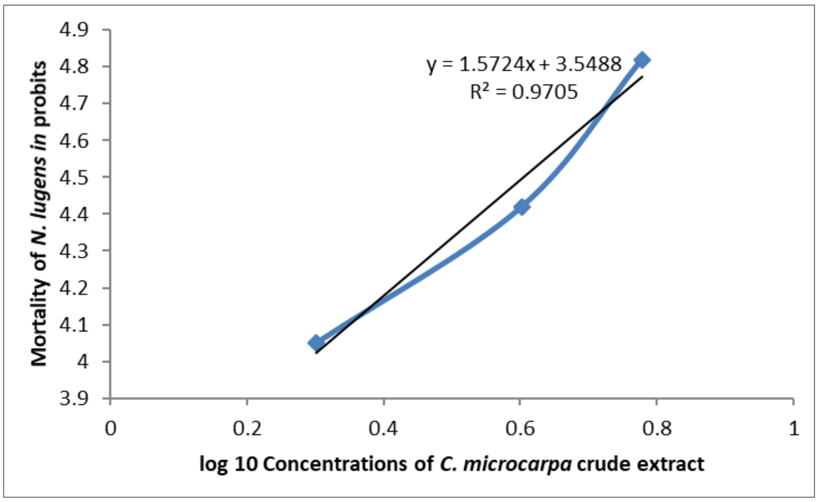

Figure 2. Probit linear regression line equation of the insecticidal activity of *C. microcarpa* peel extract against *N. lugens.*

Villa et al. (2012) in which log-probit analysis showed that increase in concentration of *C. microcarpa* extract leads to higher mortality of *A. aegypti* (Linn.) larva; thus, can be used as a cheap, common, and environmentally friendly alternative control

Conclusion

Results showed that increase in concentration of *C. microcarpa* peel extract leads to higher percent mortality of *N. lugens*. Calamansi peel extract at 8.37% can control LC50 and LC90 at 54.57% concentration. Hence, calamansi peel extract is a potential insecticide against brown planthopper and is suggested to be utilized as an organic plant-based insecticide. As water-based treatment, it is environmentally friendly and affordable approach in controlling *N. lugens*.

Further studies can be done to determine the relative potency of *C. microcarpa* to various chemical controls against *N. lugens*, including ecotoxicity and mammalian toxicity. Investigation of other parts of *C. microcarpa* such as leaves, seeds, bark, and roots as potential insecticide are also recommended. Use of different solvents and extraction techniques such as Soxhlet extraction and bioassay techniques such as leaf-dip method can also be explored. Furthermore, determining the insecticidal activity of different citrus from Rutaceae family against *N. lugens* and other rice insect pests can be explored.

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MORPHO-AGRONOMIC CHARACTERIZATION OF SELECT PHILIPPINE TRADITIONAL RICE VARIETIES

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Abstract

Assessment of agro-morphological diversity among rice germplasm is an important endeavor in any genetic resources' management and crop improvement. Discovery of desirable traits from traditional rice varieties (TRVs) and incorporating these traits in rice breeding efforts would greatly benefit rice farmers to mitigate the effect and better manage rice production in changing environmental conditions. This study provides information on the morphological characterization of TRVs used to establish each accession's genetic identity, and identified varieties with desirable traits for direct utilization and potential donors for rice improvement and assessed the extent of genetic diversity of the rice collections. There were 199 TRVs planted during the wet cropping season from 2014 to 2017 to identify morpho-agronomic characteristics using 58 traits following the standard descriptors for cultivated rice. Morpho-agronomic traits were analyzed using multivariate statistical analysis. The mean Shannon-Weaver diversity indices were H'=0.52 for all qualitative traits and H'= 0.81 for quantitative traits. An overall genetic diversity was H'= 0.66, which indicates a medium level of genetic variation among these TRVs. Rice collections that exhibited the longest panicle (>32 cm) are Sto. Nino, Speaker, Sampukoy, Salumanay, Putan_Kapa, Palaweña, Palawan, Malagkit Kapa, Madya, Ilon-ilon, Gobierno, Galo, and Canadal, while collections Tulloy, Salumanay, Pah-nga, Malagkit Kapa, Malagkit Black, Kayasakas, Diko, and Bulgar produced the heaviest grain (>35g). Several traditional rice germplasms have desirable attributes and should be further explored for varietal improvement programs.

Keywords: *Diversity, Genetic Resources, Oryza sativa, Shannon-Weaver Diversity Index.*

Introduction

The Philippines lies in one of the eight centers of crop origin and diversity recognized by the great Russian conservationist Vavilov (1951). As a center of biodiversity spot, it is a home of various rice species. Local rice cultivars have high genetic variability due to their adaptation to a wide range of agro-ecological conditions (Sarawgi and Bisne, 2007). Unfortunately, most local species diversity is not well recorded and characterized (Nurhasanah and Sunaryo, 2016): thus, less information is available on their characteristics.

Traits characterization has been one of the important steps of crop improvement. Traditionally, each accession is characterized to establish genetic identity through morpho-agronomic characters. Morpho-agronomic characters are usually used as indispensable tool to distinguish varieties of rice and other crops. Germplasm characterization is essential to provide information on the traits of accessions assuring the maximum utilization of the Philippine traditional rice germplasm collection. It is important in identifying varieties with desirable traits for direct utilization and potential donors for crop improvement and in protecting the unique traits for present and future use.

Assessment of agro-morphological diversity among conserved rice germplasm is an important endeavor in any genetic resources' management and crop improvement. Genetic diversity is commonly measured by genetic distance or genetic similarity implying either differences or similarity at the genetic level showing the relationship among genotypes. This supports the selection decision based on information about plant characteristics from the large population for crop improvement.

Effective utilization of these germplasms can be enhanced if these materials are fully characterized and evaluated for their potential use in breeding programs (Rabara et al, 2014). Discovery of desirable traits from traditional rice varieties (TRVs), and incorporating these traits in rice breeding efforts would benefit rice farmers to mitigate the effect of adverse conditions and better manage rice production in changing environments. Thus, the success of any breeding program depends on the selection of parents for hybridization (Banumathy, 2010).

This study provides information on morphological characterization of TRVs used to establish each accession's genetic identity; and identified varieties with desirable traits for direct utilization and potential donors for crop improvement and assessed the extent of genetic diversity of the germplasm collections.

Methodology

Plant Materials

List of most preferred TRVs per region was obtained from the Upland Rice Development Program (URDP). Traditional rice varieties totaling 199 were collected from different provinces of the Philippines and used in the study. Details on each accession regarding their uses and special features were collected and documented along with the seeds from respected farmers. Passport data of every collection was prepared.

Seed lot amounting to 20 g per sample was used. Seeds were sown in a raised seedbed in the field covered with carbonized rice hull. Twenty-one days old seedlings were transplanted into the field following a planting distance of 25×25 cm. There were 100 plants per plot in the field planted for characterization.

Characterization Site

The study was conducted at Philippine Rice Research Institute Central Experimental Station (PhilRice CES), Maligaya, Science City of Muñoz, Nueva Ecija (15°40' N, 120°53'E, 57.6m altitude) in the wet season from 2014 to 2017. Varieties were characterized under field conditions. Standard recommended practices for land preparation and seedling and crop establishment for rice were followed.

Morpho-agronomical Characterization

Morpho-agronomic traits of the germplasm were characterized following the procedure described in Descriptors for Rice (*Oryza* spp.) published by Bioversity International, IRRI, and WARDA in 2007. Morpho-agronomic characters were gathered from vegetative to maturity stage. Characteristics were scored based on the general appearance of the population in the plot. Ten randomly chosen rice plants for each cultivar were used on the measurement of experimental data in the field. Fifty-eight morpho-agronomic traits were selected from the descriptor list and used to characterize the germplasm (Appendix 1).

Data Analysis

Descriptive statistics such as mean, range, and sample variance were computed for the analysis of the quantitative traits. Phenotypic proportions based on the percentage of entries for the different accession were computed for the qualitative traits.

