# 2016 National Rice R&D Highlights

## APPLIED BIOLOGY CENTER FOR THE RICE ENVIRONMENT

Department of Agriculture Philippine Rice Research Institute

#### TABLE OF CONTENTS

#### Page

Executive Summary	1
I. Organisms for Enhancing Soil and Plant Nutrition	2
II. Biocontrol agents for pest management	23
III. Organisms for Enhancing Plant Tolerance to Abiotic Stresses	33
IV. Utilization of organisms from the rice environment for protein and carbon sources	39
V. Bioremediation of toxicants in rice environments	45
Abbreviations and acronymns	50
List of Tables	52
List of Figures	53

1

### Applied Biology Center for the Rice Environment

Center Director: AA Dela Cruz

#### **Executive Summary**

The potential of biological systems as means for producing alternative sources of energy, food, and protection in rice farming and for profitable rice environment-based enterprise must be fully explored and developed. Existing knowledge in Applied Biology indicate that some of organisms could be developed into technologies for improving rice farming and for profitable enterprises utilizing the rice environment. The endeavor, however, will likely transcend the usual boundary of the traditional disciplines in rice science and will necessitate multidisciplinary team approach to plan and carry out a wide range of activities before it becomes fully transferable to farmers or commercially available for public use. PhilRice has the technical capability to organize, plan, and implement activities toward this direction thus the Applied Biology Center for Rice Environment (ABCRE) was established on September 12, 2014 dedicated to the utilization and advancement of knowledge of biology applied in the rice environment and to innovations that will increase output and reduce external input for sustainable rice farming and rice-based enterprises. ABCRE aims to: (1) foster innovations focused on the factors contributing to low rice yield and low income in rice farming, (2) develop and package products that work well in the field and safe for human and environment, (3) discover venture opportunities for rice farming communities, and (4) attract disciplinary and inter-agency collaboration in the pursuit of its objectives.

In 2016, ABCRE continued the implementation of 5 projects composed of 16 studies. The projects are geared to develop technologies for beneficial organisms to enhance plant and soil health, plant tolerance to abiotic stresses, manage rice pests and diseases, and also to identify useful organisms for food/feed/industrial applications and remediation of toxicants in the rice environment. Specifically, on-going studies are determining effectiveness of certain plant-associative bacteria and fungi in increasing availability of nitrogen in soil by producing hormones or enzymes, sequester carbon and mineralize nutrient in rice production. High water temperature-tolerant Azolla species are being evaluated for other superior qualities to maximize its utilization as biofertilizer and conditioner in rice paddies. The efficacy and cost-effectiveness of microbial inocula, such as bacteria from nipa palm and an actinomycete, and of different combinations of rice straw and manures for use in upland and irrigated rice productions are being investigated. Likewise, the soil invasion of macro/microdriles are also being associated with significant changes in soil characteristics. Since some plant growth-promoting rhizospheric bacteria also have potentials to enhance plant tolerance to certain biotic and abiotic stresses, available bacterial isolates are currently being evaluated for capacity to enhance plant tolerance to drought stress. Potential

biopesticides against rice black bug and rodent pest are also being formulated. The shelf-life of powder form of spores of fungi B. bassiana and M. anisopliae and the rice-based bait for a protozoa S. singaporensis are continuously being improved for safe and easy application in field. To help reduce problems on protein deficiency and increase economic benefits of high-protein blue-green algae, the culturing of these for human and animal nutrition and for use as ingredient in feeds for aquaculture are being explored. For industrial application, the best strains of lignin-degrading bacteria, along with rice varieties with low lignin-contents, are being identified for degradation of rice biomass for bioethanol production. Earlier this year, likewise, another microbiology laboratory was fully established in the ABCRE building and has been catering to the needs of multi-diciplinary researchers and students.

#### I. Organisms for Enhancing Soil and Plant Nutrition

Project Leader: EH Bandonill

Sufficiency of rice production to feed an increasing population through the development of modern rice varieties supported by commercial fertilizers and pesticides has faced a challenge of sustainable environment. In this context, there are many beneficial organisms in the rice environment that can be be assessed and utilized. Rhizotrophic and endophytic bacteria can play a major role in substituting the commercially available N fertilizer in rice production to reduce the environment problems. In addition, bluegreen algae are abundantly available and can fix N in flooded soils while the N- fixing capability of Azolla has led to its being widely used as biofertilizer for rice. Understanding the environmental disruption, the availability of heterotrophic microorganisms to mineralize organic matters in addition to the application of inorganic fertilizer, and enough water holding capacity to support the growth of rice are very important. In addition, the use of vermicomposts has been useful in rice-based vegetable cropping system. Meanwhile, earthworm infestation needs immediate action and recommendation for management as reported by farmers. This project therefore aimed to integrate utilization of beneficial organisms for enhancing soil and plant health. On-going studies specifically aim to evaluate the effectiveness of heterotrophic biota for sustainable food production, determine the applications of beneficial microorganisms present in the rice environment as well address problems to enhance plant and soil nutrition thereby promoting growth and improve rice productivity.

## Assessment of actinomycetes for enhancing the growth and yield of upland rice

JA Cruz, JMR Bautista, CS Mabayag, LA Alejo ES Paterno

Presently, there is a low production of upland rice in the Philippines. Therefore, researchers are prompted to develop technologies to increase upland rice production. The importance of plant growth-promoting bacteria (PGPB) in agriculture is being recognized, where benefits include reduction in the use of chemical fertilizer thus conserving energy resources. Among the PGPBs, actinomycetes particularly Streptomyces are the most economically and biotechnologically valuable prokaryotes. They account for a large percentage of the soil microflora, are effective colonizer of plant root systems, and able to survive drought by forming spores. The production of growthpromoting compounds is part of the metabolism of various bacteria associated with plants, causing modifications in the morphology of roots, influencing nutrient and water absorption, and consequently promoting plant growth. It is possible that certain rhizobacteria, including actinomycete, may act as plant growth-enhancer thus this study aims to evaluate the effectiveness of actinomycetes in enhancing the growth and yield of upland rice.

#### **Activities:**

- Evaluation of the effectiveness of S. mutabilis NB3 inoculation under field conditions
- Inoculant formulation and application
- Economic analysis of the effectiveness of actinomycetes as microbial inoculant for upland rice production
- Submitted additional documents for microbial inoculant patent application to Technology Application and Promotion Institute (TAPI), Department of Science and Technology (DOST).

#### **Results:**

٠

- Under field conditions, S. mutabilis + 1/2 RRF significantly increased the grain yield of NSIC Rc192 and PSB Rc23 by 101% and by 35%, respectively, relative to 1/2 RRF alone but not significantly different from RRF, an indication of its potential to reduce fertilizer input by half.
- With the prevailing price for a kilogram of dry weight rice is PhP 20.00, the return on investment (ROI) and benefit cost ratio (BCR) were computed as profitability indicators for each treatment. Highest BCR and ROI of 2.41 and 140.99%, respectively, was obtained in ½ RRF + S. mutabilis NB3 treatment followed by RRF treatment with 2.07 BCR and 107.44%

5

#### 4 Rice R&D Highlights 2016

ROI (Table 1). For every one (1) peso investment on 1-hectare upland rice production per cropping, there will be 2.41 pesos money back in the  $\frac{1}{2}$  RRF + S. mutabilis NB3 treatment. Highest profit was obtained in  $\frac{1}{2}$  RRF + S. mutabilis NB3 treatment.

- Streptomyces mutabilis increased shoot length of rice by 28% relative to the uninoculated treatment 5 days after inoculation under laboratory conditions. The highest shoot length, 68 mm plant–1, was recorded in treatment with S. mutabilis isolate compared with uninoculated control (Figure 1).
- Inoculation with S. mutabilis NB3 increased rice root hairs at 3 days after inoculation (DAI) and at 5 DAI under laboratory conditions (Figure 2a and 2b).
- The complete requirements for patent application were already submitted.



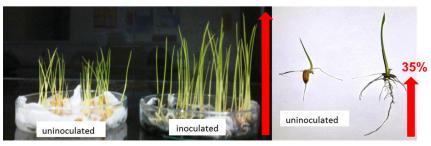
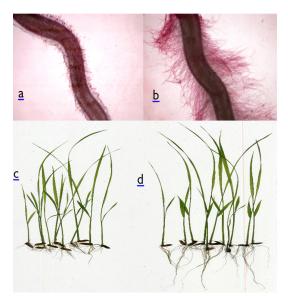


Figure 1. Effect of S. mutabilis NB3 inoculation on rice seedling growth under laboratory conditions.



**Figure 2.** Effects of S. mutabilis NB3 inoculation on rice root hairs at 3 days after inoculation (Magnification: 1000X); Upland rice seedlings as affected by Streptomyces mutablis NB3 inoculation 5 days after inoculation. (uninoculated: a and c; inoculated: b and d).

Table 1. Economic analysis of the effectiveness of actinomycetes as microbial	
inoculant for upland rice.	

	Treatment							
Particular	T1	T2	тз	T4	T5	Т6		
vield (kg/ha)	1,646.70	2,140.00	3,886.70	3,750.00	2,770.00	1,810.00		
Unit Price (Php/kg)	20.00	20.00	20.00	20.00	20.00	20.0		
Gross Income	32,934,00	42,800.00	77,734.00	75,000,00	55,400.00	36,200,0		
Less:								
Production Cost:								
a. Fertilizer	-	6,22611	12,452.22	6,47611	12,702.22	250.0		
b. Seed	2,400.00	2,400.00	2,400.00	2,400.00	2,400.00	2,400.0		
c. Pesticide	2,620.00	2,620.00	2,620.00	2,620.00	2,620.00	2,620.0		
d. Labor	14,250.00	14,625.00	15,000.00	14,625.00	15,000.00	14,250.0		
e. Machine Rental	5,000.00	5,000.00	5,000.00	5,000.00	5,000.00	5,000.0		
Total Cost	24,270.00	30,871.11	37,472.22	31,121.11	37,722.22	24,520.0		
Net Income	8,664.00	11 <i>,</i> 9 <b>2</b> 8 89	40,261.78	43,878.89	17,677.78	11,680.0		
ROI	35.70%	38.64%	107.44%	140.99%	4626%	47.63		
BCR	1.36	1.39	2.07	2.41	1.47	1.4		
Ranking	6	5	Z	1	4	3		

\*T1, zero rate fertilization; T2, ½ RRF; T3, full rate fertilization; T4, ½ RRF + inoculant; T5, RRF + inoculant, and inoculant alone, T6.

7

## **Rhizotrophic bacteria with plant nutrition potential** *XH Truong and MD Duque*

Continuous and excess use of chemical fertilizers and other agrochemicals to increase yield may lead to ground water contamination and depletion of soil nutrients. Biofertilizer (BF) composed of multiple strains of plant growth-promoting bacteria have been intensively studied worldwide. One of the major obstacles of field applicability of PGPR - biofertilizer is the development and implementation of quality control protocols to ensure its consistent efficacy on-farms. Known identity of bacterial inoculant such as N-fixers, P and K-solubilizers, growth hormones, siderophores, and antagonists is therefore critical in order to quantify and control the product quality. This study thus aimed to produce culture inoculant of beneficial bacteria isolates and assess multiple functions of PGPR-inoculant to enhance rice productivity and crop health.

