# 2015 National Rice R&D Highlights

**Genetic Resources Division** 



### TABLE OF CONTENTS

Executive Summary	Page
Genetic Resources Division	1
I. Conservation, Characterization, and Distribution of Rice Germplasm Resources	2
II. Evaluation of PhilRice Germplasm Collection for Biotic and Abiotic Stresses in Irrigated Lowland and Grain Quality	18
III. Genetic Resources Research	32
IV. Optimization of Germplasm Conservation Procedures	41
V. Conservation of Genetic Resources in the Rice Environment	43
Abbreviations and acronymns	56
List of Tables	58
List of Figures	

### GENETIC RESOURCES DIVISION

Division Head: XGI Caguiat

#### **Executive Summary**

The Genetic Resources Division (GRD) maintains its crucial role in support to crop improvement in the past years. There are lots of traits available from the vast reserve germplasm collection that could still be used in breeding programs especially in the onset of emerging challenges of current times including climate change and new pests and diseases. Projects under the division have focused on three core functions: germplasm conservation, characterization (agro-morphological, molecular and biochemical) and evaluation. GRD also caters conservation of other important organisms in the rice environment including Azolla spp., biocontrol agents and other beneficial microbes.

To ensure availability of diverse germplasm for current and future breeding programs, we implement and maintain genebank activities following the international standards which include: collection, conservation, and characterization of traditional rice varieties. Germplasm information will aid the breeders broaden the genepool of existing rice varieties. The division had acquired 943 new germplasm collections processed for conservation and subjected to slow drying condition in a container with silica gel at 15°C with approximately 40% relative humidity prior to medium and long term storage.

The utilization of vast germplasm in breeding programs needs prior information such as the evaluation of the collections against major pests, diseases and response to various abiotic stresses. For blast resistance, 212 entries were found resistant, and 68 have intermediate reactions; while 25 entries were found resistant to eight rice blast isolates. For brown planthopper resistance (BPH), 20 entries showed resistance while 109 entries have intermediate reactions. For green leafhopper (GLH) resistance, 112 entries showed intermediate reactions. In terms of abiotic stresses: 5 genotypes showed drought tolerance based on biomass weight at 12% soil moisture content; while 5 accessions have zinc tolerance.

The application of modern biotechnology tools to advance gene discovery and genetic diversity assessment in germplasm collection has been recognized in the previous years. One of the various biotechnology techniques includes the molecular marker technology to investigate the feasibility of optimizing the natural allelic richness through Multi-parent Advanced Generation Inter Crosses (MAGIC) between traditional rice varieties. The division has also ventured into bioinformatics to analyze whole genome sequences and try accelerating gene discovery of Philippine traditional varieties in collaboration with Core Facility for Bioinformatics-Philippine Genome Center and IRRI. Efforts are being made in order to put up a bioinformatics facility at PhilRice in order to address the looming demand of this tool in the current rice research and development trends. Another biotechnology tool engaged by the division is the use of genespecific markers for important traits such as the fragrance genes, wherein 14 out of 41 accessions (24 variety names) had the fgr allele. These biotechnology tools could help accelerate the genetic diversity status, gene discovery and grain quality assessment.

Aside from long-term storage and conservation of germplasm for future generations, utilization of the vast germplasm collection has been always a challenge to any genebank. Aside from strengthening interdisciplinary approaches to efficiently hasten the evaluation of germplasm for various biotic and abiotic stresses screening, the division aims to continue to widen its collaboration in terms of screening of germplasm against emerging pests, diseases, and climate change effects. The division, through field visits dubbed as "GRD Walkthrough: Unitas in Diversitates, and formal discussions and agreements with local and national agencies assures availability, accessibility and safety of these vast germplasm collection.

The valuable accomplishments of PhilRice genebank with its vast collection may reveal important germplasm materials for a more resilient, high-yielding rice varieties with pest and disease resistance and tolerance to abiotic stresses, and with good eating quality.

### I. Conservation, Characterization, and Distribution of Rice Germplasm Resources

Project Leader: MC Ferrer

The Philippines being one of the countries with a wide biodiversity index, locally grown rice varieties have been part of the culture, traditions, and heritage. Collection and conservation of these rice germplasm will facilitate protection of genetic wealth, thus safeguarding Philippine rice germplasm rich diversity. Ex-situ conservation in genebanks provides a safe storage system for the germplasm to ensure seed availability and survival.