Qualitative and quantitative data were analyzed using the Numerical Taxonomy and Multivariate Analysis System (NTSYS, version 2; Rolf, 1990). Multivariate cluster analyses were performed separately for qualitative and quantitative data sets. Clustering using the sequential, agglomerative, hierarchical, and non-overlapping type (SAHN) was done based on the unweighted pair group method, arithmetic average (UPGMA). A simple matching similarity coefficient was used for qualitative analysis and Euclidean distance coefficient for quantitative traits analysis.

Standardized Shannon-Weaver Diversity Index was computed following the formula (Shannon and Weaver, 1949):

$$H' = \frac{-\sum_{i=1}^{n} Pi(\log_2 * Pi)}{\log_2 (n)}$$

Where: H'= Shannon-Weaver Index

n = number of phenotype descriptors for character

Pi= proportion of the total number of entries belonging to the i^{th} class.

Where: H' was classified as the following arbitrary rating scale:

High diversity	:(H'=0.67-1.00)
Moderate diversity	:(H'=0.34-0.66)
Low diversity	:(H'=0-0.33)

Results and Discussion

Qualitative Traits Characterization

Qualitative characters are mostly genetically controlled and less influenced by the environment; thus, considered as the most important characters to differentiate accessions in the collection. Table 1 presents the calculated Shannon diversity index (H') for each descriptor. High diversity (H'= 0.73 to 0.99) was observed in culm kneeing ability, culm lodging resistance, leaf blade intensity of green color, endosperm type, leaf blade pubescence, lemma apiculus color, caryopsis shape, panicle main axis attitude, apiculus color (late), stigma color, panicle shattering, caryopsis color, and lemma and palea color (early). Moderate diversity (H'=0.37 to 0.65) was observed in panicle threshability, culm habit, culm internode anthocyanin, flag leaf attitude of the blade (early), leaf senescence, lemma and palea (late), panicle exsertion, awn color (late), panicle secondary branching, spikelet fertility, sterile lemma color, awn distribution, awn color (early), leaf sheath anthocyanin coloration, ligule color, culm underlying internode color, panicle branches attitude, and leaf blade attitude (late). Low diversity (H'=0.04 to 0.24) was observed in leaf blade anthocyanin distribution, basal leaf sheath color, leaf anthocyanin coloration, collar color, auricle color, and culm nodes anthocyanin. The accessions were monomorphic (H'=0) in leaf blade attitude, ligule shape, and culm underlying node color. A moderate degree of variation exists within the collection for qualitative traits as reflected by the mean diversity index of 0.52.

Table 1. Qualitative descriptors showing the Shannon diversity indices (H').

Descriptors	H'	Diversity Classification
Culm kneeing ability	0.99	High
Culm lodging resistance	0.91	High
Leaf blade intensity of green color	0.90	High
Endosperm type	0.86	High
Leaf blade pubescence	0.85	High
Lemma apiculus color	0.85	High
Caryopsis shape	0.77	High
Panicle main axis attitude	0.77	High
Apiculus color (late)	0.77	High
Stigma color	0.77	High
Panicle shattering	0.76	High
Caryopsis pericarp color	0.76	High
Lemma and pale color (early)	0.73	High
Panicle threshability	0.73	Moderate
Culm habit	0.65	Moderate
Culm (internode anthocyanin)	0.64	Moderate
Flag leaf attitude of blade (early)	0.61	Moderate
Leaf senescence	0.57	Moderate
Lemma and palea (late)	0.56	Moderate
Panicle exsertion	0.54	Moderate
Awn color (late)	0.54	Moderate
Panicle secondary branching	0.53	Moderate
Spikelet fertility	0.50	Moderate
Sterile lemma color	0.48	Moderate
Awn distribution	0.48	Moderate
Awn color (early)	0.45	Moderate
Leaf sheath anthocyanin coloration	0.42	Moderate
Ligule color	0.40	Moderate
Culm (underlying internode color)	0.40	Moderate
Panicle branches attitude	0.38	Moderate
Attitude of blade (late)	0.37	Moderate
Leaf blade distribution of anthocyanin	0.24	Low
Basal leaf sheath color	0.22	Low
Leaf anthocyanin coloration	0.21	Low
Collar color	0.14	Low
Auricle color	0.11	Low
Culm anthocyanin of nodes	0.04	Low
Leaf blade attitude	0.00	Uniform
Ligule shape	0.00	Uniform
Culm (underlying node color)	0.00	Uniform
Mean Diversity Index	**0.52**	**MODERATE**

Cluster Analysis

Cluster was analyzed to find out the relationship among the cultivars. Genetic relatedness among cultivars was grouped (Figure 1) based on morpho-agronomic characters. Genotypes having a similar local name and collected from the same locations such as Awot 12800 and Awot 12802 from North Cotabato; Pinilisa 14764 and Pinilisa 14773 from Isabela; and Milagrosa 14276 and Milagrosa 14279 from Palawan were clustered together with 97% level of similarity in terms of qualitative characters. It was observed that Black Rice 12699 and Black Rice 12836 from South and North Cotabato respectively, clustered together with 0.97 similarity coefficient.

Results showed that Azucena (Table 2) from Sultan Kudarat (12812-A), Batanes (16052), and Negros Oriental (14259) clustered together with 0.68 similarity coefficient (Figure 2), while another Azucena from Sultan Kudarat emerged as the most distinct as it formed a separate cluster from the Azucena cultivars. This morphological divergence can be attributed to differences in awn color, leaf pubescence, and lodging resistance. The three Pinilisa from Isabela were 98% similar in terms of qualitative traits, while these were 84% similar with Pinilisa of Zamboanga. Although there were variances in these varieties, they might be genetically similar (Nurhasanah and Sunaryo, 2016).

Variation was observed in some cultivars with the same names collected from different locations (Figure 2). Most of the Dinorado collections are from North Cotabato that clustered together with Dinorado from Zamboanga with 0.68 similarity coefficient while distinct differences with Dinorado were observed from Misamis Occidental.

There were some varieties with different names that are closely clustered together. It was noted that Inumay (13916) from Maguindanao and Azucena (14759) from Sultan Kudarat were 92% similar in terms of qualitative traits.

The cluster analysis of preliminary group cultivars was based on similarities and inter-cluster morphological variation. However, diversity analysis based on phenotypic values may not be a perfect