#### Activities:

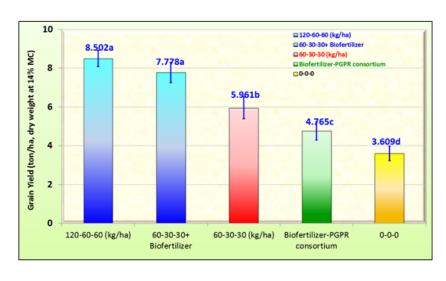
- Production of PGPR inoculant
- Experimental set up in CES
- Statistical analysis of data derived based on 5 sqm crop cut per treatment (ANOVA and Duncan's Multiple Range Test using IRRISTAT)

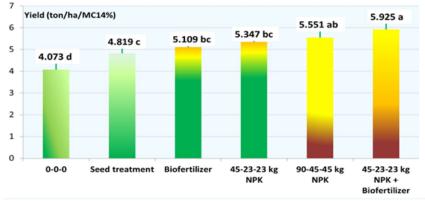
#### **Results:**

- In both field trials of DS and WS 2016, NSIC Rc238 applied with one half recommended NPK rate plus PGPRbased biofertilizer produced the highest yield among other treatments (Figure 3, a and b). This contributed to savings of about 50% of recommended inorganic NPK fertilizer).
- Findings indicated that the application of 90-45-45 NPK/ha during WS was an overshoot because the plants lodged and were slightly infected with bacterial leaf blight resulting to lower attainable yield.
- Seed treatment yield was comparable to one half recommended rate (45-23-23kg NPK/ha) and plant applied with PGPR-based biofertilizer.
- Single application of inorganic fertilizer or PGPR-based biofertilizer and the combination enhanced the grain yield higher than the untreated negative control.
- Highest plant height was obtained from the recommended

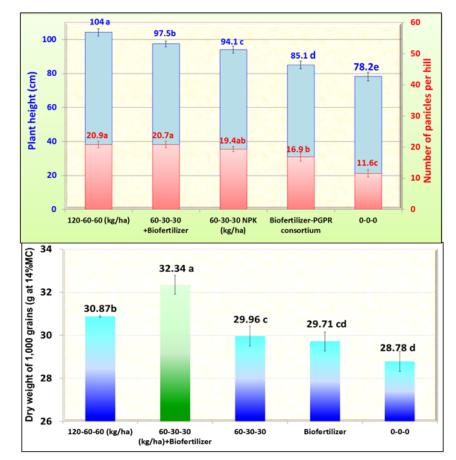
rate for both seasons (WS data not shown).

- In contrast, dry weight of 1,000 grains obtained from PGPR-inoculant was the heaviest (Figure 4).
- Correct application of PGPR-based biofertilizer can save 50% of inorganic fertilizer without compromising the yield.





**Figure 3.** Grain yield (t/ha at 14% MC) of NSIC Rc238 applied with inorganic fertilizer and pGPR-based biofertilizer: a - 2016DS, CES; b – 2016WS, CES.



**Figure 4.** Efficacy of PGPR-inoculant on plant height and number of panicles per hill (a) and on dry weight of 1,000 grains at 14% MC (b) of NSIC Rc238. DS 2016, CES.

#### Algalization of rice paddies

EH Bandonill, HM Corpuz, NRL Sevilla, JCA Cacerez, MD Malabayabas, GO San Valentin, MR Martinez-Goss

Blue-green algae (BGA), one of the major biomasses that easily bloom in paddy fields and open ponds, are recognized for their ability to fix atmospheric N in flooded rice soils. Meanwhile, there is a growing concern about the adverse effect of excessive use of chemical fertilizers on soil biological properties associated with soil productivity. BGA may offer economically- and ecologically-sound alternative to chemical fertilizers in increasing agricultural productivity through algalization technology where algae is inoculated by farmers into the rice paddies and their growth is enhanced while intercropped with rice. The technology has been tested in field trials in experimental farms and showed positive results in both grain yield and economics. However, results had been only confined in India and there is no information on the use of this technique in the Philippines. Thus, this study aimed to exploit BGA as cheap and renewable source of biofertilizer for rice cultivation.

#### Activities:

- Algae Propagation
- Nitrogen Fixation Ability Determination
- Pot Experiment

#### **Results:**

- Based on visual observation, algal bloom was fastest in the non-spherical type of algae such as Anabaena variabilis (Ns71Ph), N. Maahas (Ab23Ph) and Nostoc A UPLB (Ns07Ph) and least for the spherical type Nostoc commune (Ns21Ph). Thus, the algal flakes collected from the propagated BGAs followed similar trend with Ab23Ph having the highest yield (12.09g) followed by Ns07Ph (9.61g), Ns71Ph (7.58g), and least by the spherical type, Ns21Ph (6.88g). The nitrogen content ranged from 1.00-1.14% (Figure 5).
- In the initial microscope counts, Ns71Ph had relatively higher values in terms of length of filaments (125.31 um), number of vegetative cells (VC=20.25), while higher for the spherical Ns21Ph in terms of diameter of vegetative cell (102.34) and heterocysts (1.60) using BG11-N media (no nitrogen). However, in the final counting, it was Ab23Ph which obtained longest length of filaments = 77.19um, heterocyst = 2.0; akinetes in BG11 = 0.35, diameter of VC = 6.36in BG11 and 5.80in BG11-N).

- In terms of initial ammonia content, Ab23Ph again obtained higher values in both BG11 (with nitrogen) and BG11-N media, respectively (1.47, 0.08ppm). Similar trend was observed in the final ammonia analysis where Ab23Ph had higher ammonia content at 2.66, 0.31 ppm in both BG11 and BG11-N media, respectively. This was followed by Ns71Ph (0.38, -2.49ppm) and lastly by Ns21Ph (-5.33, -0.08ppm).
- In the parallel test conducted, highest amount of ammonia nitrogen (0.85 and 0.72 ppm) was produced by Ns07Ph while Ns71Ph generated the highest nitrate nitrogen (317.4 and 0.05 ppm) when inoculated in BG11 and BG11-N, respectively.
- Based on the initial results, the non-spherical BGA, Ns71Ph was selected as the algae to be used in mass propagation. Enough amount of algal flakes (79.04 g) was collected prior to setting up of the initial pot experiment (Table 2). Ab23Ph will also be mass propagated in the succeeding activities.
- Comparable plant height (100.6; 102.4cm), number of tillers (9; 10), and leaf greenness (37.3; 36.2) were observed from treatments using 100% NPK and 50:50 algae:NPK, respectively during active tillering of PSB Rc82 (Table 3).

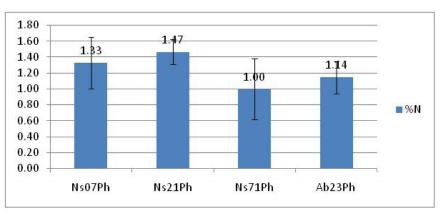


Figure 5. Nitrogen analysis of propagated flakes from the 4 BGA strains.

**Table 2.** Ammonia nitrogen and nitrate nitrogen produced by BGA in BG11 and BG11-N liquid media.

	Ammonia Content (ppm)						
	Ammonia	a Nitrogen	Nitrate	Nitrogen			
Algae	(p	pm)	(ppm)				
	BG11	BG11 <sup>-</sup> N	BG11	BG11⁻N			
Blank	0.82	0.70	340.0	0.05			
Ns71Ph	0.78	ND	317.4	0.05			
Ns21Ph	ND	0.69	297.5	0.04			
Ns07Ph	0.85	0.72	258.7	0.04			
Ab23Ph	0.49	0.70	171.5	0.05			

ND - not detected

Table 3. Agronomic data of PSB Rc82 during active tillering stage.

		Values	
Treatment	Plant Height (cm)	No. of Tillers	Greenness
1	88.5±5.4ª	7±1	36.1±0.4
2	100.6±3.4 <sup>b</sup>	9±1	37.3±1.2
3	102.4±1.9 <sup>b</sup>	10±2	36.2±1.2
4	92.3±3.2ª	9±2	34.9±1.1

T1-no fertilizer (control), T2-100% NPK, T3-50% NPK and 50% algae, T4-100% algal fertilizer

 $^{1}/\text{Means}$  in column followed by a common letter are not significantly different based on Tukey's\_b test at á =0.05

Hormonal and nutrient properties of rice straw-based vermicompost MC Quimbo, MS Cabrera, BT Salazar, GO San Valentin (PhilRice Los Baños)

PB Sanchez (UPLB)

Vermicomposting had been gaining popularity in recent years due to its positive effects on soil fertility, microbial diversity, and significant yield improvement on various crops. Commercial production of vermicompost, vermicast, vermi-tea and vermiculture are blooming off-farm business ventures due to high demand and income generation. Rice straw which is commonly burned in the field by Filipino farmers for disposal amounts to about 3,232kg/ha/year (BAS, 2006). This vast amount which has an approximate C:N ratio of 60 and contains about 0.58% N, 0.10% P, and 1.38% K (Mamaril et al. 2009) can be converted to vermicompost and utilized as organic fertilizer/conditioner for various crops. There are available literatures on vermicomposting using rice straw and some impacts of vermicompost to rice production. However, the studies were only few and mostly based on personal experiences. This research focused on verifying the efficacy of the vermicompost from rice straw to soil characteristics, growth hormonal properties, and microbial population. It tried to address the constraints that may surface on vermicomposting rice straw, thereby, improving the quality of vermicompost produced.

#### Activities:

- Another vermicomposting trial was conducted from January to April 2016 with 5 treatment combinations: rice straw (RS) only, RS + Azolla, RS + ipil-ipil, RS + cow manure and RS + carabao manure) to verify the results of the previous trial.
- Chemical laboratory analyses (pH; C:N ratio; total N, P and K) of raw materials, vermicompost, and vermicast were conducted at certain stages of decomposition from the 1st season trial.
- Plant growth hormone and microbial analyses of vermicomposting products from the 1st season trial were completed.

#### **Results:**

- The summary of finding is shown in Table 4.
- The production of vermicompost and vermicast from RS + cow manure was significantly higher than RS only, RS + ipil-ipil and RS + Azolla but comparable with RS + carabao manure. Vermicompost production from all rice straw combinations was significantly higher than RS only.
- The highest significant recovery of vermicompost and vermicast from initial substrates used was obtained from RS only.
- RS + animal manures had significantly higher recovery of vermicast compared to RS + plant manures. No significant difference in percent recovery of vermicompost was observed between animal and plant manures.
- Rice straw has the widest C:N ratio of 73. Ipil-ipil had the highest N content and the lowest C:N ratio (14). Cow and carabao manures have the highest amount of total P2O5. In terms of K content, cow manure also had the highest.
- The pH of the vermicomposts were not significantly different from each other and ranged from 5.3 to 6.86.

#### 14 Rice R&D Highlights 2016

- The total N of RS + ipil-ipil (2.27%) was considerably higher than the other treatments and the lowest is RS only (2.08).
- The trends in the parameters measured for vermicasts are similar to the vermicomposts, although the pH values were slightly lower compared to the vermicomposts.
- The C:N ratios were not significantly different among the vermicasts but the highest was obtained from RS + carabao manure (17.28) while RS + Azolla gave the lowest (1.92%).
- Two weeks after vermicomposting, total C decreased in all treatments, with RS+Azolla having the lowest C content (17.34). In week 4, total C in RS only, RS + ipil-ipil and RS + carabao manure (33.4, 31.26 and 31.05 respectively) were not significantly different from each other but different from RS + Azolla (7.62) and RS + cow manure (20.99).The total C among treatments were not significantly different at week 8 and 10.
- Addition of ipil-ipil resulted in significantly highest total N compared to other treatments all throughout the vermicomposting process. Similarly with total *C*, the total N at week 10 was not significantly different among treatments.
- The C:N ratio of all treatments decreased from week 2 until week 10. RS only had the highest C:N ratio all throughout the vermicomposting process while RS + ipil-ipil had the lowest.
- All hormones found in vermicompost and vermicast showed no significant differences among different treatment combinations. ile RS + Azolla produced one of the highest hormones in vermicompost and vermicast, it produced the lowest hormones in vermitea. The highest kinetin content was also found in RS + cow manure, followed by RS + ipil-ipil which was the lowest hormone producer in both vermicompost and vermicast.
- The IAA contents of vermitea from RS + ipil-ipil, RS + cow manure and RS + carabao manure were the highest compared to all other RS + substrate combinations, be it vermicompost, vermicast or vermitea.
- The kinetin contents of vermicompost ranging from 50 to 72 ppm had a similar trend as IAA contents of vermicompost, although kinetin was 10-fold smaller than IAA. The highest

kinetin contents were found in vermicomposts, followed by vermicast and vermitea.