The Genetic Resources Division (GRD) serves as the national repositories of rice germplasm. It supports breeding of rice cultivars by providing germplasm materials for research as well as parental stocks. The division collects and conserves rice genetic resources to ensure the future generations of available materials needed to build better rice plants. Currently, GRD holds 15,257 collections and 7,129 of which are assigned as accessions, identifying them as unique among the registered collections. The collections include modern, traditional varieties, foreign introductions, hybrid parental lines, breeding lines, and wild rice relatives. Preservation and documentation of these germplasm is of paramount importance for direct

use and may source of useful genes that serve as building blocks for the improvement of rice varieties. Rice germplasm must be efficiently harnessed and properly assessed through morpho-agronomic characterization to identify potential donor parents in breeding to meet the demand for rice consumption.

Given the sheer number of germplasm, highest management practices are to be pursued. Continuity and long-term program is essential to conservation and maintenance of rice germplasm. Regeneration is conducted to replenish germplasm with low viability and seed stocks. Viability and storage conditions monitoring is done regularly to ensure viable conserved germplasm. Conservation efforts at different research station of PhilRice were implemented in synchrony with the activities and procedures done at PhilRice Genebank for better management and enhanced utilization. With the advent of the Plant Variety Protection Act, access, exchange and benefit sharing of rice germplasm conserved requires legal instrumentalities such as the Standard Material Transfer Agreement (SMTA) will be instituted for the protection of the Philippine rice genetic resources.

#### Collecting and acquisition of new germplasm materials

Plant germplasm is a vital resource in generating plant types having desired traits that help in increasing food production and thus improve the level of malnutrition. Traditional varieties prove greater genetic variability and can furnish useful traits to broaden the genetic base of crop species. However these traditional genetic resources are gradually disappearing due to the introduction of improved varieties, socio-economic changes in agriculture, rapid urbanization and etc.

The initial step in genetic conservation is the collection and assembly of crop germplasm. Germplasm collections exist to conserve the genetic diversity of these crop species and their wild relatives. Exploration and collection comprise a difficult and challenging phase of genetic conservation. A systematic approach to germplasm collection program is necessary to insure that the maximum range of diversity of germplasm will be collected in a cost- and time-efficient manner. PhilRice, through GRD, collects and conserve rice genetic resources. Rice improvement programs rely on the vast genepool represented in genebanks for the source of genes and novel alleles needed to build better rice. The wider selection and diversity of materials can be utilized for varietal improvement if more rice germplasm accessions and information available. Collecting activities prioritized the underrepresented provinces and tribal area and stored at PhilRice' own genebank. The collecting activities are closely linked to conservation and use.

3

#### 4 Rice R&D Highlights 2015

### Highlights:

- In 2015, GRD had acquired 943 germplasm. Among the collected materials, 26 Advanced/improved cultivar, 27 are breeding lines, 178 traditional. Total of 707 gemplasm were received for safety duplication of rice germplasm from PhiliRice Batac (27) and PhilRice Midsayap (685). The origin of newly acquired rice germplasm was shown in Figure 1.
- 100% seed files of acquired rice varieties were prepared for future reference.



Figure 1. Number of acquired germplasm with corresponding sources of collection from January to December 2015.

### Regeneration and conservation of rice germplasm

MIC Calayugan, CQ Cortaga, MC Ferrer, MVG Embate, MD Duldulao and LM Perez

Rice genetic resources, comprising landrace varieties, modern and obsolete varieties, genetic stocks, breeding lines, and the wild rice relatives, are the basis of world food security. However, increase in modern variety use has caused narrowing of genetic diversity among rice cultivars that possess valuable traits of for survival to environment changes.

The long-term preservation of rice genetic resources is the principal aim of GRD. The ex-situ conservation provides a safe storage system for these germplasm materials under optimal storage conditions that is efficiently managed and accessible to users. Conservation of these genetic stocks requires a continuous supply of genes of interest to the users as breeding activity progresses (Chang, 1976), therefore there is a need to conserve rice cultivars for future use. PhilRice genebank conserves 15,257 collection of rice germplasm. The facilities of the genebank ensure the long-term preservation of this important diversity. The value of conserved germplasm can be assessed in terms of useful traits for rice breeding and the economic impact that germplasm utilization has on rice production and productivity.

Regeneration of genebank collections is necessary due to decreasing seed viability as well as diminishing amount of seeds overtime through active distribution. According to Upadhyaya (2013), regeneration aims to increase the quantity of seed accession and restore the maximum viability of seed collection. Seed multiplication is the best way to revitalize stocks to maintain the genetic integrity of germplasm collection. Germplasm conservation plays a key role on the integration of technological developments in the field of molecular genetics, genomics, cryopreservation and geographic information system to further facilitate conservation and utilization of genetic stocks. The study aims to conserve rice germplasm resource for medium and long term storage and rejuvenate low stocks and low viability rice germplasm for conservation and distribution.