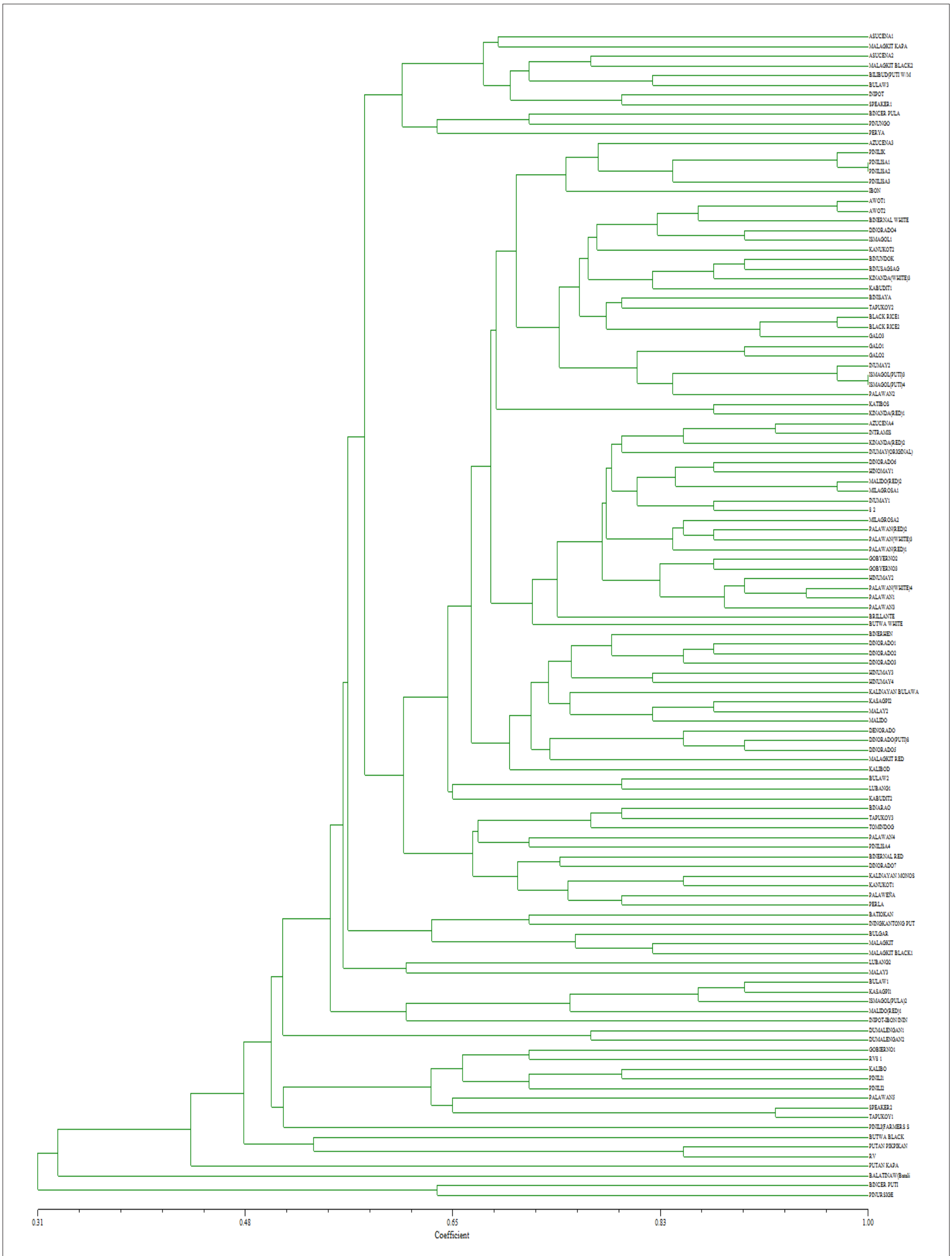

Figure 1. UPGMA dendrogram showing the relationships of traditional rice varieties as showed by qualitative characters.

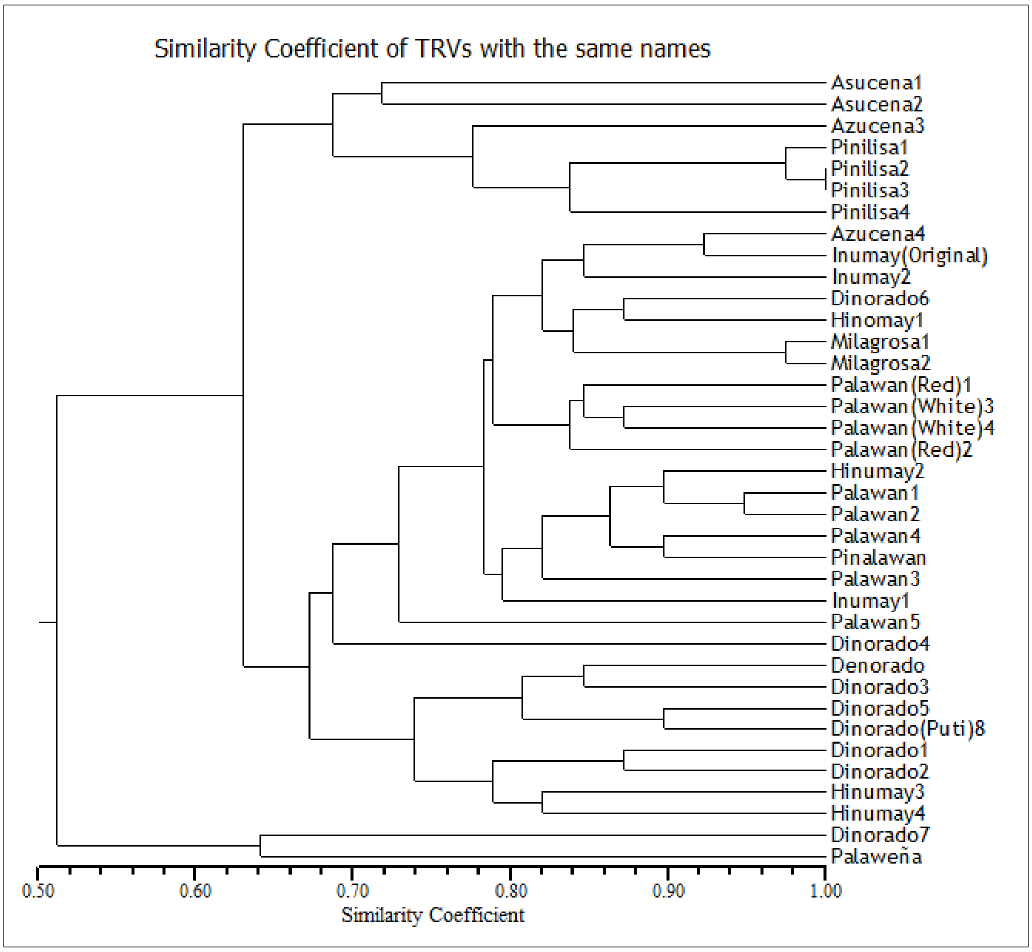

Figure 2. UPGMA dendrogram showing the relationships of traditional rice with same names as shown by qualitative characters.

representation of natural groupings of cultivars due to environmental effects (Li et al, 2002). Thus, it is necessary to subject molecular markers to validate phenotypic data to reduce environmental effects and allow quantitative prediction of genotype values.

Table 2. List of selected varieties with the same names.

Cultivar Name	Collection Number	Source
Asucena 1	12812-A	Sultan Kudarat
Asucena 2	16052	Batanes
Azucena 3	14259	Negros Oriental
Azucena 4	14759	Sultan Kudarat
Dinorado	14277	Palawan
Dinorado 1	12798-A	North Cotabato
Dinorado 2	12798-B	North Cotabato
Dinorado 3	12801-A	North Cotabato
Dinorado 4	12801-B	North Cotabato
Dinorado 5	14072	Zamboanga del Norte
Dinorado 6	14760	North Cotabato
Dinorado 7	15993	Misamis Occidental
Dinorado (Puti)8	13914	Maguindanao
Hinomay 1	15381	Davao Oriental
Hinumay 2	12799-A	North Cotabato
Hinumay 3	12799-B	North Cotabato
Hinumay 4	12804-A	12804-A
Inumay 1	13919	Maguindanao
Inumay 2	13921	Maguindanao
Inumay (Original)	13916	Maguindanao
Milagrosa 1	14276	Palawan
Milagrosa 2	14279	Palawan
Palawan 1	12731	Apayao
Palawan 2	12850	Iloilo
Palawan 3	14240	Ifugao
Palawan 4	14262	Negros Oriental
Palawan 5	15875	Nueva Vizcaya
Palawan (Red) 1	14246	Ifugao
Palawan (Red) 2	14762	Isabela
Palawan (White) 3	14247	Ifugao
Palawan (White) 4	14763	Isabela
Palaweña	15816	Sultan Kudarat
Pinalawan	15241	Batangas
Pinilisa 1	14764	Isabela
Pinilisa 2	14773	Isabela
Pinilisa 3	14774	Isabela
Pinilisa 4	15260	Zambales

Table 3. Descriptive statistics and Shannon diversity indices (H') of Quantitative descriptors.