- The AA contents of vermicompost ranging from 49 to 78 ppm also followed similar trend as IAA and kinetin contents of vermicompost, with values generally within the same range as kinetin (50 to 72ppm). RS + ipil-ipil remained to be the lowest in vermicompost and vermicast while RS + Azolla was the lowest in vermitea. Similarly, the AA contents were found in in the order: vermicomposts > vermicast > vermitea.
- The trend for GA contents was also similar to the other hormones but the quantities were 10-100x that of the hormones from vermicompost and vermicast. In vermitea, the GA contents were comparable to the IAA contents of the same RS + substrate combination.
- RS only and RS + Azolla produced the highest GA in vermicompost but the lowest in vermitea.

٠

- Results of bacterial count expressed in colony-forming units/ gram (CFU/g) in vermicompost ranging from 18 x 106 to 39 x 106 showed that RS + Azolla had significantly higher CFU/g than RS + cow manure but not significantly different from RS only, RS + carabao manure and RS + ipil-ipil. The highest CFU/g among the vermiteas was also obtained from RS + ipilipil. The bacterial CFU/g were in the order: vermicompost > vermicast > vermitea.
- In vermicompost, the CFU/g of RS + ipil-ipil was significantly higher than RS + Azolla, RS + cow manure and RS + carabao manure but it was not significantly different from RS only. In vermicast, RS + ipil-ipil was significantly higher than all of the treatments.
- The CFU/g of vermitea from RS only and RS + Azolla were higher than both vermicompost and vermicast, but the highest CFU/g among vermiteas was recorded from RS + ipil-ipil.

#### Table 4. Summary of Findings.

abie 4. Summary of Findings.	
RS + Animal manure combination	RS + Green manure combination
<ul> <li>higher production and recovery of vermicast</li> <li>higher P and K contents which subsequently led to higher total NPK (3.3-3.6%)</li> <li>RS + Cow manure – produced the highest hormonal content (IAA, Kinetin, AA and GA) in vermitea</li> <li>RS + carabao manure – produced the</li> </ul>	<ul> <li>higher production and recovery of vermicompost</li> <li>higher microbial population (bacteria and fungi) in vermicompost, vermitea and vermicast</li> <li>RS + Ipil-ipil have higher N content; tended to have the lowest hormonal content (IAA, Kinetin, AA and GA) in both vermicompost and vermicast</li> </ul>
highest Kinetin and GA in vermicast	<ul> <li>RS + Azolla had the lowest hormonal contents in vermitea.</li> <li>RS + Azolla and RS only produced the highest hormones in vermicompost</li> </ul>

#### Selection of azolla for heat tolerance and superior qualities as biofertilizer and conditioner in flooded rice environment CLC Mondejar, GO San Valentin, TC Fernando, AA Dela Cruz

Numerous studies conducted have demonstrated the effectiveness of azolla as biofertilizer in rice production. Despite the effort of the Philippine government to promote azolla in 1980s, only very few fields with azolla can be found nowadays. The PhilRice continues the R&D research on Azolla in support of the organic agriculture program. One of the studies is selection of heat tolerant strains. The research on azolla aims to make the biofertilizer a stable part in rice farming system catering not only the favorable, but also the lowland ecosystem which may not be favorable to various strains such as high temperature environment. Azolla cover in rice paddy will not only increases the nitrogen uptake, but will also lower the temperature of pond water. In the Philippines, the warmest temperature was recorded at 37.5 °C (PAGASA, 2013). Some azolla can still survive at temperature ranges from 5 to 45 °C (NAAP, 1987).

#### Activities:

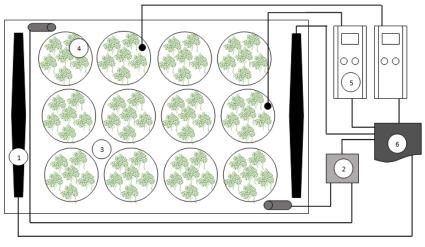
- Screening for high floodwater tolerant A total of 12 zolla accessions were incubated in a customized culture baths (Figure 5) for 10 days at an average floodwater temperature of 36°C. An initial weight of 2.0 grams was used in the 2nd and 3rd incubation. Characteristics such as growth rate, spore production and low P requirement were likewise examined.
- Selected azolla strains were evaluated along with the rice plants in the field. The difference in the soil and floodwater temperature with azolla over without azolla will be determined. The growth of azolla in Los Baños will be compared with azolla in Nueva Ecija, an area with warmer temperature.

#### Applied Biology Center for the Rice Environment 17

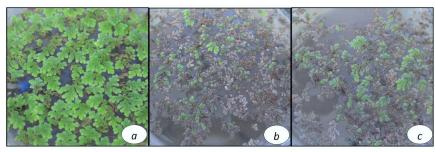
#### **Results:**

.

- Ambient, floodwater and soil temperature in the field at Los Baños, Laguna were monitored starting from 2014 WS to 2016 DS. The year 2015 had the highest temperature recorded in the field. In the month of May, floodwater temperature reached to 40°C with the average floodwater temperature of 29°C. Majority of the azolla propagated in the field did not survive except Azolla mexicana # 2024, Azolla mexicana # 2002 and Azolla mexicana # 2026. These accessions continued to grow and even produced spores.
- Under field condition, the doubling time of the three selected azolla accession play around from 5 to 8 days. The survived during summer when the floodwater temperature reaches to 40°C and with direct sunlight were observed to produce spores in the field.
- During incubation in the customized waterbath (Fig. 6), Azolla mexicana # 2033 showed the highest growth rate value, followed by Azolla mexicana # 2024 and Azolla microphylla # 4099 while Azolla mexicana # 2026, Azolla mexicana # 2030 and Azolla microphylla # 4098 had negative growth rate values which denoted a reduction in biomass. After 10 days, senescence of the fronds for Azolla microphylla # 4114 and # 4119 were observed. Other accessions demonstrated healthy fronds (Fig. 7) though the two accessions did not undergo a reduction in biomass.
  - All of the accessions showed a much lower growth rate compared to the standardized growth rate obtain during a pot experiment. Table 5 shows that both Azolla microphylla # 4114 and # 4119 portrayed senescence in the 2nd and 3rd incubation. These two azolla accessions were not tolerant to high floodwater temperature. Azolla mexicana # 2030 showed lowest growth rate for the last two incubation and a reduction in the biomass at the first incubation. Azolla mexicana # 2030 is not recommended for warm regions. Azolla mexicana # 2002, #2024, # 2026, # 2033, and Azollla microphylla # 4098, # 4099, # 4113, and # 4123 were consistent with their performance. These accessions except Azolla mexicana # 2002, #2024 & # 2026 can further be evaluated in the field to check their performance.



**Figure 6.** Customized culture baths used in screening under controlled floodwater temperature. (1) heater, (2) aerator, (3) hot water, (4) pots with soil-and-water medium, (5) data logger and (6) source of electricity.



**Figure 7.** Response of different azolla accessions after 10 days of incubation at 36°C average floodwater temperature. (a) Azolla microphylla # 4124, (b) Azolla microphylla # 4114 and (c) Azolla microphylla # 4119.

Table 5. Growth rate (g/day) of eleven azolla accessions from April 14 to May	
27, 2016 in PhilRice Los Baños Azolla Nursery.	
Growth rate (g/day)	

Diant Coursels	Growth rate (g/day)					
Plant Sample	1 <sup>st</sup> Incubation	2 <sup>nd</sup> Incubation	3 <sup>rd</sup> Incubation			
Azolla mexicana # 2002	0.06	0.19	0.19			
Azolla mexicana # 2024	0.14	0.26	0.25			
Azolla mexicana # 2026	-0.12	0.20	0.13			
Azolla mexicana # 2030	-0.07	0.07	0.10			
Azolla mexicana # 2033	0.16	0.20	0.27			
Azolla microphylla # 4098	-0.02	0.18	0.22			
Azolla microphylla # 4099	0.14	0.23	0.17			
Azolla microphylla # 4113	0.03	0.23	0.16			
Azolla microphylla # 4114	0.03	0.26	0.12			
Azolla microphylla # 4119	0.08	0.23	0.21			
Azolla microphylla # 4123	0.12	0.17	0.21			
Azolla microphylla # 4124	0.02	0.26	0.17			

Biology and effect of red earthworm to the rice plant CB Codod, EC Martin, ER Tiongco

In the Philippines, vermiculture is very popular as source of organic fertilizer and protein. However, its effect on native earthworm population as well as its potential harm to pond cultures and rice paddies, if the population is not well managed, are not yet studied (Mohaganet. Al, 2013). Rice crops could tolerate earthworm densities of 140 m-2 but suffer significant yield loss when population is ten times higher, and complete loss at 700 m-2 (Barrion and Litsinger in 1994). Farmers from Pangasinan, Kalinga, Ilocos Norte, and Mt. Province reported apparent earthworm damage in their rice crops. Accordingly, the earthworms' burrowing activity caused leaks in rice levees and destroyed terrace walls. Other farmers said that earthworm castings covered emerging rice seedlings which lead to seedling mortality with surviving seedlings having longer succulent stems. Thus, it is an alarming concern in the rice seedbed that needs proper attention and appropriate action. This study therefore aims to assess the distribution of red earthworminfested rice paddies at Pangasinan, Kalinga, Ilocos Norte, Nueva Ecija, and Mt. Province; study the biology of the red earthworm; and determine their direct or indirect effect to the rice plant.

#### Activities:

- Examined the earthworm impact to rice seedlings.
- Studied the behavior of eartworms under laboratory and screenhouse conditions.
- Examined the toxicity of common synthetic pesticides and other farm inputs through laboratory bioassay and screen house experiment.

#### **Results:**

٠

.

٠

- Pontoscolex corethrurus (Muller, 1857) is most problematic on the seedbed. They cover the emerging seeds with thick mud castings resulting in seedlings with longer roots and stem which are succulent and hard to pull. In some instances, mortality was observed on seedlings that were heavily covered with mud castings. In May 2016, reduction in emergence of seedlings in soil with 45, 90, and 180 earthworms (equivalent to 2,500- 10,000 m<sup>-2</sup>) was recorded at 2.41, 1.20, and 4.82%, respectively. Seedling emergence in soil with 360 earthworms (20,000 m<sup>-2</sup>) had 18.07% reduction (Table 6).
  - Seven days after the experiment was set up, earthworm mortality of 12.5, 37.5, and 41.67% was observed in test tubes with 2.5cm, 5cm, and 10 cm water level. Dead earthworms surfaced on the soil with pale color and disintegrated skin starting from the posterior part. Mortality was evident with the presence of pungent odor from the tubes with dead earthworms. No earthworm died in the test tubes with 0 to 0.5cm water level.
  - The levees in the pail with 10 cm flood water level were loosened and destroyed by the burrowing activities of earthworms (Figure 8). Thick casting was obvious on the side of levees flooded at 3 to 5cm and was otherwise present at the base of the levee with 0 to 1cm water level. The earthworms flocked in paddy levees and other elevated areas in the field to have access to oxygen and survive the flooded condition.
- A hundred percent mortality was observed on earthworms treated with niclosamide molluscicide 15 minutes after treatment application. Mortality of 70 to 100% was observed after 24 hr regardless of the rate of chemical applied. Mortality was not observed on earthworms applied with metaldehyde molluscicide and synthetic fertilizers (urea, ammonium phosphate, and complete). Dead earthworms did not react when touched using forceps and their skin disintegrated starting from their tail.
  - Under screen house conditions, paddy soil applied with niclosamide molluscicide and carbofuran had the least number of earthworms after 14 days from treatment application with an average number of 4.75 and 5 earthworms per pot. The highest number of earthworms at 11.50 and 10.75 per pot was recovered from soil applied with N and P-K fertilizers. In terms of individual weight (g), fresh weight of earthworms

treated with fertilizer (N-P and P-K) and pesticides (butachlor, pretilachlor and chlorpyrifos) had higher weight of 0.1318-0.1844 g than the control with 0.0844g (Table 7). Earthworms applied with carbofuran had the least weight of 0.07g. Treatments 2 and 3 had the most number of cocoons (average of 2.75 per pot).