### Highlights:

Regeneration

- Exactly 2651 collections were selected for regeneration in 2015 cropping seasons (Table 1). The germplasms were selected based on the level of viability or germination rate and seed stocks. Germination rate of the germplasms sown for regeneration were 69.7% (1796). The number of entries with sufficient harvest in the two cropping seasons (DS and WS) was only 71.17% (1242). The 28.82% with less harvest will be scheduled again for seed multiplication.
- The data obtained can serve as basis of planning for regeneration of rice germplasm to achieve sufficient seed stocks.

### Conservation

- A total of 994 new collections from 2014 to 2015 were processed for conservation (Table 2).
- Regenerated materials from 2014 cropping seasons and 2015 dry season which included 548 accessions/ collections were processed for conservation.
- A total of 982 accessions/ collections including original 2013 to 2014 collections and regenerated germplasm in 2014 cropping seasons were tested for viability (Table 3).

#### 6 Rice R&D Highlights 2015

• Viability data showed that 92% (907) were  $\geq$ 85% indicating high viability while 8% (75) showed < 85% indicating that the latter were candidates for regeneration.

Table 1. Summary of regenerated rice germplasm in 2015 cropping seasons.

Season	Total sown	Live	Harvests	Sufficient
2015 dry season	1225	779	762	675
2015 wet season	1426	1017	983	567
TOTAL	2651	1796	1745	1242

**Table 2.** Summary of processed rice accessions/ collections in 2015 for conservation.

Year	No. of vacuum packed and stored
ORIGINAL/ NEW COLLECTIONS	
2015	961
2014	33
REGENERATED MATERIALS	
2015 Dry Season	85
2014 Wet Season	461
2014 Dry Season	2
TOTAL	1,424

### Table 3. Results of viability test conducted in 2015.

Year	Season	High Viability (>85%)	Low Viability (<85%)
2015	ORIGINAL/ NEW	96	69
	Dry Season	52	0
	ORIGINAL/ NEW	1	3
2014	Dry Season	258	0
	Wet Season	500	3
TOTAL		907	75
OVERALL TOTAL		982	
% HIGH / LOW VI	ABILITY	92%	8%

#### Germplasm distribution and information management

MD Duldulao, MC Ferrer, MIC Calayugan and XGI Caguiat

The Genebank Documentation System (GEDS) is a relational database management system (RDBMS) developed to document, manage, and centralize the large quantities of data of all germplasm conserved in the genebank. Data include passport data; agro-morphological characterization; grain quality, biotic and abiotic stresses evaluations; viability conditions; and seed inventory. GEDS maintains accurate, reliable and up-to-date rice germplasm information, thus, facilitates ease of data search and retrieval for better access and use of germplasm.

PhilRice genebank regulates the release of seeds that can be used in research, breeding methods and genetic improvements to support the utilization of rice genetic resources. This is accompanied with Standard Material Transfer Agreements (SMTAs) that defines the terms and conditions for use of germplasm agreed upon between PhilRice and receiving party and vice versa. Germplasm data request is offered to rice breeders, researchers, and individuals for their germplasm/ traits of interest. The conservation of rice genetic resources is a success if germplasm is utilized.

- The GEDS containing important rice germplasm data was updated: passport data (960 collections), characterization data (172 entries for 2014DS and 547 entries for 2014WS), grain quality data (885 collections/ accessions), rice tungro field evaluation data (967 collections/accessions) and inventory data (4,074 accessions).
- Exactly 422 seed requests covering 1,582 rice accessions/ collections with a total of 4,079 seed packets have been distributed to both PhilRice and non-PhilRice individuals. The purpose of such requests was mostly intended for farmer's use and research/ hybrid rice breeding. (Figure 2)
- In terms of request for germplasm information, 29 data requests have been catered and provided to PhilRice staff for research and breeding purposes.
- To properly document release and receipt of rice germplasm, 21 SMTA's and 10 PhilRice MTA-GUD's have been issued covering 831 accessions/ collections. SMTA is being used to protect intellectual properties or rights over the rice varieties being provided to non-PhilRice staff. PMTA-GUD serves as an attachment to the SMTA for additional conditions on the transfer of breeding lines.