Descriptor	Mean	Sd	Minimum	Cultivar Name	Maximum	Cultivar Name	H'	Diversity Classification
Caryopsis length	5.87	0.78	3.22	Perya (15415)	7.88	Mako Odyam (16009)	0.28	Low
Caryopsis width	2.26	0.27	1.6	Malido (12655)	3.15	Pah-nga (15869)	0.88	High
Grain length	8.31	1.04	5.24	Perya (15415)	10.75	Guyod (12803)	0.86	High
Grain width	2.71	0.38	1.9	Malido (12655)	3.98	Pah-nga (15869)	0.88	High
Culm diameter	7.56	1.25	4.6	75 days (14257)	11	Pinungo (16025)	0.77	High
Culm length	114.14	17.25	64	Tomindog (14261)	158	Bincer Pula (15804)	0.83	High
Culm number	11	2	7	Palawan (White) (14247) Speaker (12796), Tapukoy (15903), Tipak (14267)	22	75 days (14257)	0.82	High
Flag leaf length	33.74	6.77	16	Pinilisa (15260)	51	Agoo (16013)	0.86	High
Flag leaf width	1.85	0.3	1.2	Azucena (14259, Hinumay 12799-A)	2.76	Gobierno (15241)	0.88	High
Filled grains	1.49	52	8	Gabay (Alo-al) (15231)	296	Domapong (15414)	0.84	High
Unfilled grains	33	24	6	Fortuna (16020)	140	Malay3 (12810-a)	0.73	High
1000 grain weight	24.5	0.56	12.45	Hinumay (12799-a)	43.55	Malagkit Black (16056)	0.84	High
Leaf blade length	50.33	8.82	28.2	Tomindog (14261)	80.8	Tanggiling (15620)	0.85	High
Leaf blade width	1.31	0.21	0.76	Pinili (16018)	1.88	Tumindog (Mabinay)	0.84	High
Ligule length	13.89	3.34	7	Malay3 (12810-a)	24.6	Tanggiling (15620)	0.85	High
Panicle length	27.41	2.82	19.26	Pinili (16018)	35.18	Palawan (14240)	0.84	High
Panicle number	11	2	7	Palawan (White) (14247) Speaker (12796)	24	Pinungo (16025)	0.83	High
Plant height	141.94	18.38	85.96	Tomindog (14261)	185.98	Maligaya (15908)	0.83	High
Sterile lemma length	2.21	0.51	1.06	Perya (15415)	6.76	Putan Pikpikan (15462)	0.85	High
						Mean Diversity	**0.81**	**HIGH**

Quantitative Traits Characterization

Statistical analyses of 19 quantitative traits are presented in Table 3. Variations in characters were observed in different rice collections. Standardized Shannon-Weaver diversity indices for quantitative traits showed that the collections were highly diverse (H'=0.81). The average grain length of the germplasm characterized was 8.13 mm. The number of tillers ranged from 7 to 22; the highest number of tillers peaking at 75 days. Plant height varied from 86 to 186 cm. In addition, majority (74%) of the varieties were >142 cm tall. The 1,000 seed weight observed ranged from 12.45 to 43.55 g with Hinumay recording the lightest 12.45 g and Malagkit Black the the heaviest (43.55 g) weight.

Selection of Rice Germplasm

TRVs in this study exhibited interesting characteristics that can be useful for farmers, breeders, and stakeholders. Short plant stature has been the major target of improvement of lodging resistance in rice because it confers lower susceptibility to lodging and higher harvest index (Ookawa et al., 2010). TRVs such as 75 Days, Butwa White, Malay2, Tipak, Tomindog had the shortest plant height of less than 100 cm (Table 4). Grain size is an important quality parameter and rice grain can be categorized as extra-long, long, medium, and short (Akram et al., 1995). Long grains (>10 mm length) were observed on Agoo, Asucena, Bulgar, Canadal, Gos, Guyod, Mako odyam, Malagkit_Black, Malay2, Palaweña, Putan_Kapa, and Tapul/Alik-Ik. The rice collections that exhibited the longest panicle (>32 cm) are Sto. Nino, Speaker, Sampukoy, Salumanay, Putan kapa, Palaweña, Palawan, Malagkit Kapa, Madya, Ilon-ilon, Gobierno, Galo, and Canadal. Collections such as Tulloy, Salumanay, Pah-nga, Malagkit Kapa, Malagkit black, Malagkit black, Kayasakas, Diko, and Bulgar produced the heaviest grain (>35 g) among the cultivars. Variations in culm diameter were recorded and could be utilized in the crop improvement program. The thickest culm diameter >9.8 cm was observed in varieties Kayampog, Malan, Sto. Nino, Malagkit Black, and Pinungo.

In terms of qualitative traits, some of the Philippine traditional varieties have glabrous leaves (Table 5). Glabrous leaves are usually fed to ruminants. In this study, 3 Buwan, Batiokan, Binarao, Binerhen, Binernal_Red, Butwa_White, Dinorado, Diko, Gabay(Alo-Al), Gininto, Hinumay, Inamper, Japanese, Kalanay, Kalinayan_Bulawanon, Kalinayan_Monos, Kanukot, Kawitan, Kawot, Makiraga, Malagkit, Malay2, Malido, Mindoro, Palaweña, Samuel, Tapukoy, Tayak_Pula, and Tumindog (Mabinay) have glabrous leaves. Moreover, very strong lodging resistance was observed on Amayan, Bilibud (Puti), Domapong, Inamper, Kaboyo, Pah-nga, and Tipono.

Conclusion and Recommendations

Morpho-agronomic characterization can be used to establish the genetic identity of each accession. This will serve as a reference for identity verification, validation, and is used in duplicate identification and elimination. The study showed sufficient divergence for various qualitative and quantitative traits. The 199 traditional rice varieties showed variability for most of the characteristics, especially for the quantitative traits. Several TRVs possess desirable traits that can be used for rice improvement program.

Clusters were analyzed to find out the relationship among the cultivars. Genetic relatedness among cultivars was grouped based on several morpho-agronomic characteristics. Results indicated that TRVs, which have similar name and collected from the same and different locations formed in the same cluster. Variations were observed in some TRVs with the similar and different names from different locations that formed into the same group. The cluster analysis provided preliminary data on groupings of the TRVs based on similarity and inter-cluster morphological variation. However, diversity analysis based on phenotypic values may not be a perfect representation of natural groupings of cultivars due to environmental effects. Thus, it is necessary to provide molecular marker data to validate phenotypic data and reduce environmental effects that will allow quantitative prediction of genotypic values.

To maximize the selection of parentals and widen the use of the rice germplasm, existing collections should be further characterized and evaluated for their potential use in breeding programs. Broadening the genetic base using diverse germplasm in varietal improvement is essential in developing new climate resilient rice varieties.

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RICE VARIETY IMPROVEMENT STRATEGIES AT THE PHILIPPINE RICE RESEARCH INSTITUTE (PHILRICE)

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Abstract

Research and development are key to progress. Rice, as a staple food for Filipinos should increase its productivity and income through research, particularly natural sciences related to variety improvement and development. The development of appropriate and climate-resilient modern rice varieties remains an integral aspect in achieving this desired outcome. The primary focus of breeding has been high yield potential. High-yielding rice varieties have provided consumers more food, but the changing socioeconomic landscape of farmers and consumers now calls for varietal options that strike a good balance among elements such as higher yield, insect pest and disease resistance, resilience to extreme weather conditions, farm machine compatibility, nutrient value, and good eating quality. The breeding strategies of Philippine Rice Research Institute (PhilRice) are now anchored in Agriculture 4.0. The aim is to revolutionize the varietal development system of the Philippines by leveraging available technology to navigate the difficult terrains grappling the rice industry at present and in the future.

Keywords: *Breeding, Development, Resilience, Rice, Variety Release.*

Introduction

Rice remains one of the most important crops in the Philippines. In 2018, Philippine population reached 105.6 million with each individual consuming 110 kg of rice annually (Philippine Statistics Authority, 2018). The rising population and standard of living calls not only for ensuring food security and income but also sustaining the environment.

Water scarcity and climate change present major challenges to our food producers. The El Niño (drought) phenomenon in 2016 and southwest monsoon enhanced by typhoons in 2018 cost the agriculture sector PhP 727 and PhP 897 million worth of losses, respectively (NDRRMC, 2018). An excerpt from the book, Understanding the Philippine Rice Industry (Litonjua et al., 2019), showed that the slight dips in rice production in 2010 (15.77 Mt) and 2015-2016 (18.15 and 17.63 Mt) were brought about by drought in the northern part of Luzon. Meanwhile, natural calamities contributed to the slight descent of rice harvest area in some years from 2000 to 2018.