Table 6. Effect of different number of earthworms on rice seedlings at 20	
DAS.	

No. of earthworm in #4 clay pot (176.71cm <sup>2</sup> )	% Seedling Emergence at 20 DAS	% Reduction in Seedling emergence relative to % emergence in T1	SHOOT LENGTH (cm)	ROOT LENGTH (cm)	SHOOT DW (mg)	ROOT DW (mg)
0	92.22 a	-	43.17	16.35 a	124.67 a	39.00 a
45	90.00 a	2.41	43.80	11.50 b	91.33 b	21.00 ь
90	91.11 a	1.20	45.07	11.68 b	77.67 b	21.00 ь
180	87.78 a	4.82	42.15	10.43 cb	84.33 b	19.48 b
360	75.56 b	<u>18.07</u>	<b>41</b> .35	8.32 c	80.00 b	<u>15.07</u> ь

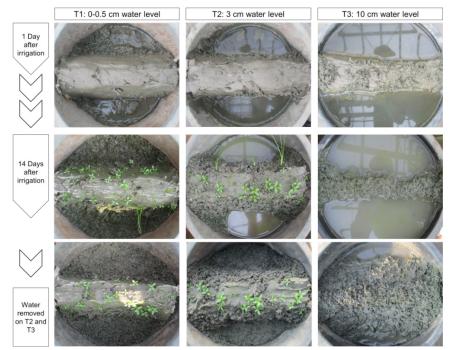


Figure 8. Loosening of levees by earthworms in relation to flooding.

Treatments	Active	Number of	Fresh wt.	Dry wt.	Number of		
Treatments	ingredient	earthworms	TTESTT WL.	Diy wi.	cocoons		
T1	Niclosamide	4.75 b	0.0873 bc	0.0166 ab	0.50 b		
T2	Butachlor	8.25 ab	0.1844 a	0.0184 ab	2.75 a		
Т3	Petilachlor	8.00 ab	0.1497 a	0.0136 ab	2.75 a		
T4	Chlorpyrifos	7.75 ab	0.1536 a	0.0136 ab	0.50 b		
T5	Carbofuran	5.00 b	0.0700 c	0.0106 b	2.00 ab		
T6	N-P-K	7.00 ab	0.0746 bc	0.0113 b	0.75 b		
17	Ν	11.50 a	0.0841 bc	0.0137 ab	1.00 ab		
T8	N-P	8.75 ab	0.1318 ab	0.0194 a	2.00 ab		
Т9	P-K	10.75 a	0.1608 a	0.0162 ab	1.75 ab		
T10	Control	8.25 ab	0.0844 bc	0.0139 ab	1.75 ab		

Table 7	7. Effec	t of syntl	netic farm	inputs to	earthw	orms w	hen app	lied	to
potted	paddy	soil with	ı earthwoi	ms at 14	days aft	er treat	ment ap	plic	ation.

### II. Biocontrol agents for pest management

Project leader: JT Niones

Microorganisms with their various mechanisms of establishing a positive host plant interaction can improve crop nutrition and the ability of crops to resist biotic and abiotic stress. Microorganisms such as plant growth promoting rhizobacteria (PGPR) and mycorrhizal fungi maintain multiple positive interactions with their plant hosts promoting plant growth via increased nutrient availability, by influencing plant hormone balance and the production of stress-protective compounds, and associative plant host protection against diseases, nematode and insect pests. This project generally aims develop technologies for beneficial organisms used for control of rice pests and diseases and eventually help reduce use of herbicides and pesticides. Specifically, studies under this project aims to (1) Formulate Beauveria bassiana (bals.) vuill. and Metarhizium anisopliae in a form that optimizes its efficacy, stability, safety and ease of application against rice bug; (2) Evaluate the efficacy of bacterial endophyte, Bacillus sp against sheath blight pathogen of rice; (3) Develop a rice-based bait for effectively delivery of S. singaporensis to rodent pest; and (4) Determine the disease resistance enhancing ability of selected strains of PGPR against rice blast disease and rice sheath blight.

#### Development and optimizing the production of powder form of fungal biological control agents for some major insect pests of rice (ABC-003-001)

GF Estoy and BM Tabudlong

The use of microbial control provides an alternative measure to insect pest population below damaging level. The Metarhizium anisopliae and Beauveria bassiana are entomopathogenic fungi proven to be effective against some major insect pests of rice. These organisms are being massproduced using different agricultural culture media. Production of the fungi in powdered form is practical and easier to apply in the field. Thus, the study aims to formulate and optimize the production of B. bassiana and M. anisopliae powder form, determine the effective rate of the fungus against rice bug, identify the best method of application and determine the costeffectiveness ratio of the powder form as fungal biological control agents of the some major rice insect pests.

#### **Results:**

Seven (7) culture media such as cracked corn, corn grits, sorgum, palay grain, milled rice, oatmeal and ordinary flour in powder form were evaluated for efficient formulation of B. bassiana and M. anisoplie. Results showed that powder form of the fungus on different formulation yielded a concentration of 107-108 conidia mL-1 suspension.

• To optimize the production of fungal conidial spores, M. anisopliae were produced in cracked corn and oatmeal while Beauveria bassiana in oatmeal and corn grits. Powder form the fungus yielded a conidial concentration of 108 conidia mL-1 suspension. Furthermore, powder fungal spores in oil formulation incubated in the laboratory was on-going. Evaluation of the fungal powder form against major pests in comparison with insecticide will be done under field condition to determine the cost-effective application ratio and optimize field application protocol (Table 8).

Table 8.	Production	of fungal	spores on	different	powder	formulation.
----------	------------	-----------	-----------	-----------	--------	--------------

Powder Substrates	Production of	Production of Fungal spores				
	Metarhizium anisopliae	Beauveria bassiana				
Cracked corn	7.7 x 10 <sup>8</sup> conidia mL <sup>-1</sup>	2.1 x 10 <sup>8</sup> conidia mL <sup>-1</sup>				
Palay grain	2.3 x 10 <sup>8</sup> conidia mL <sup>-1</sup>	1.1 x 10 <sup>7</sup> conidia mL <sup>-1</sup>				
Corn grits	3.5 x 10 <sup>8</sup> conidia mL <sup>-1</sup>	3.5 x 10 <sup>8</sup> conidia mL <sup>-1</sup>				
Sorghum	1.0 x 10 <sup>8</sup> conidia mL <sup>-1</sup>	1.2 x 10 <sup>8</sup> conidia mL <sup>-1</sup>				
Oatmeal	6.0 x 10 <sup>8</sup> conidia mL <sup>-1</sup>	4.2 x 10 <sup>8</sup> conidia mL <sup>-1</sup>				
Milled rice	3.2 x 10 <sup>8</sup> conidia mL <sup>-1</sup>	1.8 x 10 <sup>8</sup> conidia mL <sup>-1</sup>				
Ordinary Flour	1.7 x 10 <sup>8</sup> conidia mL <sup>-1</sup>	1.3 x 10 <sup>7</sup> conidia mL <sup>-1</sup>				

#### Utilization of Bacillus spp. and mycorrhiza as plant growth promoter and for sheath blight disease management in rice EB Gergon and AJ Santos

Sheath blight (ShB) caused by Rhizoctonia solani is one of the most economically important diseases of rice causing significant grain yield reduction. One successful formulation of biopesticide uses Bacillus sp. which has resistant life stage-like endospores that can survive adverse conditions. On the other hand, biofertilizers such as root inoculant that contain vesicular arbuscular mycorrhizal fungi (VAM) can address the high cost of inorganic fertilizer while preventing soil-borne pathogens such as R. solani from infecting the plants or helping plants tolerate the negative effects of root pathogens. Thus, the application of VAM can be a good alternative method for farmers to reduce fertilizer requirement and protect plants from soil-borne diseases. This study generally aims to develop biopesticide against the disease that can be mass produced and inherently safer to use and environment friendly. This study specifically evaluated a Bacillus sp. isolated from rice seed and VAM as rice growth promoters and biological control agents against sheath blight disease.

#### Activities:

٠

- Evaluation of antagonistic activity of Bacillus isolates by disc diffusion assay. The inhibition zones (IZ) around the discs after 72 hours at 28±2°C were measured.
- Evaluation of the effectiveness of VAM inoculant and Bacillus EG130 and EG108 on growth and yield of NSIC Rc240 under screenhouse and field conditions.
- Efficacy trials of VAM inoculant and Bacillus EG130 and EG108 against rice sheath blight under naturally infested field. Disease incidence and Relative Lesion Height (RLH) were assessed.

#### **Results:**

- Out of the 111 Bacillus isolates initially screened using multiple culture assay, 46 grew profusely ( $\geq$  2.0cm) inhibiting the mycelia of R. solani. Further screening using disc assay method showed that isolates EG130 and EG108 markedly inhibited R. solani after having profused growth of  $\geq$  5cm.
- Seed bacterization with EG130 and EG108 at any concentration significantly increased the root and shoot length of rice seedlings at 14 DAS (Figure 9). However, the total biomass of EG130 at 107 and EG108 at 108 concentrations were not significantly different with the control.
- Figure 10 shows that VAM-treated plants fertilized at full rate exhibited the highest shoot length (36.08 cm) and root length (31.59cm) having percent changes of 10.95 and 5.51%, respectively. Panicle length was also increased by 5.34% and 7.09% in VAM-treated plants fertilized at full and half rates, respectively. Rice plants inoculated with VAM and fertilized at any rates significantly increased the grain weight ranging from 64.77 to 75%. Highest grain weight was obtained with VAMtreated plants without any fertilizer applied (1.54g/panicle). The, highest root mass was obtained from VAM-treated plants fertilized at full rate (5.58g) and lowest at unfertilized plants (3.61g).
  - Multiple comparisons showed that, from 60 DAS up to 102 DAS, ShB incidence in plants treated with EG130, EG108 and VAM were consistently lower compared to untreated plants (Table 9). At 102 DAS, ShB incidence was significantly reduced by 45.63, 33.77 and 32.89% in VAM, EG130 and EG108– treated plants, respectively. Lowest ShB incidence (19.77%) was observed in plants treated with VAM inoculant. However,

#### 26 Rice R&D Highlights 2016

plants treated with EG130 and EG108 were not significantly different from VAM inoculated-plants having percent disease incidences of 24.08% and 24.40%, respectively. The untreated plants, having percent disease incidence of 36.36%, exhibited the highest ShB incidence.

- Plants treated with EG130, EG108 and VAM inoculant significantly reduced the sheath blight lesions by 28.20, 20.77 and 29.60% at 102 DAS, respectively. However, statistical analysis revealed that EG130 and VAM-treated plants were not significantly different from EG108-treated plants having RLH values of 37.92% and 41.84%, respectively. On the other hand, severity of ShB symptoms was highest in untreated plants as it exhibited RLH value of 52.81%.
- Unfortunately, none of the treatments used (EG130, EG108, VAM) did enhance the plant height and no. of tillers of rice 125 DAS as compared to untreated plants.
- Data on grain yield and yield components are still being processed. Percentage of seed discoloration will also be assessed to determine the intensity of damage of ShB on the seeds of treated plants.



**Figure 9.** Shoot (left) and root (right) length of rice seedlings as affected by seed bacterization at 14 DAS. Isolate 130 at 106 dilution (a). Isolate 130 at 107 dilution (b). Isolate 130 at 108 dilution (c). Isolate 108 at 106 dilution (d). Isolate 108 at 107 dilution (e). Isolate 108 at 108 dilution (f). Control (sterile dH2O) (g).