#### 8 Rice R&D Highlights 2015

- 23 SMTA's with 18 IRRI-OMTA and CMTA attachments for the seeds requested by PhilRice researchers were accepted through the GRD Head and is currently being monitored. IRRI MTA attachments are for materials classified as non-sensitive and sensitive germplasm respectively.
- A new system function of the GEDS database has been developed for tagging and selecting multiple records/ entries in the inventory system.



### Purpose of Germplasm Seed Requests

**Figure 2.** Utilization of the germplasm seed requests received and processed in 2015.

#### Germplasm inventory

MC Ferrer, MIC Calayugan, MV Embate, MD Duldulao, DF Villanueva, JN Castro, JT Marturillas, JM MVino, MG Redondo and LM Perez

Germplasm banks play a crucial role in the conservation and use of biodiversity. Preservation of genetic integrity and prolonging the longevity is the main goal of germplasm conservation. Conservation of plant genetic resources (PGR) is not limited to attaining and physically possessing the materials (collection and storage) but also includes ensuring the existence of these under viable conditions and with their original genetic characteristics intact.

The GRD as national repository for rice germplasm, collects and conserve rice genetic resources to ensure the future generation of available materials needed to build better rice plants. To improve the seed quality of conserved genetic resources, the genebank has prioritized the seed identity verification and viability monitoring of its germplasm collection. Every new seedlot produced after a cycle of regeneration should be screened and compared against its parent or most original sample, to assure maintenance of genetic integrity. A detailed inventory system was done to ensure the germplasm's genetic integrity is preserved with sufficient viable stocks through the application of standard conservation techniques.

- In 2015, stocks for Collection 6000 to 9045 (not yet assigned with accession number) were extracted from different storage location (drying room, medium-term, and long-term storages) for inventory.
- To ensure that the conserved germplasm are same as the original collection, seed identity were verified through cross-checking with available seed files, planting plans and panicle files. Comparison between the seed lot and the seed file was done to verify the identity of the seed lot and the status of the seed quality (i.e. mix, mismatch, infected and etc) were also noted. Based from the 943 verified collections, 1284 seed packets were handled. It was noted that 795 collections were match with the original seed files, 41 were mismatch, while 107 were without seed files and still need re-authentication.
- Proofread/ double checked labels of verified accessions (PRRI000001 to PRRI007129) stored in active set storage.

### Germplasm characterization

MIC Calayugan, MC Ferrer, MVG Embate and LM Perez

The rice varieties nurtured by farmers for generations have an inherent genetic value because of their adaptation to different farming conditions. Genetic diversity is the foundation of plant breeding programs. Characterization of each germplasm is done to establish genetic identity based on its agro-morphological characters. According to Upadhyaya et al (2008), germplasm characterization aims to describe each accession to establish genetic identity to avoid genetic erosion; classify collection using sound criteria; identify potential traits; develop interrelationship between or among environmental groups of cultivars and estimate range of variation. Knowledge of these traits, their genetic and stability under different conditions enhances the value of conserved germplasm. Standard descriptors for rice of Bioversity International (2007) were used to characterize and identify the materials to efficiently harness, properly assess and identify potential parents in breeding to produce quality traits of the varieties to help meet rice self-sufficiency. Characterization and systematic study of germplasm is not only important in identifying potential donors for crop improvement but also important in protecting the unique traits for present use (Parikh et al. 2012). Over the decades, the germplasm collection in Philrice-GRD has been systematically characterized for a range of morphological and agronomic traits that facilitate conservation, as well as selection of suitable phenotypes by farmers and breeders.

- Fifty-eight quantitative and qualitative agro-morphological traits by Bioversity International (2007) were observed and recorded. These characteristics were identified priority in support for breeding and diversity analysis.
- Exactly 628 entries were characterized in 2015 cropping seasons (Table 4).
- In 2015 dry season, exactly 166 entries were characterized at vegetative stage while 162 at reproductive and postharvest stage. In 2015WS, total of 532 entries characterized during vegetative stage while only 424 were characterized during reproductive and post-harvest. Some varieties were photoperiod sensitive/ late maturing and transferred to screenhouse.
- The data obtained were uploaded in the germplasm database to be accessible for researchers to serve as basis for selection of target traits for breeding.