However, the comprehensive report also showed that from 2000-2018, the country enjoyed an overall increase in production in all ecosystems by 6.68 Mt or an annual growth rate of 2.38%. The highest recorded production was in 2017 with 19.28 Mt, made possible by favorable weather conditions. Widened irrigation, adoption of hybrid varieties, training of rice farmers, use of high-quality seeds, and machine ownership contributed to production growth in 2000 onwards (Bordey, 2010). Paddy rice yield also jumped from 3.07 t ha^{-1} in 2000 to 3.97 t ha^{-1} in 2018. Irrigated areas were consistently more productive than the non-irrigated farms that include the uplands and stress-prone environments. In 2018, the irrigated areas averaged 4.37 t ha^{-1} and only 3.12 t ha^{-1}, elsewhere.

As water is a very critical input in rice production, investing in irrigation facilities help boost the competitiveness of our farmers. The factors that positively and significantly affected yield were quantities of material inputs, labor, adoption of technologies such as machine and high-quality seeds, access to irrigation system free of charge, and production management practices of farmers (Bordey et al., 2017). However, planting hybrid seeds and accessing large-scale irrigation systems could give 45% and 21% more yield, respectively. Government efforts may focus on these factors to substantially boost yield.

Increasing Filipino farmers' competitiveness is also a must as the Philippines enter the free rice trade regime starting 2019. By law of supply and demand, the increase in rice supply in the country brought by the liberalization of rice importation would lead to lower rice prices in the local market. Data from Philippine Statistics Authority show that *palay* prices have declined since January 2019, even before the implementation of Republic Act No. 11203 better known as the Rice Tarrification Law (RTL) on March 5, 2019. As of July 2019, average domestic price of dry *palay* has dropped to PhP17.87 per kg from PhP19.83 per kg in January 2019 with local prices ranging from PhP12.8 (in Cavite) to PhP 22.00 per kg (in Bulacan and Guimaras). While this would benefit consumers, local rice producers are expected to be adversely affected scaring them from further engaging in the industry.

Consolidating these findings and projecting future needs, the Department of Agriculture, through the initiative of the Philippine Rice Research Institute (PhilRice), adopted a new vision enshrined in the Philippine Rice Industry Roadmap 2030: Rice-secure Philippines. This means having safe and nutritious rice available and accessible at affordable price at all time. To help make this happen, PhilRice embarked on a mission to improve the competitiveness of the Filipino Rice Farmer and Rice Industry and transform it to be more profitable, resilient, and sustainable through responsive, balanced, environmentally sound and partnership-based research, development and extension (PhilRice Strategic Plan, 2016).

On the research side, the development of appropriate modern rice varieties remains an integral aspect in achieving this desired outcome. For more than five decades, plant breeding has produced many high-yielding crop varieties that helped address the food supply conundrum. By optimizing the rice plant's morphology and physiology, and incorporating desirable traits, cultivators are able to produce more using less resources.

For many years, the primary breeding focused on high yield potential. High-output varieties have afforded consumers more food, but the changing socioeconomic landscape of both farmers and rice consumers now calls for varietal options that strike a good balance among elements such as yield, insect pest and disease resistance, resilience to extreme weather conditions, farm machine compatibility, nutrient value, and eating quality.

The breeding strategies of PhilRice are now anchored in Agriculture 4.0. The aim is to revolutionize the varietal development system of the Philippines by leveraging available technology to navigate the difficult terrains grappling the rice industry at present and in the future. It advocates a stronger and more cohesive variety development structure to keep up with the needs of the times as well as global challenges. Rice breeders are charged with a tall order: come up with varieties that can yield double, and are climate resilient, pest and disease resistant, palatable, and nutritious.

Strategies

Plant breeders, more than ever, must work closely in specific units and wide array of disciplines such as genetic resource, crop protection, agronomy and physiology, rice chemistry, socio-economics, business development, molecular biology, and genetics to succeed in raising the yield frontier in both experimental farms and farmer fields. A panel of senior breeders and consultants conducted a workshop and discussed the incorporation of new breeding techniques and methods to efficiently implement in the breeding program. At PhilRice, it identified three units and two sub-units to be implemented in the division.

Division

PhilRice Plant Breeding and Biotechnology Division (PBBD) was created to ensure stable and sustainable rice production through the development of high-yielding, pest and abiotic stress resistance, and good grain quality rice varieties that can adapt to different rice growing ecosystems in irrigated, rainfed, and upland. The following sections outline the strategies by which this objective is being carried out:

Proposed Units:

Rice Breeding - This includes both conventional and non-conventional systems.

Sub-units:

Conventional Breeding - The classical breeding system features purely conventional or traditional approach such as pedigree, modified bulk, single seed descent, population improvement, plant selection, and yield trials.

Non-conventional Breeding - Non-conventional breeding uses molecular markers as modern tools in developing varieties. Marker-assisted selection (MAS) and marker-assisted backcrossing (MAB) are pursued for traits that are economically important and have diagnostic markers. Traits with MAS-proven markers are bacterial leaf blight resistance, tungro resistance (from ARC11554), submergence (*sub1* gene), drought (Moroberekan and Malay 2), high temperature tolerance (Nagina 22 and Dular), and beta carotene synthesis. On the other hand, markers that are being studied for MAS application are those for blast resistance, tungro resistance (from Utri Merah), salinity tolerance (Pokkali), aroma, and amylose content. Non-conventional approaches are often combined with classical breeding to shorten the breeding cycle.

Breeding by mutagenesis - Mutagenesis creates population of different genotypes without hybridization. Traits of interest are identified through population screening of the target traits. Traits associated with biotic and abiotic stresses, value adding, high yield, and grain quality are identified.

Molecular Biology and Genetics - Molecular biology and genetics unit will be provided for the basic research and molecular studies of DNA, RNA, and protein as tools in line development in terms of routine genotyping, gene discovery, plant physiology, and other biotechnological tools. Activities will be integrated with data management systems. Basic research on molecular (DNA, RNA, protein) marker development, QTL mapping, gene expression, gene discovery and function, genetic engineering will be incorporated. Enhancing yield capacity will be explored through genome-wide selection. Gene discovery, including genetic analysis of complex traits, will be pursued for yield and yield components, resistance/tolerance to biotic and abiotic stresses.

Database Management - Much of breeding support relates to data acquisition and management. As such, a Database Management Unit that provides biometric support will be established.

3.1. Data management and connectivity:

a. Pedigree and trait data management system
b. Molecular data management
c. Seed information management system
d. Laboratory information management system
e. NCT (National Cooperative Test) data management system

Digital labeling and data acquisition

Statistics support - B4R (IRRI free software) and ongoing data management support system are being developed.

Breeding Themes

Plant breeders partner closely with experts from a wide array of disciplines to stand a better chance at being successful in raising the yield frontier in both experimental farms and farmer fields. The four themes and seven sub-theme projects are implemented under the division (Table 1).

Product Development Workflow

The division takes an industry-inspired paradigm with three major nodes: Trait Discovery, Line Development, and Variety Delivery (Figure 1). Trait Discovery covers basic concepts and new breeding techniques, application of high throughput molecular markers, novel gene discovery, stress tolerance and resistance, statistical methods, and rice physiology and

Table 1. Breeding themes for variety development.