**Figure 10.** Top. Comparison between lowland rice with full fertilizer rate (left), half fertilizer rate (center) and 0 rate (right) 90 days after transplanting; Bottom: Effect of mychorrhizal inoculation on the roots of rice at 0, half, and full fertilizer rates at 90 days after transplanting.

**Table 9.** Effects of EG130, EG108 and VAM on the relative lesion height (RLH) of rice plants infected with sheath blight from 60 DAS up to 102 DAS in the field.

	RELATIVE LESION HEIGHT (%)								
- Treatment	60 DAS	67 DAS	74 DAS	81 DAS	88 DAS	95 DAS	102 DAS	% reduction at 102 DAS	
EG130	24.99 <sup>b</sup>	24.78 <sup>b</sup>	29.63 <sup>b</sup>	31.87 <sup>b</sup>	34.73 <sup>b</sup>	41.61 <sup>ab</sup>	41.84 <sup>b</sup>	20.77	
EG108	32.36ª	31.62ª	31.17 <sup>b</sup>	31.31 <sup>b</sup>	29.02°	34.52°	37.18 <sup>b</sup>	29.60	
VAM	28.76 <sup>ab</sup>	27.71 <sup>b</sup>	31.30 <sup>b</sup>	33.27 <sup>b</sup>	33.30 <sup>bc</sup>	38.86 <sup>cb</sup>	37.92 <sup>b</sup>	28.20	
Control	28.79 <sup>ab</sup>	33.62ª	41.10ª	42.30ª	44.98ª	47.84ª	52.81ª	-	

\*Values with the same letter within a column are not significantly different at P < 0.05 according to LSD

**Development of bait base for the delivery of Sarcocystis singaporensis** *UG Duque and MC Ablaza* 

Rodent pest has been a problem in agriculture, destroying different kinds of crops at different stages of production. Biological method through the use of a protozoon, Sarcocystis singaporensis presents as an alternative to manage this pest. This protozoon has been found to be highly specific to Rattus and Badicota genera only, where most rodent pest in the Philippines belongs to. This study aims to develop a bait base that can effectively deliver S. singaporensis to rodent pest. Currently, different rice-based baits are being tested for effectiveness and cost-efficiency. In addition, harvesting and purification of feces from pythons are routinely being done for the mass production of S. singaporensis.

#### Activities:

- Mass production of S. singaporensis.
- Palatability testing and virulence evaluation.

#### **Results:**

- Four bait bases with different ingredient proportions were already developed and being evaluated for rat acceptance (Table 10) and cost.
- Infection of rats with S. singaporensis using gavage needle were done in 34 apparently healthy rats.
- Observed effects of S. singaporensis ingection in rats include development of white spots in the eyes, aborted embryos, distorted lungs and intestines, raised fur and gradual weakening.
- Body weights of infected rats significantly declined 12 days after feeding, which continued to decline on the succeeding days under controlled conditions where favorable conditions were provided (Figure 11).

**Table 10.** Average daily consumptions after 5 consecutive days of feeding using choice test method on all possible treatment combinations. P values were derived using Student's t-test.

Male	Ave da consumpt	,	p val (0.0		Female	Ave da consumpt	,	p valu (0.05	
T1 VS T2	100	100	1	ns	T1 VS T2	100	100	1	ns
T1 VS T3	97.88	77.35	0.09	ns	T1 VS T3	100	100	1	ns
T1 VS T4	100	68.64	0.04	*	T1 VS T4	100	48.65	0.01	*
T2 VS T3	100	75.89	0.1	ns	T2 VS T3	100	100	1	ns
T2 VS T4	100	100	1	ns	T2 VS T4	100	93.18	0.19	ns
T3 VS T4	100	77.24	0.1	ns	T3 VS T4	51.93	86.09	0.05	ns



**Figure 11.** Body weight of R. tanezumi taken at 3 days interval for 24 days period. Blue line indicates control or untreated while the orange line is the treated. This shows that treated rats' body weight continue to decline after feeding as indicator of deteriorating body condition.

## Disease resistance inducing ability of selected isolates of plant growth promoting bacteria

JT Niones and JA Poblete

There is a growing body of evidence that some strains of plant growth-promoting microbes can also protect plants from infecting pathogens either directly or indirectly via induction of plant defense responses. Several PhilRice researchers have identified rhizobacteria and mycorrhizal fungi imparting growth-promoting activity to the rice plant but the disease resistance inducing ability of these organisms are yet characterized. Thus this study specifically aims to determine the disease resistance enhancing ability of Streptomyces mutabilis and Bacillus sp.. against rice blast disease and rice sheath blight. Once disease resistance enhancing ability is determined, the mechanisms of plant immune responses, including that of defense related- gene expression changes and cellular defense responses triggered by plant growth promoter rhizobacterial isolates will be characterized. If proven that the root-colonizing and plant growth-promoting activities of these organisms are also associated with plant protection against diseases then these can offer better opportunity for optimizing crop productivity.

#### Activities:

- Revival and culture maintenance of Bacillus sp. and Pyricularia oryzae.
- Evaluation of antagonistic activity of Bacillus sp. isolate through dual culture assay with P. oryzae and R. solani.
- Evaluation of the effect of Bacillus sp. on plant resistance against rice leaf blast and rice sheath blight.

#### **Results:**

- Bacillus sp. showed inhibitory activity against mycelial growth of P. oryzae and R. solani (Figure 12).
- Rice seedlings infected with Bacillus sp. had lower rice blast disease incidence as compared with those Bacillus sp.- free seedlings (Figure 13).
- Rice seedlings bacterized with Bacillus sp. had significantly lower number of spores per diseased lesion than Bacillus sp-free plants (Figure 14).
- Rice seedlings infected with Bacillus sp. had lower relative length of sheath blight lesions as compared with those Bacillus sp.- free seedlings (Figure 15).



B

**Figure 12.** Inhibitory activity of Bacillus sp. against P. oryzae and Rhizoctonia solani. A) Bacillus sp. (Right) inhibits the mycelial colony of P. oryzae (left). B) Bacillus sp. (right) inhibits the mycelial colony R. solani (left).

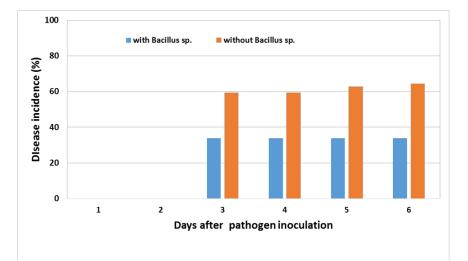
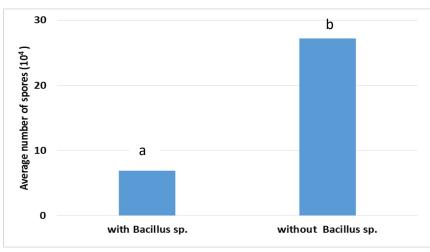
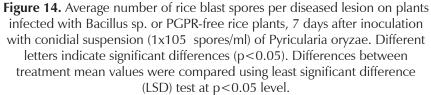
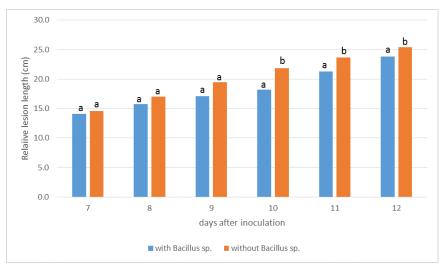


Figure 13. Percent rice blast blast disease incidence of plants infected with Bacillus sp. or PGP-free rice plants.







**Figure 15.** Relative length of sheath blight lesion on plants infected with Bacillus sp. or PGPR-free rice plants , starting at 7 days after inoculation with Rhizoctonia solani . Different letters indicate significant differences (p<0.05). Differences between treatment mean values were compared using least significant difference (LSD) test at p<0.05 level.

# III. Organisms for Enhancing Plant Tolerance to Abiotic Stresses

Project Leader: JA Cruz

Environmental stresses such as drought, temperature, salinity, air pollution, heavy metals, pesticides, and soil pH are major limiting factors in crop production because they affect almost all plant functions. Rhizobacterial populations of stressed soils are adapted and tolerant to stress which can be screened for isolation of efficient stress-tolerant, plant stress homeostasisregulating bacteria (PSHB) strains that can be used as inoculant for crops grown in stressed ecosystems. Production of exopolysaccharides (EPS) and accumulation of endogenous osmolytes (proline and glycine betaine) under varying stress conditions are significant strategies adopted by bacterial strains for successful survival in plant rhizosphere. These microbes can provide excellent models for understanding the stress tolerance, adaptation and response mechanisms that can be subsequently engineered into crop plants to cope with climate change induced stresses. Thus, this project aims to develop technologies for beneficial organisms inocula used to enhance plant tolerance to abiotic stresses.

## Isolation and Screening of Plant Growth Promoting Bacteria as Potential for Enhancing Drought Tolerance of Upland Rice

JA Cruz, JMR Bautista, TC Fernando, RL Ordonio, AA Dela Cruz, and ES Paterno

Upland rice production in the Philippines is challenged by many abiotic stresses. Amongst any other environmental stress, drought is considered the single most devastating environmental stress which decreases crop growth. This adverse effect may be attributed to decreased enzyme activities, loss of turgor, and decreased energy supply due to water insufficiency. To address drought stress in rainfed upland environment, plant growth-promoting rhizobacteria (PGPR) in the form of microbial inoculant became a very important component in the integrated plant nutrient management systems, particularly in rainfed areas, where farmers tend to rely either on "no-cost" or "low-cost" inputs. Inoculation of plants with organisms that have plant growth-promoting capabilities such as 1-aminocyclopropane-1-carboxycylic (ACC)-deaminase, indole-3-acetic acid (IAA), and phosphate solubilization may increase drought tolerance of rice growing in this droughtaffected areas. Many PGPR is currently being used to increase growth of rice yet very few information regarding PGPR for improving drought-tolerance of rice was generated. This study aims to screen for drought tolerant PGPR and examine their performance under drought conditions.

#### Activities:

- Drought tolerance of 46 selected potential drought-tolerant rhizobacteria was further tested using 25% polyethylene glycol (PEG) 8000 (water potential of -7.5 bars), a more severe stress. The inoculum was grown in arginine-glycerol-salt (AGS) broth medium for 7 days and 0.25ml of this culture was inoculated to pregerminated seeds of rice variety PSB Rc23 sown in sterilized test tube containing 0.25 ml 25% S-P solution (PEG in SNAP solution). Simplified nutrient addition program (SNAP) solution was obtained from the Institute of Plant Breeding, University of the Philippines Los Banos. Solution was prepared following the manufacturer's directions.
- At 14 days after sowing (DAS), plant height was measured and plant response to drought in terms of leaf rolling and leaf tip drying was observed.
- Four isolates were selected from among 46 isolates based on their effects on total plant length at 25% PEG.

#### **Results:**

- Less leaf rolling was observed in rice inoculated with IS 4-7, AP 3-7, AP 2-1, CS 10-12, and IS 8-9 isolates (Figure 16), with 25% PEG (water potential of -7.5 bars). Leaf rolling and leaf tip drying are indications of improved water assimilation in plants.
- Inoculation of rice plants with IS 4-7, AP 3-7, CS 10-12, and IS 8-9 isolates increased total plant length by 8% to 23% at 14 days after sowing (Figures 17 and 18).