Season	Entries	Vegetative	Reproductive	Post-Harvest
2015DS	166	166	162	162
		collections	collections	collections
2015WS	532	532	424	424
		collections	collections	collections
TOTAL		698	586	586

**Table 4.** Summary of characterized rice germplasm in 2015 dry and wet season.

### Conservation and management of rice genetic resources in PhilRice Los Baños

WB Abonitalla, LV Guittap, TM Masajo, TH Borromeo and S Bon

This study is a joint activity of PhilRice Los Baños and UPLB. Materials were processed and stored in the Seed Processing and Seed Storage facility at the branch. Germplasm materials were characterized using the standard descriptors prescribed for rice. Seeds with low stocks were multiplied, while accessions with low viability were rejuvenated. Seed processing and packaging in aluminum sachet is in progress. Duplicate samples were shared with the PhilRice CES genebank as safety duplicates. Main users of the collection were breeders at UPLB and the hybrid breeding project at Los Baños branch. The collections were available to all PhilRice staff by request.

The rice germplasm maintained at the station is close to about 2400 accessions consisting of 70% varietal collections (mostly traditional varieties) and 30% selections and elite lines. Wide hybridization derived lines, TGMS lines, promising hybrid pollen parents, and highly selected NCT lines are in the collection. Also stored at PhilRice Los Baños were the parentals of public released hybrids. The accessions were maintained in short-term storage at 15°C and 50% to 60% RH mainly kept in the cold storage room. The seeds were processed for medium-term storage (in freezers).

It is essential to develop/upgrade the system of handling germplasm materials since breeding depends on good seed management, with seed and data retrieval system properly in place. Conservation and maintenance guarantee seed availability for breeders' needs. Similarly, original seeds should be securely kept to serve as check in case problems in identity arise as back-up seed file. Moreover, breeders require not only the seeds but also information presented in a manner that will allow them to identify lines potentially useful to programs. Data on characterization and evaluation are the essential link between conservation and use of stored germplasm. Germplasm work at PhilRice Los Baños was implemented in synchrony with the activities and procedures done at CES genebank.

### Highlights:

- 125 lines were assembled during the year 2015 (112 NCT lines; 11 Inbred and 2 Breeding lines).
- 100 germplasm accessions were viability tested.
- A total of 98 lines breeding lines seed multiplied (50 line during 2015DS and 48 during WS).
- 50 breeding lines planted and characterized during 2015DS.
- 50 observation nursery (ON) lines were packed during the year.
- Full characterization of 330 lines were conducted during 2015DS.
- 100 lines were manually cleaned and packed in aluminum foil for medium term storage.

## Collecting, conservation and management of traditional varieties in Mindanao

GD Balleras, JMNiones, SJ Labarosa, JLG Espina, MC Ferrer and LM Perez

Crop diversity has a key role to play in helping the upland farmers improve their livelihoods while protecting the environment and their health. Mindanao farmers cultivated huge hectares of popular traditional and indigenous varieties. However, many areas are still untouched and undiscovered in which plenty of traditional, indigenous and even wild rice relatives might be found. PhilRice is mandated to collect, conserved and protect all traditional and indigenous rice varieties in our country. The utilization of traditional and indigenous varieties is of great help in the varietal development, deployment and promotion since these traditional and indigenous varieties are already adopted and accepted by the local farmers. This study aims to proper collect, document and conserves traditional germplasm in Mindanao, and duplicates these to Genetic Resources Division at PhilRice CES.

### Highlights:

- A total of 730 germplasm materials were sent to PhilRice CES wherein 543 of which are traditional varieties. In 2015, exactly 81 traditional rice varieties from Alamada, North Cotabato were collected (Table 5).
- Seed viability test were conducted on germplasm with enough seed samples (a total of 20 samples), while collections with limited seed were forwarded to GRD for embryo rescue. Germinated seeds from seed viability test conducted were transplanted into plastic pails for seed increase purposes.