Theme	Sub-theme	Priority Traits
Resource use efficiency and yield maximization	Irrigated lowland	DS transplanted, direct-seeded (8-10 t ha^{-1}) WS transplanted, direct-seeded (6-8 t ha^{-1})
	Rainfed lowland	Transplanted, direct-seeded (4-6 t ha^{-1})
Biotic stress resistance and abiotic stress tolerance	Irrigated lowland	Rice tungro virus, green leaf hopper, bacterial leaf blight, sheath blight, blast, brown plant hopper, rice black bug, stemborer, high and low temperature, anaerobic germination, drought, submergence, lodging, shattering, viviparity, plant morphology and archetype, nitrogen use efficiency (NUE)
	Rainfed lowland and upland	Drought, submergence (stagnant, flood), salinity, blast, aerobic rice, and two to three combinations of the abiotic stress
Value-adding systems	Specialty rice	Nutrient-dense (Vitamin A, iron, zinc), specialty rice (waxy, pigmented, aromatic, tropical japonica, nutraceutical, industrial, brown rice, yield enhancement (milling recovery, head rice recovery, chalkiness). Higher milling and head rice recovery breeding is also being proposed.
Basic research	Advanced rice science and new breeding techniques	Molecular breeding, genomics, physiology/phenomics, crop management information system, statistics

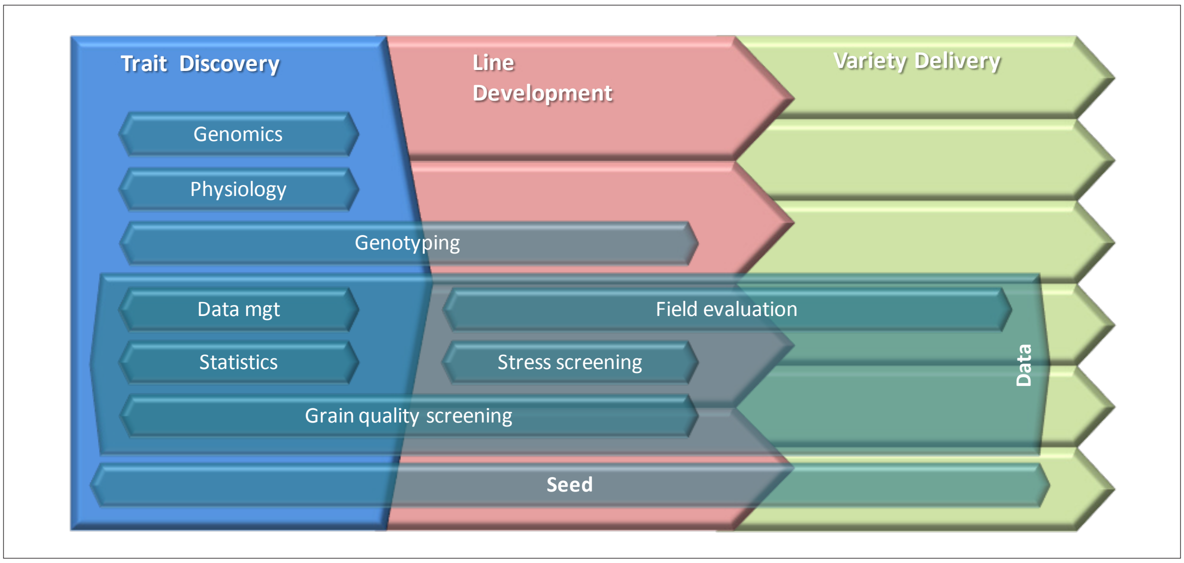

Figure 1. A simplified overview of the variety development workflow from design, through development to delivery.

quality. Line Development takes care of hybridization, generation advance, multi-location tests, technology optimization, and application of combined breeding methods. The variety delivery component includes product characterization, upscaling, promotion, and providing customer support system.

Technical Working Group

An in-house Technical Working Group (TWG) creates a stronger and more coordinated nationwide effort. The TWG provides general direction, oversees breeding and testing, managing problems and issues, and implements plans of the PhilRice varietal development agenda. A more collective effort enhances efficient use of resources and puts the entire group in a better position to succeed. The in-house TWG holds its regular meeting during the National Rice R4D Conference. It is comprised of representatives from the following disciplines and offices:

Trait Discovery	**Variety Delivery**
Molecular genetics Plant genomics Plant physiology	Working seed management Commercial seed production Promotion and positioning Crop management information system
Line Development	**Breeding Stations**
Irrigated (transplanted and direct seeded) Rainfed (transplanted and direct seeded) Value-adding (micro-nutrient dense) Grain quality evaluation	PhilRice Central Experiment Station PhilRice Los Banos

Data management system
Screening
Biotic stresses (Table 4)
Abiotic stresses Submergence Drought Salinity High temperature Low temperature Combination of 2-3 abiotic stresses

Inter-Station Line Development

PhilRice branch stations are strategically located to serve regional needs of farmers, rice growers, and consumers. While most of the breeding work is being done at the Central Experiment Station (CES), it is also important to equip branch stations with the capability to develop varieties that are inherently adapted to their respective area of responsibilities, and thus as support breeding stations. The branch stations also provided genetic materials directly coming from specific farmers and locations and become part of the collections by the Genetic Resource Division at the CES.

The probability of discovering adapted materials is higher if breeding lines undergo selection right at the target environments themselves or the branch stations and surrounding farmers' fields. Branch stations with ongoing breeding support activities, and are therefore already relatively equipped, are San Mateo, Isabela; Los Baños, Laguna; and Central Mindanao University, Cotabato. Much support is needed on the other hand for PhilRice stations in Batac, Ilocos Norte; Murcia, Negros Occidental; and Basilisa, Agusan Del Norte. Initial segregating materials and training are provided by PhilRice-CES and PhilRice-Los Baños. The focus of breeding stations according to sub- breeding theme is presented in Table 2.

Generation advance in the breeding stations or shuttle breeding, general yield trials (GYT), and advanced yield trials (AYT) per breeding theme are set up across stations so materials may be readily tested in multiple environments prior to National Cooperative Tests (NCT) nomination. The resulting selection from the GYTs are submitted by each breeding station; these are then consolidated by the TWG and categorized per theme. For example, PhilRice-CES, PhilRice-Isabela, and PhilRice-Los Baños, all deeply involved in hybrid rice breeding, submit their promising entries to the TWG for multi-location testing, and all three breeding stations have a common GYT from season to season. After GYT, the promising entries are entered into AYT where breeding lines that passed the criteria for yield, pests and diseases, and grain quality are nominated to the NCT. A general diagram showing the flow of breeding materials between divisions and breeding stations during line development is shown in Figure 2.

Variety Release and Promotion

Up to the present, new varieties of rice are tested by the Rice TWG through the NCT; recommended for release by National Seed Industry Council (NSIC); and granted final seal of approval by the Secretary of Agriculture. Throughout this long process, a genotype undergoes 10 years of design and development and another two years of large seed multiplication and delivery before it is finally released and used by the farmers as a variety. Simplified diagram is shown in Figure 3 while the detailed stage-by-stage process flow is shown in Table 3.

A second route is being proposed in releasing a variety: a very promising selection, after multi-location testing in GYT and Multi-location Yield Trial (MYT), are submitted to the Bureau of Plant Industry (BPI) for Plant Variety Protection (PVP) application. After passing the distinctness, uniformity, and stability test (DUST), the variety becomes a new release without having to be recommended by NSIC; therefore, can be then heavily promoted by PhilRice for nationwide or location-specific adoption. Through this option, the variety delivery process is shortened by at least two years. NCT (which includes NCT-1 and Multi Adaptive Trial or MAT for irrigated lowland inbreds) take more than two years to complete, whereas PVP

Table 2. Breeding stations and their focus sub-themes (IL-Irrigated Lowland; HY-Hybrid; RL-Rainfed Lowland and Upland; SR-Specialty Rice; ARS-Advance Rice Science).