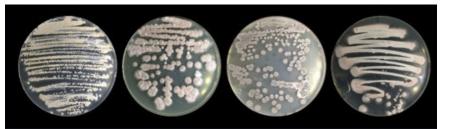
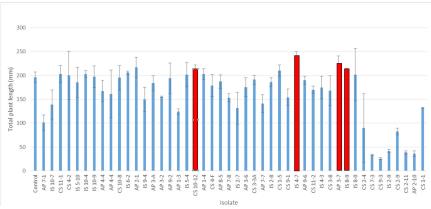
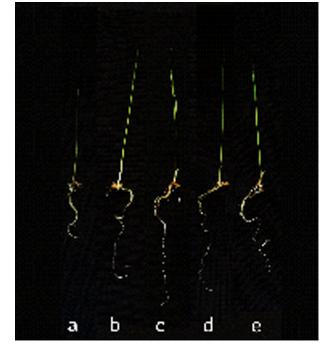


Figure 16. Isolates (a) IS 4-7, (b) AP 3-7, (c) CS 10-12, and (d) IS 8-9 isolates grown in AGS agar medium.



**Figure 17.** Total plant length of PSB Rc23 seedlings as affected by forty-six selected isolates at 25% PEG concentration, a more severe drought stress or lower water potential = -7.5 bars, 14 days after sowing (DAS). Note: Initial screening started with 140 isolates.



**Figure 18.** Upland rice as affected by inoculation with each of the four selected isolates: (a) Uninoculated, (b) IS 4-7, (c) AP 3-7, (d) CS 10-12, and (e) IS 8-9 at 14 DAS.

#### **Potential Fungi for Acquired Drought Tolerance** *XH Truong and MD Duque*

Endophytes include all organisms living in the internal tissues of their hosts during a period of their life. Endophytic fungi are living inside the plants without causing any apparent symptoms. A single endophyte produces several bioactive metabolites that are useful to pharmaceutical and agricultural industries. Endophytes can influence soil stability directly by their mycelia networks in the soil as well as indirectly altering roots and physical conditions of the host plants. Piriformospora sp. is a mycorrhizae-like fungus, cultivable in axenic cultures, where it forms chlamydospores asexually. This fungus is symbiotic with several plant species. This is of great interest that it has plant growth-promoting characteristics and contributes to their host plants including water stress or drought tolerance, and plant protection. This study aims to evaluate rice seeds inoculated with root endophyticfungi Piriformospora sp. that possibly can tolerate water stress during the reproductive growth stage.

#### Activities:

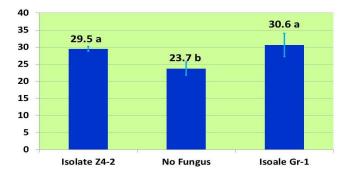
- Maintenance of Piriformospora sp isolated from rice and grass in dry land in Central Luzon.
- Examination of fungal drought tolerance in the greenhouse using NSIC Rc238 as test variety. Treatment was replicated three times and laid out in a RCBD.
- Examined daily the plant response to water deficit. A visual score of the degree of leaf rolling or folding was recorded on sample leaves using a 1 to 5 scale with 1 being the first evidence of rolling and 5 being a closed cylinder (IRRI rating scheme for drought tolerance).

#### **Results:**

- Seeds of NSIC Rc238 that were inoculated with Piriformosa sp, conferred water stress tolerance through a 30-day period during the reproductive stage (57 to 87 DAS). Under regular rice culture, the panicle production of NSIC Rc238 inoculated with Piriformosa sp, was enhanced (Figure 19),
- Under water stress, a scheme for scoring leaf rolling and dried up spikelets suited for NSIC Rc238 was developed (Figure 20);
- Using the scheme for stress evaluation, Figure 21 and 22

showed that NSIC RC238 inoculated with the endophytic fungal isolate Z4-2 conferred the water stress tolerance during the reproductive stage;

However, the grain yield and biomasses of plant tissues during the ripening stage are being monitored.



.

**Figure 19.** Under non-water stress, panicle production of NSIC Rc238 enhanced by seed treated with endophytic fungal isolate Z4-2 vs negative check vs seed treated with isolate GR-1.



**Figure 20.** Leaves and panicles of NSIC Rc238 affected by 30d withheld irrigation during reproductive developmental stage (57 to 87 DAS): Leaf rolling 2 is considered as threshold tolerant stress of panicle development.

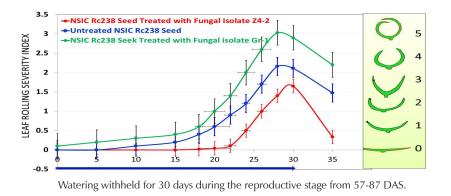


Figure 21. NSIC Rc238 seed treated with fungal isolate Z4-2 showed the lowest leaf rolling/dried up spikelets.



**Figure 22.** Under water stress: Watering withheld for 30 days from 57 to 87 DAS, seed treated with fungal isolate Z4-2 and Gr-1 enhanced the panicle production of NSIC Rc238.

# IV. Utilization of organisms from the rice environment for protein and carbon sources

Project Leader: RL Ordonio

Increasing the amount of food, feed and raw materials for the ever-increasing population and mitigating the fast depletion of natural resources remain a daunting challenge in many developing countries like the Philippines. The rice environment is known to host numerous species of microorganisms that when utilized, might provide solutions to address these concerns. To this end, this project aims to develop technologies for beneficial organisms useful in product development. On-going studies are exploring the use of a nitrogen-fixing blue-green algae common to rice paddies, as a source of food and feed supplement. Its robustness under stresses, and its low maintenance and input requirement make this exotic crop a potential additional source of livelihood for farmers. Another is the use of lignindegrading bacteria from cow/carabao dung as catalysts for the conversion of rice lignocellulosic biomass that are usually burned, into bioethanol that can be used as alternative environment-friendly fuel or gasoline additive; hence, maximizing the benefits derived from rice farming and mitigating the worsening problem on global warming. Together, these studies will deliver outputs that have environmental, nutraceutical and industrial significance.

## Ecological basis for persistence and production of Nostoc commune in the rice paddies (ABC-005-001-CES)

AA Dela Cruz, TC Fernando, ES Avellanoza, JC Yabes, MM Goss and GO San Valentin

During the 1980s to the early 2000s, Nostoc commune (aka "tabtaba") was intercropped with rice and existed in large biomasses. It was consumed as traditional food by the llocanos as source of protein or as feed for farm animals. It has originally been explored as a nitrogen source in rice fields. However, it recently disappeared, if not, decreased in terms of area inhabited. The cultural practices of farmers might have created variations in the soil and water conditions, along with the dramatic change in environmental condition brought about by climate change, which eventually affected the populations of Nostoc. To be able to restore its production level, this study generally aims to determine the ecological factors that affect the persistence and reproduction of N. commune in the rice paddies. Specifically, this study aim to characterize the existing growing conditions where it grows prolifically and eventually design scalable systems for its production using farm resources.

#### Activities:

- Optimization of use of farm resources for pond culture of N. commune: Three 1 x 1 m culture ponds were established in PhilRice CES in May, each with the following treatments: T1 = soil and water only; T2 = soils and water, enhanced with 5g solophos (0-18-0); T3 = soil and water, enhanced with 5g solophos (0-18-0) + 5g complete fertilizer (14-14-14).
- Outdoor culture of N. commune in glass containers: T1 was supplied with soil-water medium, T2 and T3 with additional enhancement of solophos (0-18-0) at 3ppm and 6ppm, respectively. BG-11 synthetic medium served as control (T4). Four grams of fresh spherical N. commune (approximately 10 pcs) were added into each glass containers with 3L culture media.
- Survey of areas where N. commune is still existing and revisited areas that were previously reported with abundant bloom.
- On-farm culture of N. commune in selected areas in llocos Norte: Three 1x1m<sup>2</sup> cages were laid on the field and inoculated with 55g crushed N. commune

#### **Results:**

- Figure 23 shows the growth pattern of N. commune from week 8 to week 24 in culture ponds established in PhilRice CES, along with the average temperature and percent relative humidity (%RH) recorded during the production period (May-November). In November, when the production was at peak, the average temperature was 30oC and RH was 66%. With reference to the observations gathered in 2015, the slight variations in the temperature and % RH did not affect the growth and production of N. commune in PhilRice CES.
- Tiny spherical N. commune started to develop in culture ponds a month after inoculation. After 28 weeks, a total of 2,985g N. commune was harvested from T2, while 325g and 175g were harvested from T3 and T1, respectively.
- Spherical N. commune with sizes  $\geq$  5mm were observed in T2 starting week 8. On the other hand, it was only in week 24 that it was observed in T3, and at week 28 in T1.
- Likewise, outdoor culture of N. commune using different culture media is currently being tested in glass containers

(28x18x18cm) to examine the effect of various culture media on heterocyst formation, the site for nitrogen-fixation.

- A plentiful bloom of N. commune in the rice paddy (Figure 24 ) was documented in Sitio Ballit, Brgy. Sta. Matilde, Pasuquin, llocos Norte in September. About 500 to 1000g fresh samples were collected in each of the 3 1x1m<sup>2</sup> quadrats sampled.
- Figure 25 shows the areas surveyed for presence of N. commune. Municipal Agriculturists and few farmers in Ilocos Norte (Batac City, San Nicolas, Burgos, Bangui, Bacarra, Sarrat, Vintar, Dumalneg, Pagudpud, Pasuquin, and Piddig), and other areas marked with green, testified that it is still being found in hilly areas or rice paddies near mountains but their occurrence has already dwindled. Areas marked with yellow color were previously reported with abundant algal bloom but none was found anymore when the sites were revisited.
- On-farm culture of N. commune was initially established in San Pedro, Pasuquin and Batac Ilocos Norte. Monitoring of persistence and growth is on-going.



**Figure 23.** Growth pattern of N. commune from week 8 to week 24 in culture ponds established in PhilRice CES (GT1= soil and water only; GT2= soils and water, enhanced with 5g solophos (0-18-0); GT3= soil and water, enhanced with 5g solophos (0-18-0) + 5g complete fertilizer (14-14-14); Average water pH (pHT1, pHT2, pHT3), average air temperature (Temp), percent relative humidity (%RH).



Figure 24. A. A plentiful bloom of N. commune in the rice paddy (Fig. ) documented in Sitio Ballit, Brgy. Sta. Matilde, Pasuquin, Ilocos Norte; B. Sampling of specimen from a  $1x1m^2$  quadrat.



**Figure 25.** Areas in Ilocos Region, Cagayan Valley and Mountain Province surveyed for presence of N. commune (Green – N. commune is still existing but occurrence has already dwindled; Yellow – areas previously reported with abundant bloom but none was found during the inspection).

## Identification of rice lignin-degrading bacterial strains and screening of low-lignin rice

RL Ordonio, JA Cruz and TC Fernando

As an agricultural country which mainly produces rice, the Philippines seasonally produces a great amount of rice straw, which is a form of lignocellulosic biomass. Such biomass is an attractive raw material for the production of bioethanol, one way of mitigating global warming. However, in order to efficiently utilize rice straw, lignin, which encases cellulose fibers, must first be degraded to aid saccharification. Although plant biomass can be chemically treated to get rid of lignin, the process requires hazardous chemicals or high temperatures that pose a hazard to the environment and workers. The use of microbial inoculants is a feasible and practical way of processing lignocellulosic biomass for bioethanol production. However, this entails careful screening and characterization of local microbial isolates to prove their effectiveness. To complement this, it should be important to identify rice varieties with optimal lignin content for utilization in bioethanol production., This study therefore aims to isolate lignindegrading microorganisms from cow/carabao manure, test their effectiveness in degrading rice lignocellulosic biomass and rank popular high yielding varieties in terms of lignin content.

#### Activities :

- Isolation and screening of lignin-degrading bacteria from fresh cow and carabao manure.
- Chemically analysis and profiling of popular high yielding varieties based lignin content.

#### **Results:**

- A total of 16 lignin-degrading bacteria were isolated, 9 of which were found to be thermophilic.
- Of the 9 isolates, 7 were positive for IAA production, showing that these isolates may be used to promote plant growth.
- Two isolates (6R-4 & 3-R-2-Sp) were able to solubilize phosphorus to a form available for plants.
- Also, one isolate (6R-4) was found to hydrolyze starch, a trait that is useful for rice biomass degradation and saccharification.
- Siderophore production was also detected in 6R-4, indicating that it can be used as a soil fertility enhancer and as a biocontrol agent.