Station	IL	HY	RL	SR	ARS
CES	▪	▪	▪	▪	▪
PhilRice Ilocos			▪		
PhilRice Isabela		▪	▪		
PhilRice Los Baños		▪	▪	▪	▪
PhilRice Bicol	▪				
PhilRice Negros	▪			▪	▪
PhilRice Agusan	▪		▪		
PhilRice Cotabato	▪		▪		▪

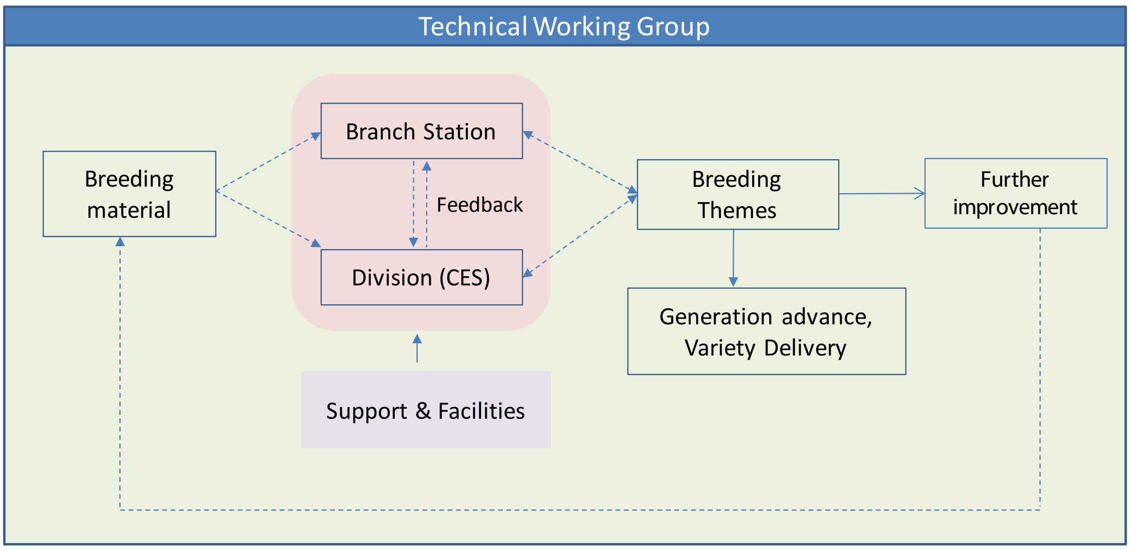

Figure 2. General workflow of breeding material during line development.

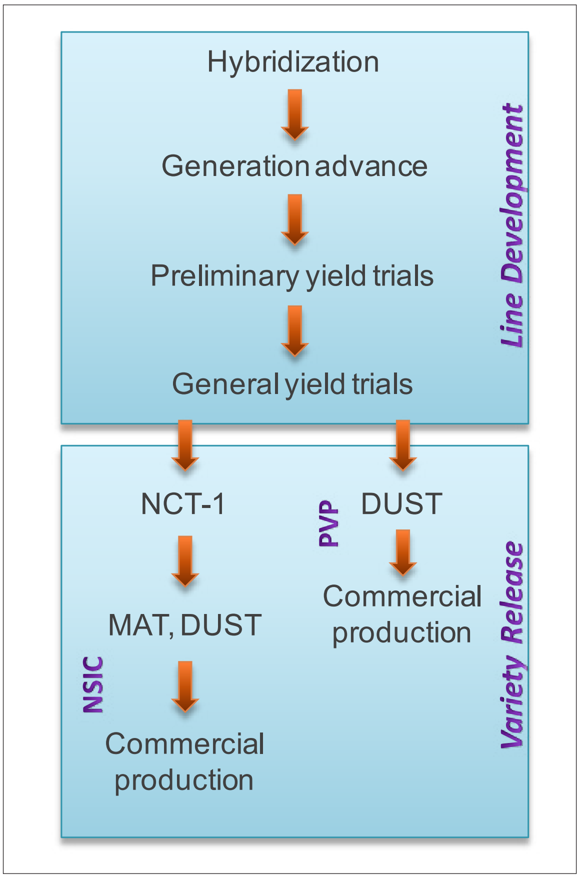

Figure 3. Schematic diagram of varietal development and release. Newly released varieties are entered into Participatory Varietal Selection (PVS)

requires only one season of DUST (and another half-year for processing of documents).

Working Seed Storage and Management

At the heart of a profitable commercial breeding program is the seed. Seed handling and multiplication therefore is one of the cornerstones in this variety development business. Working seed, as used in this document, refers to seeds of breeding populations in active development by breeders. Plant Breeding and Biotechnology Division (PBBD) will work closely with Germplasm Resources Division (GRD) to ensure proper seed health practices are followed during post-harvest stages in preparation for the following season, and with Business Development Division (BDD) for seed storage of advanced lines (i.e., entries in multi-location trials). Inventory and movement of seed between researchers and between stations are integrated with the data management system as part of inter-station connectivity.

Centralized Screening

Precision breeding not only calls for revolutionary molecular techniques but also accurate measurement of the phenotype as well. Screening is very critical for grain quality, resistance to insect pests and diseases, and tolerance to abiotic stresses, and is an undertaking that involves different divisions and stations (Figure 2). The complexity of the whole process thus entails careful standardization of methodologies, and accurate integration of data and seed management systems.

An operational procedure for each trait is being developed and will be implemented. For high-quality data, screening in these three major areas (i.e., grain quality, biotic stress, abiotic stresses) will be the focus of facility improvement and build-up in the Line Development node. Each centralized screening facility will be initially funded through external projects or with PhilRice management help, but will adopt the charge-back system for cost recovery and maintenance of operations. Figure 4 describes the abiotic stress tolerance breeding strategy at the Plant Breeding and Biotechnology Division.

Table 4 shows the summary of traits to be screened and the roles of CES divisions and branch stations. For some traits, more than one station is involved in the screening to allow for ecotype (race) differences in pathogens or insects, and to account for environmental differences among branch station locations.

Advanced Rice Science

Advanced laboratory support is provided to Trait Discovery and Line Development in terms of routine genotyping, gene discovery, molecular genetics, plant physiology, and grain quality. Activities will be integrated with the Crop Biotechnology Center (CBC).

1. Genotyping, molecular genetics, and marker-assisted breeding

Marker-assisted selection is pursued for traits that are economically important and have diagnostic markers. Enhancing yield capacity is explored through genome selection (GS). A central genotyping facility will be set up for target gene assay and genome SSR assay to serve as a clearing house for samples to be brought to International Rice Research Institute or other international outsource facility for genome SNP genotyping.

Traits with MAS-proven markers are bacterial leaf blight resistance, tungro resistance (from ARC11554), submergence tolerance (*sub 1 gene*), and beta carotene synthesis. On the other hand, markers that are being studied for MAS application are those for blast resistance, tungro resistance (from Utri Merah), drought (Moroberekan), salinity (Pokkali), high temperature (Nagina 22), aroma, and amylose content.

2. Genomics

Gene discovery, including genetic analysis of complex traits, is pursued for yield and yield

Table 3. Detailed stages in design, development, and delivery of a rice variety.

Node	Stage	Activity	Key players*
Trait Discovery	**Variety R&D** (6 years minimum)		
	0D	Product design (yield, biotic and abiotic resistance, grain quality)	PBBD, CPD, RCFSD, GRD
Line Development	0R.1, 0R.2	Hybridization	PBBD
	0S1 (F_2) to 0S5 (F_6)	Selfing and selection (including MAS) generations for inbreds	PBBD, CPD
	0C	Testcrossing for hybrids	PBBD
	1	Preliminary observational nurseries	PBBD
	2	Advanced observational nurseries	PBBD
	3	Preliminary yield trials	PBBD
	4	Advanced (general, pre-NCT) yield trials, coding system, seed increase	PBBD, CPD, RCFSD, ASPPD, ISD, Branch Stations, Cooperators
	Variety Testing and Registration - PVP Route (1 year)		
	5A.1	DUST, fingerprinting, seed increase	PBBD, BDD-ITSO, TMSD
	5A.2	PVP accreditation, commercial production	PBBD, BDD-ITSO, BDD
	Variety Testing and Registration - NSIC Route (3 years)		
	5B.1	NCT (1st year), seed increase	PBBD, CPD, RCFSD, Cooperators
	5B.2	NCT (2nd year), seed increase	PBBD, CPD, RCFSD, ASPPD, GRD, BDD, Cooperators
	5B.3	MAT (1 year), DUST, basic seed production	PBBD, CPD, RCFSD, ASPPD, GRD, BDD, Cooperators
	5B.4	NSIC approval, commercial production	PBBD, RTWG, NSIC
Variety Delivery	**Variety Launch, Sale, and Stewardship** (less than 3 to more than 10 years)		
	6	Product launch, promotion, initial sale into the market	BDD, SED, TMSD, RSS, SeedNet, DA-RFOs
	7	Rapid growth in market share, promotion, and active stewardship	BDD, SED, SeedNet, DA-RFOs
	8	Plateau in market share, promotion and stewardship	BDD, SED, SeedNet, DA-RFOs
	9	Declining promotion, sale and stewardship	BDD, SED, SeedNet, DA-RFOs
	Variety Decline and Phase-out (1-2 years)		
	10	Phase-out and clearing of inventory	BDD, SeedNet, DA-RFOs

ASPPD – Agronomy, Soils, and Plant Physiology Division; BDD – Business Development Division; BDD-ITSO – Innovation and Technology Support Office; CPD – Crop Protection Division; DA-RFOs – Department of Agriculture- Regional Field Offices; GRD – Genetic Resources Division; ISD – Information Systems Division; NSIC – National Seed Industry Council; PBBD – Plant Breeding and Biotechnology Division; RCFSD – Rice Chemistry and Food Science Division; RSS – Rice Seed Systems; RTWG – Rice Technical Working Group; SeedNet – Seed Network; SED – Socioeconomic Division; TMSD – Technology Management and Services Division.

components, blast resistance, and drought tolerance. Mass screening to discover novel alleles for herbicide tolerance and resistance to biotic and abiotic stresses will be conducted for Genebank accessions.