#### 44 Rice R&D Highlights 2016

- Two isolates (3-R-2-Sp & Car 2a) were assayed for the presence of Lignin Peroxidase enzyme (LiP) using methylene blue dye. Decolorization of methylene blue dye was used as an indicator of the oxidation ability of ligninolytic enzyme produced by the potential lignin degrading bacterial strains.
- Using Biolog Gen III system, 2 of the 9 isolates were identified as Bacillus amyloliquefaciens (3-R-2-Sp) and Bacillus cibi (3R-2S). 6R-4 was determined as Actinomycete sp. based on its morphology.
- Result on the lignin profiling of different varieties showed that NSIC Rc300\*\* had the highest lignified area (33.4%) while NSIC Rc29\* had the lowest (13.3%) (Figure 26).

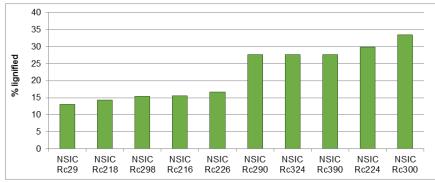


Figure 26. Lignified area estimated from the stained stem cross sections of different released rice varieties.

### Bioremediation of toxicants in rice environments

Project Leader: JC Magahud

Rice environments can be enriched with toxicants due to regular application of farm inputs, burning of rice straw, and deposition of industrial wastes. Toxicants are threats to the functions of rice areas to human population, which is to produce enough nutritious rice with safe levels of contaminants. As such, there is a need to assess levels of these toxicants. Moreover, high levels should be reduced if they exceed the limits. Organisms can reduce toxicant levels in rice fields. Furthermore, they can be used as indicators of presence or level of toxicants because biological parameters are sensitive to toxicants, and standard laboratory techniques are very expensive and timeconsuming. The general objective of the project is to reduce the levels of toxicants in rice environments. Specifically, it aims to develop techniques of using indicator organisms in assessing the levels of toxicants, and identify organisms that can reduce levels of toxicants.

#### **V. Bioremediation of toxicants in rice environments** Project Leader: JC Magahud

Rice environments can be enriched with toxicants due to regular application of farm inputs, burning of rice straw, and deposition of industrial wastes. Toxicants are threats to the functions of rice areas to human population, which is to produce enough nutritious rice with safe levels of contaminants. As such, there is a need to assess levels of these toxicants. Moreover, high levels should be reduced if they exceed the limits. Organisms can reduce toxicant levels in rice fields. Furthermore, they can be used as indicators of presence or level of toxicants because biological parameters are sensitive to toxicants, and standard laboratory techniques are very expensive and timeconsuming. The general objective of the project is to reduce the levels of toxicants in rice environments. Specifically, it aims to develop techniques of using indicator organisms in assessing the levels of toxicants, and identify organisms that can reduce levels of toxicants.

## Abundance of macro and microdriles as indicator for degradation of paddy soils

JC Magahud, SLP Dalumpines and CC Cabusora

Natural factors as well as continuous high-yield cropping can result in degraded conditions of agricultural soils. Examples of degraded soils are those that are strongly acidic, low in nutrient levels, and those that contain high amounts of pesticide residues. Degraded paddy soils usually produce low rice yields and can threaten the health of rice consumers. However, standard laboratory techniques for assessing soil degradation levels are expensive and time-consuming. As such, there is a need for alternative cost-effective techniques. One promising technique is the use of macro and microdriles because their abundance and biomass are related to soil fertility and health. This study aims to compare the efficiency and practicality of methods for determining macro and microdrile abundance. This study also aims to assess the relationship of the organisms' abundance with rates and frequency of pesticide and fertilizer applications, and soil fertility and pollution levels. Field assessments and laboratory experiments are being done to accomplish the objectives.

#### 46 Rice R&D Highlights 2016

#### Activities:

- Bulk collection and culture of Lumbriculus variegatus.
- Testing of Lumbriculus variegatus as indicator of pesticide and heavy metal concentration or presence.
- Assessment of techniques for counting Lumbriculus variegatus in the field.

#### **Results:**

- Four bulk collections of L. variegatus were performed. Collections were done under the heaps of rice straw in the field, where L. variegatus are naturally abundant. They were transported to screenhouse, and placed into plastic pails under a temperature of 28 to 30°C. After a few weeks, worms were used for the experiments.
- Lumbriculus variegatus were cultured in aerated aquaria. Presoaked strips of brown papers were placed on the bottom of the aquarium, and worms were fed with sinking feeds. Number of earthworms increased by 4% from 0 until 14 days of incubation in cultures with initial number of 30 (Table 11a). Number of earthworms increased by 7% from 14 until 28 days of incubation in cultures with initial number of 60. Total biomass of earthworms also increased by 5% from 14 until 28 days of incubation in cultures with initial number of 60 (Table 11b).
- Mortality of immature L. variegatus generally increases with increasing concentrations of butachlor at 0—948 mg/kg after 7 and 14 days of exposure (Figure 27a).
- Mortality of immature L. variegatus also increases with increasing concentrations of niclosamide at 0—521 mg/kg after 7 and 14 days of exposure (Figure 27b).
- Mortality of immature L. variegatus also increases with increasing concentrations of copper at 0—1000 mg/kg after 7 days of exposure (Figure 28).
- One person needs to spend 50 minutes to count L. variegatus using hand sorting method in 20cm x 20cm x 15cm soil volume.

**Table 11a.** Number of surviving earthworms after 14, 28 and 42 days of culture in aerated aquaria.

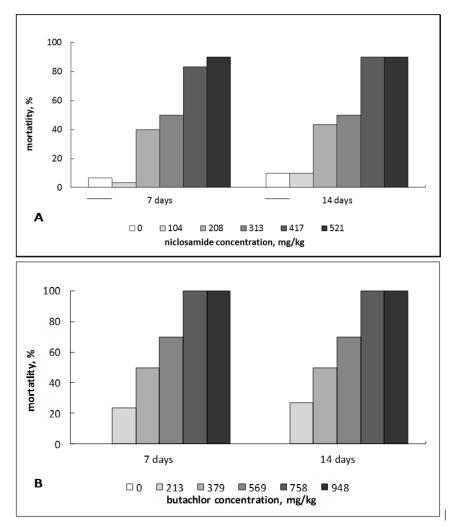
Initial Number of Worms	Number of Worms after 14 days⁺	% Increase (+) or Decrease (-)	Number of Worms after 28 days⁺	% Increase (+) or Decrease (-)	Number of Worms after 42 days⁺	% Increase (+) or Decrease (-)
30	30.0	0	32.0	+7	31.3	-2
60	62.3	+4	60.3	-3	59.3	-2

<sup>+</sup>average of 3 replications

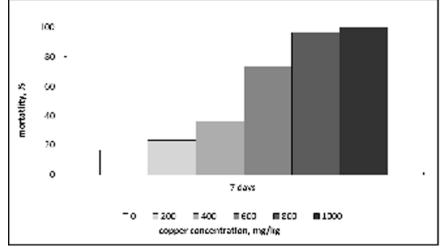
**Table 11b.** Biomass of surviving earthworms after 14, 28 and 42 days of culture in aerated aguaria.

Initial Number	Biomass after	<b>Biomass after</b>		Biomass after	% Increase (+)
of Earthworms	<b>14 days</b> , g <sup>+</sup>	28 days, g <sup>+</sup>	or Decrease (-)	<b>42 days</b> , g⁺	or Decrease (-)
30	2.03	2.04	0	1.85	-9
60	4.17	4.39	+5	3.43	-22

<sup>+</sup>average of 3 replications



**Figure 27.** Percent mortality of immature L. variegatus in varying concentrations and exposure time to butachlor (a) and niclosamide (b)



**Figure 28.** Percent mortality of immature L. variegatus in varying concentrations and exposure time to copper.

#### Abbreviations and acronymns

ABA – Abscicic acid Ac – anther culture AC – amylose content AESA - Agro-ecosystems Analysis AEW - agricultural extension workers AG – anaerobic germination AIS – Agricultural Information System ANOVA - analysis of variance AON – advance observation nursery AT – agricultural technologist AYT - advanced yield trial BCA - biological control agent BLB – bacterial leaf blight BLS – bacterial leaf streak BPH – brown planthopper Bo - boron BR – brown rice BSWM - Bureau of Soils and Water Management Ca - Calcium CARP - Comprehensive Agrarian Reform Program cav – cavan, usually 50 kg CBFM – community-based forestry management CLSU - Central Luzon State University cm - centimeter CMS – cystoplasmic male sterile CP – protein content CRH - carbonized rice hull CTRHC - continuous-type rice hull carbonizer CT – conventional tillage Cu – copper DA – Department of Agriculture DA-RFU - Department of Agriculture-Regional Field Units DAE – days after emergence DAS – days after seeding DAT – days after transplanting DBMS - database management system DDTK – disease diagnostic tool kit DENR – Department of Environment and Natural Resources DH L- double haploid lines DRR – drought recovery rate DS – dry season DSA - diversity and stress adaptation DSR – direct seeded rice DUST - distinctness, uniformity and stability trial DWSR – direct wet-seeded rice EGS – early generation screening EH – early heading

EMBI – effective microorganism-based inoculant EPI – early panicle initiation ET – early tillering FAO – Food and Agriculture Organization Fe – Iron FFA – free fatty acid FFP – farmer's fertilizer practice FFS – farmers' field school FGD – focus group discussion FI – farmer innovator FSSP - Food Staples Self-sufficiency Plan g – gram GAS – golden apple snail GC – gel consistency GIS – geographic information system GHG – greenhouse gas GLH - green leafhopper GPS – global positioning system GQ - grain quality GUI – graphical user interface GWS - genomwide selection GYT – general yield trial h – hour ha – hectare HIP - high inorganic phosphate HPL – hybrid parental line I - intermediate ICIS – International Crop Information System ICT – information and communication technology IMO - indigenous microorganism IF – inorganic fertilizer INGER - International Network for Genetic Evaluation of Rice IP – insect pest IPDTK - insect pest diagnostic tool kit IPM – Integrated Pest Management IRRI – International Rice Research Institute IVC – in vitro culture IVM – in vitro mutagenesis IWM – integrated weed management JICA – Japan International Cooperation Agency K – potassium kg – kilogram KP – knowledge product KSL – knowledge sharing and learning LCC – leaf color chart LDIS - low-cost drip irrigation system LeD – leaf drying LeR – leaf rolling lpa – low phytic acid LGU – local government unit

PI – panicle initiation

LSTD - location specific technology development m – meter MAS - marker-assisted selection MAT – Multi-Adaption Trial MC - moisture content MDDST - modified dry direct seeding technique MET – multi-environment trial MFE – male fertile environment MLM - mixed-effects linear model Mg – magnesium Mn – Manganese MDDST - Modified Dry Direct Seeding Technique MOET - minus one element technique MR - moderately resistant MRT – Mobile Rice TeknoKlinik MSE – male-sterile environment MT – minimum tillage mtha-1 - metric ton per hectare MYT - multi-location yield trials N – nitrogen NAFC – National Agricultural and Fishery Council NBS - narrow brown spot NCT – National Cooperative Testing NFA – National Food Authority NGO – non-government organization NE – natural enemies NIL – near isogenic line NM – Nutrient Manager NOPT - Nutrient Omission Plot Technique NR - new reagent NSIC - National Seed Industry Council NSQCS – National Seed Quality Control Services OF - organic fertilizer OFT – on-farm trial OM - organic matter ON – observational nursery OPAg - Office of Provincial Agriculturist OpAPA – Open Academy for Philippine Agriculture P – phosphorus PA – phytic acid PCR – Polymerase chain reaction PDW - plant dry weight PF – participating farmer PFS – PalayCheck field school PhilRice - Philippine Rice Research Institute PhilSCAT – Philippine-Sino Center for Agricultural Technology PHilMech – Philippine Center for Postharvest Development and Mechanization PCA – principal component analysis

PN – pedigree nursery PRKB – Pinoy Rice Knowledge Bank PTD – participatory technology development PYT – preliminary yield trial QTL - quantitative trait loci R - resistant RBB – rice black bug RCBD - randomized complete block design RDI - regulated deficit irrigation RF – rainfed RP – resource person RPM – revolution per minute RQCS – Rice Quality Classification Software RS4D – Rice Science for Development RSO – rice sufficiency officer RFL – Rainfed lowland RTV – rice tungro virus RTWG – Rice Technical Working Group S – sulfur SACLOB - Sealed Storage Enclosure for Rice Seeds SALT – Sloping Agricultural Land Technology SB – sheath blight SFR – small farm reservoir SME – small-medium enterprise SMS - short message service SN – source nursery SSNM - site-specific nutrient management SSR – simple sequence repeat STK – soil test kit STR - sequence tandem repeat SV – seedling vigor t – ton TCN – testcross nursery TCP – technical cooperation project TGMS – thermo-sensitive genetic male sterile TN – testcross nurserv TOT – training of trainers TPR – transplanted rice TRV - traditional variety TSS - total soluble solid UEM – ultra-early maturing UPLB – University of the Philippines Los Baños VSU – Visayas State University WBPH – white-backed planthopper WEPP - water erosion prediction project WHC – water holding capacity WHO - World Health Organization WS – wet season WT - weed tolerance YA – yield advantage Zn – zinc ZT – zero tillage

#### List of Tables

List of Figures

rates at 90 days after transplanting.

	Page		Page
<b>Table 1.</b> Economic analysis of the effectiveness ofactinomycetes as microbial inoculant for upland rice.	5	<b>Figure 1.</b> Effect of S. mutabilis NB3 inoculation on rice seed- ling growth under laboratory conditions.	4
<b>Table 2.</b> Ammonia nitrogen and nitrate nitrogen produced by BGA in BG11 and BG11-N liquid media.	12	<b>Figure 2.</b> Effects of S. mutabilis NB3 inoculation on rice root hairs at 3 days after inoculation (Magnification: 1000X);	5
<b>Table 3.</b> Agronomic data of PSB Rc82 during active tilleringstage.	12	Upland rice seedlings as affected by Streptomyces mutablis NB3 inoculation 5 days after inoculation. (uninoculated: a and c; inoculated: b and d).	
Table 4. Summary of Findings.	16	Figure 3. Grain yield (t/ha at 14% MC) of NSIC Rc238 applied	8
<b>Table 5</b> . Growth rate (g/day) of eleven azolla accessions from April 14 to May 27, 2016 in PhilRice Los Baños Azolla	19	with inorganic fertilizer and pGPR-based biofertilizer: a - 2016DS, CES; b – 2016WS, CES.	
Nursery.		<b>Figure 4.</b> Efficacy of PGPR-inoculant on plant height and	9
<b>Table 6.</b> Effect of different number of earthworms on riceseedlings at 20 DAS.	20	number of panicles per hill (a) and on dry weight of 1,000 grains at 14% MC (b) of NSIC Rc238. DS 2016, CES.	
<b>Table 7.</b> Effect of synthetic farm inputs to earthworms when           applied to potted paddy soil with earthworms at 14 days after	22	<b>Figure 5.</b> Nitrogen analysis of propagated flakes from the 4 BGA strains.	11
treatment application.		Figure 6. Customized culture baths used in screening under	18
<b>Table 8.</b> Production of fungal spores on different powder           formulation.	24	controlled floodwater temperature. (1) heater, (2) aerator, (3) hot water, (4) pots with soil-and-water medium, (5) data logger and (6) source of electricity.	
Table 9. Effects of EG130, EG108 and VAM on the relative	27		18
lesion height (RLH) of rice plants infected with sheath blight from 60 DAS up to 102 DAS in the field.		<b>Figure 7.</b> Response of different azolla accessions after 10 da of incubation at 36°C average floodwater temperature. (a) Azolla microphylla # 4124, (b) Azolla microphylla # 4114	
Table 10. Average daily consumptions after 5 consecutive	29	and (c) Azolla microphylla # 4119.	
days of feeding using choice test method on all possible treatment combinations. P values were derived using Student's t-test.		<b>Figure 8.</b> Loosening of levees by earthworms in relation to flooding.	21
<b>Table 11a.</b> Number of surviving earthworms after 14, 28 and42 days of culture in aerated aquaria.	47	<b>Figure 9.</b> Shoot (left) and root (right) length of rice seedlings as affected by seed bacterization at 14 DAS. Isolate 130 at	26
<b>Table 11b.</b> Biomass of surviving earthworms after 14, 28 and42 days of culture in aerated aquaria.	47	106 dilution (a). Isolate 130 at 107 dilution (b). Isolate 130 at 108 dilution (c). Isolate 108 at 106 dilution (d). Isolate 108 at 107 dilution (e). Isolate 108 at 108 dilution (f). Control (sterile dH2O) (g).	
		<b>Figure 10.</b> Top. Comparison between lowland rice with full fertilizer rate (left), half fertilizer rate (center) and 0 rate (right) 90 days after transplanting; Bottom: Effect of mychorrhizal inoculation on the roots of rice at 0, half, and full fertilizer	27

Rice R&D Highlights 2016

#### **List of Figures**

29

31

31

32

32

34

35

35

Figure 11. Body weight of R. tanezumi taken at 3 days interval for 24 days period. Blue line indicates control or untreated while the orange line is the treated. This shows that treated rats' body weight continue to decline after feeding as indicator of deteriorating body condition.

Figure 12. Inhibitory activity of Bacillus sp. against P. oryzae and Rhizoctonia solani. A) Bacillus sp. (Right) inhibits the mycelial colony of P. oryzae (left). B) Bacillus sp. (right) inhibits the mycelial colony R. solani (left).

Figure 13. Percent rice blast blast disease incidence of plants infected with Bacillus sp. or PGP-free rice plants.

Figure 14. Average number of rice blast spores per diseased lesion on plants infected with Bacillus sp. or PGPR-free rice plants, 7 days after inoculation with conidial suspension (1x105 spores/ml) of Pyricularia oryzae. Different letters indicate significant differences (p < 0.05). Differences between treatment mean values were compared using least significant difference (LSD) test at p < 0.05 level.

Figure 15. Relative length of sheath blight lesion on plants infected with Bacillus sp. or PGPR-free rice plants, starting at 7 days after inoculation with Rhizoctonia solani . Different letters indicate significant differences (p<0.05). Differences between treatment mean values were compared using least significant difference (LSD) test at p < 0.05 level.

Figure 16. Isolates (a) IS 4-7, (b) AP 3-7, (c) CS 10-12, and (d) IS 8-9 isolates grown in ACS agar medium.

Figure 17. Total plant length of PSB Rc23 seedlings as affected by forty-six selected isolates at 25% PEG concentration, a more severe drought stress or lower water potential = -7.5bars, 14 days after sowing (DAS). Note: Initial screening started with 140 isolates.

Figure 18. Upland rice as affected by inoculation with each of the four selected isolates: (a) Uninoculated, (b) IS 4-7, (c) AP 3-7, (d) CS 10-12, and (e) IS 8-9 at 14 DAS.

**List of Figures** 

	Page
<b>Figure 19.</b> Under non-water stress, panicle production of NSIC Rc238 enhanced by seed treated with endophytic fungal isolate Z4-2 vs negative check vs seed treated with isolate GR-1.	37
<b>Figure 20.</b> Leaves and panicles of NSIC Rc238 affected by 30d withheld irrigation during reproductive developmental stage (57 to 87 DAS): Leaf rolling 2 is considered as threshold tolerant stress of panicle development.	37
<b>Figure 21.</b> NSIC Rc238 seed treated with fungal isolate Z4-2 showed the lowest leaf rolling/dried up spikelets.	38
<b>Figure 22.</b> Under water stress: Watering withheld for 30 days from 57 to 87 DAS, seed treated with fungal isolate Z4-2 and Gr-1 enhanced the panicle production of NSIC Rc238.	38
<b>Figure 23.</b> Growth pattern of N. commune from week 8 to week 24 in culture ponds established in PhilRice CES (GT1= soil and water only; GT2= soils and water, enhanced with 5g solophos (0-18-0); GT3= soil and water, enhanced with 5g solophos (0-18-0) + 5g complete fertilizer (14-14-14); Average water pH (pHT1, pHT2, pHT3), average air temperature (Temp), percent relative humidity (%RH).	41
<b>Figure 24.</b> A. A plentiful bloom of N. commune in the rice paddy (Fig.) documented in Sitio Ballit, Brgy. Sta. Matilde, Pasuquin, Ilocos Norte; B. Sampling of specimen from a 1x1m <sup>2</sup> quadrat.	42
<b>Figure 25.</b> Areas in Ilocos Region, Cagayan Valley and Mountain Province surveyed for presence of N. commune (Green – N. commune is still existing but occurrence has already dwindled; Yellow – areas previously reported with abundant bloom but none was found during the inspection).	42
<b>Figure 26</b> . Lignified area estimated from the stained stem cross sections of different released rice varieties.	44
<b>Figure 27.</b> Percent mortality of immature L. variegatus in varying concentrations and exposure time to butachlor (a) and niclosamide (b)	48
<b>Figure 28.</b> Percent mortality of immature L. variegatus in varying concentrations and exposure time to copper.	49



PhilRice Central Experiment Station; Maligaya, Science City of Muñoz, 3119 Nueva Ecija Tel: (44) 456-0277 • Direct line/Telefax: (44) 456-0112 • Email: prri.mail@philrice.gov.ph PhilRice Text Center: 0920-911-1398 • Websites: www.philrice.gov.ph; www.pinoyrice.com

#### **BRANCH STATIONS:**

PhilRice Agusan, Basilisa, RTRomualdez, 8611 Agusan del Norte; Telefax: (85) 343-0768; Tel: 343-0534; 343-0778; Email: agusan.station@philrice.gov.ph PhilRice Batac, MMSU Campus, Batac City, 2906 Ilocos Norte; Telefax: (77) 772-0654; 670-1867; Tel: 667-1508; Email: batac.station@philrice.gov.ph PhilRice Bicol, Batang, Ligao City, 4504 Albay; Tel: (52) 284-4860; Mobile: 0918-946-7439; Email: bicol.station@philrice.gov.ph PhilRice Isabela, Malasin, San Mateo, 3318 Isabela; Mobile: 0908-895-7796; 0915-765-2105; Email: isabela.station@philrice.gov.ph PhilRice Los Baños, UPLB Campus, Los Baños, 4030 Laguna; Tel. (49) 536-8620; 501-1917; Mobile: 0920-911-1420; Email: losbanos@philrice.gov.ph PhilRice Midsayap, Bual Norte, Midsayap, 9410 North Cotabato; Tel: (64) 229-8178; 229-7241 to 43; Email: midsayap.station@philrice.gov.ph PhilRice Negros, Cansilayan, Murcia, 6129 Negros Occidental; Mobile: 0932-850-1531; 0915-349-0142; Email: negros.station@philrice.gov.ph PhilRice Field Office, CMU Campus, Maramag, 8714 Bukidnon; Mobile: 0916-367-6086; 0909-822-9813 Liaison Office, 3rd Floor, ATI Bldg, Elliptical Road, Diliman, Quezon City; Tel: (02) 920-5129

#### SATELLITE STATIONS:

Mindoro Satellite Station, Alacaak, Sta. Cruz, 5105 Occidental Mindoro; Mobile: 0908-104-0855 Samar Satellite Station, UEP Campus, Catarman, 6400 Northern Samar; Mobile: 0948-800-5284