3. Physiology

In addition to genomics, enhanced yield capacity beyond today's attainable levels will have to come from improved nitrogen use efficiency and harvest index. Numerous past and current studies involving NUE (Nitrogen Use Efficiency) and source-sink relationship were conducted to improve the attainment of yield potential of elite varieties, involving a few genotypes at a time. However, NUE has never been a selection target during line development of hundreds of genotypes, and harvest index though selected for to some degree, is very tedious to assess. Breeders and plant physiologists will have to work together to discover techniques that are straightforward and less costly, to ultimately allow NUE and harvest index become major traits for selection during line development.

Root parameters like growth in general and elongation response to drought stress are also a physiologic aspect that ultimately contributes to stabilizing and even enhancing the yield. ASPPD will intensify research work on root characteristics that enable the plant to adapt to drought stress. Functional

Table 4. Trait screening by division and branch station.

Station or Division	Biotic Stress	Abiotic Stress	Grain Yield and Quality
PBBD	BLB, blast [SH (screenhouse)], tungro	Salinity (SH), drought (SH and field), submergence, high and low temperature, aerobic condition adaptation, anaerobic germination, direct-seeded	
ASPPD			Yield potential, NUE, WUE
CPD	Tungro, YSB, GLH, BPH, BPH		
RCFSD/ASL			Milling, physical, physico-chemical characteristics
PhilRice Ilocos		Drought, high temperature	
PhilRice Isabela	Blast (field)	Salinity [Off station (OS)], high temperature (OS), low temperature (OS)	
PhilRice Los Baños	Blast	aerobic condition adaptation	
PhilRice Bicol	RBB	Salinity (OS)	
PhilRice Negros	Tungro, blast		
PhilRice Agusan	WSB	Low solar radiation, Zn-deficiency, submergence	
PhilRice Cotabato	Tungro, panicle blight, WSB, sheath blight, RBB	Drought	

stay green characteristic and photosynthetic rate are also yield-enhancing traits that need to be investigated at the physiological level.

4. Grain quality

Rice Chemistry and Food Science Division (RCFSD) is gearing towards value-adding aspects of the rice grain such as aroma, vitamins (e.g., folate), minerals, and metabolites. This is mainly in congruence with Goal 2 of the PhilRice Strategic Plan (i.e. reduced incidence of poverty and malnutrition).

Biometrics Unit

Much of the breeding support relates to data acquisition and management. As such, a Biometrics Unit will be established as a tripartite cooperation between the different divisions. The main tasks of the Biometrics Unit, are as follows:

1. Data management and connectivity

   a. Pedigree and trait data management system

The new data management system (ongoing) will be used as the platform for the integration of pedigree and trait data from researcher to researcher (e.g., breeder, entomologist, and agronomist) and from breeding station to breeding station. The Biometrics Unit will spearhead the formulation of a naming convention for crosses, pedigree lines, traits and nurseries, and a uniform format for data and field books.

   b. Seed information management system

At the national level, seeds of GYT and AYT entries and other breeding materials will be moving from station to station. In each breeding station, breeders and stress screening experts deal with a large number of breeding lines every season. This movement and inventory of working seed will be made more systematic by integrating ICIS (International Crop Information System) with barcoding and database management systems.

   c. Laboratory information management system

An information management system for analysis of laboratory samples will also be put in place so that data (e.g., marker, N content, grain quality) from the laboratory are unified with data from morpho-agronomic tests and stress screening.

4. Digital labeling and data acquisition

Breeding materials will be labeled digitally with the aid of barcoding equipment, which must be also integrated with ICIS and the new data management system that will be adopted. To minimize chances for errors, data gathering will be done using a handheld data logging device. The Biometrics Unit will be tasked to set up this system and make it useful for the intended users in PBBD, ASPPD, CPD, RCFSD, and branch stations.

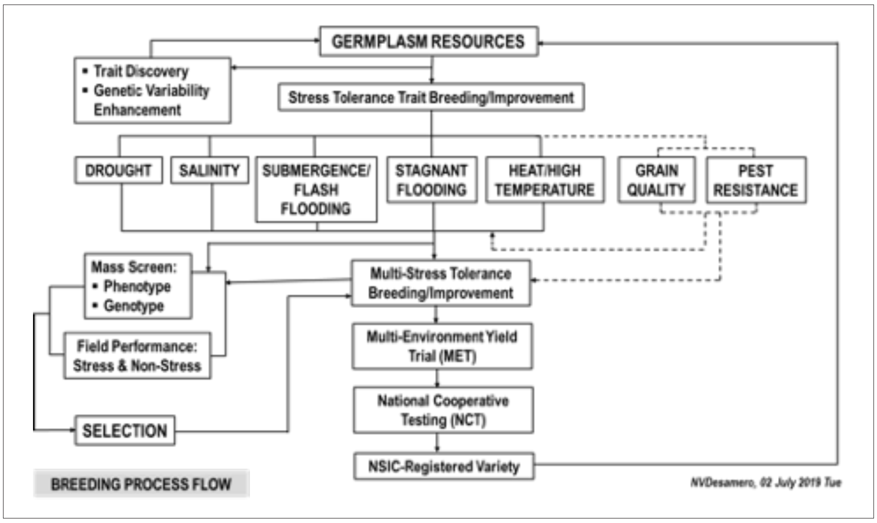

Figure 4. Schematic diagram of the abiotic stress tolerance breeding strategy at PhilRice (Desamero 2019, unpublished).

5. Statistics support

Support in terms of data analysis, statistical consulting, and training will be provided by the Biometrics Unit to all breeding stations. An operational procedure covering types of analysis and costing to help with overhead costs will be implemented.

6. Crop management information system

Matching the yield potential of a variety with what a specific environment can offer to attain that yield is an endeavor that requires soil, climate, and pest profile characterization of testing and target sites, which are all necessary pieces of information to better plan crop care and management. Breeding lines are evaluated in testing sites while varieties will be promoted in target sites. With GIS support, PBBD will be working closely with the ASPPD to develop precision management systems for varieties to perform optimally in their intended areas of release.

Partnership with SCUs

Faculties of state universities and colleges and (SUCs) across the country offer a much wider array of expertise and modern equipment. PhilRice continues to strengthen its partnership with SUCs to pursue advanced researches in biochemistry, statistics, and remote-sensing, as well as downstream applications of product testing in the farmers' fields. PhilRice reasserts its presence in the academic community by offering thesis projects, co-adviser, and mentor of MS and PhD students, OJTs (national and international), and internships to students; thereby increasing its potential to draw in more and better talents.

Breeding Manual

A breeding manual is being crafted by a team of experts for further review. The main objective is to provide a guide for training new rice breeders who will be hired by PhilRice. It will also serve as a tool for learning and applying the methods for students, teachers, and breeding personnel at PhilRice conducting their breeding activities.

Acknowledgments

The authors are grateful to PhilRice division heads, project and study leaders, branch stations, researchers, and field workers for their support in the conduct of studies and analyses on rice variety development. Thanks is due to the Office of the Deputy Executive Director for Research and staff for managing the edits of the manuscript and consolidating data for improvement. The manuscript is fully supported by DA-PhilRice funds for smooth implementation of the projects in rice breeding.

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