DUEC CI di	Neder News		Madata Masa
PMES Code	Variety Name	PMES Code	Variety Name
PMES 1064	Tomo	PMES 1105	Balakayo
PMES 1065	Dinolores	PMES 1106	Maria gakit
PMES 1066	Karutak	PMES 1107	Kapunsa
PMES 1067	Kalinayin	PMES 1108	Urabon
PMES 1068	Kalanay	PMES 1109	Kalubid
PMES 1069	Balete	PMES 1110	RF47
PMES 1070	Malay 2	PMES 1111	Speaker
PMES 1071	Kalusi	PMES 1112	Gregory
PMES 1072	Kawilan	PMES 1113	Kinalamawawal
PMES 1073	Azucena	PMES 1114	Pulota papakan
PMES 1074	Zamboanga White	PMES 1115	Katawil
PMES 1075	Kaputol	PMES 1116	Maam Bing Upland
PMES 1076	Kutibos 1	PMES 1117	Malunding
PMES 1077	Kapusan	PMES 1118	Without awn RTU resistance
PMES 1078	Pilit KC	PMES 1119	RTU resistance
PMES 1079	Kutibos White	PMES 1120	Mubpan
PMES 1080	Banisika	PMES 1121	Marabit
PMES 1081	Swarna Sub1	PMES 1122	Biday
PMES 1082	Suakong	PMES 1123	B. Bontong
PMES 1083	Wag-wag	PMES 1124	Palawan
PMES 1084	Urano	PMES 1125	TK 303
PMES 1085	Kutibos Red	PMES 1126	Balima Puti
PMES 1086	Kaputol	PMES 1127	Seratus Hasit 36
PMES 1087	Pilit Tapol	PMES 1128	Utri Merah
PMES 1088	Jasmin 1	PMES 1129	Narim PL-9
PMES 1089	Kaliso	PMES 1130	Kalinayan
PMES 1090	Baris	PMES 1131	Tjempo Kisik
PMES 1091	Bolao	PMES 1132	Ketan Nangka
PMES 1092	Manisi	PMES 1133	Maria Bochi
PMES 1093	Narra G	PMES 1134	Basmati
PMES 1094	Makabangon	PMES 1135	Monobenekan
PMES 1095	Smagol	PMES 1136	Habigan DW8
PMES 1096	Pangoraman	PMES 1137	Pragathi 13
PMES 1097	Banisi	PMES 1138	RT-87
PMES 1098	Pangoraman	PMES 1139	Azucena
PMES 1099	Pakpakan	PMES 1140	Awot
PMES 1100	Upland Rice	PMES 1141	Pure Dinorado
PMES 1101	Ginalos	PMES 1142	Hinumay
PMES 1102	Balete	PMES 1143	Malay 2
PMES 1103	Bagong Bayan	PMES 1144	Dinorado Dagko
PMES 1104	Mitaw-mitaw	PMES 1145	Kanuni Dinorado

### Table 5. List of germplasm collected in 2015 at PhilRice Midsayap.

### Germplasm collecting and management in PhilRice Batac

JM Solero, AY Alibuyog, MC Ferrer and LM Perez

Although modern high-yielding varieties presently dominate the lowland rice paddies, traditional rice varieties continue to be planted by farmers in diverse ecosystems throughout the country. Farmers opt to plant traditional varieties for various reasons: adaptability, resistance to extreme climatic conditions, tolerance to pests, minimal external input requirements excellent grain and eating quality among others. A variety that has been grown in a particular locality for a long period eventually adapts, at some level, to biotic and abiotic stresses.

It is a common practice, though, of farmers growing traditional varieties to use planting materials from their produce without any conscious selection and systematic purification process. This resulted to variety mixture and loss of seed vitality. The most serious problem is when seeds of a variety are totally wiped out in a locality. Some farmers in Ilocos Norte have reported that they could no longer plant certain traditional varieties that they had been growing for a long time because their last standing crop was completely destroyed by natural calamities. Unless a move is done to help conserve these traditional rice varieties, they may soon be completely obliterated from the agro-ecosystem.

- Forty new germplasm were added to the collections. The new collections had been registered and encoded at the database, along with their passport data. A collection number had been assigned as identification number for each collection. To date, 238 TRVs were already collected and registered at the database.
- All (238) collections had seed files in coin envelopes, 76 were packed in foil packets at 20g each, 71 TRVs with seed samples in vials for display and lecture purposes and 65 with panicle samples vacuum sealed in clear polyethylene plastic.
- 194 out of the 238 TRVs were tested for viability. Results showed that 114 TRVs had good viability while the rest (80 TRVs) had poor viability. Viability testing of the remaining 44 TRV collections will be done in 2016.
- Fifteen TRVs were currently dried to 6-7 % MC using silica gel at the station's storage room. Due to limited silica gel at the storage room, 65 TRVs were also sent to PhilRice-CES genebank to facilitate drying.

- Seeds of 60 TRVs were requested for various R&D endeavors. Most of the TRVS requested were upland varieties with 100 g to 2 kg seed per request depending on the purpose. Eight TRVs were returned to the source site through the DA-LGU for 2015 WS planting. The aim of the project is to have the source site a starter source of purified seeds for them to mass produce for utilization of more farmers.
- To cater to the needs of other stakeholders, seed increase for TRVs with limited seeds was also done in 2015WS. A total of 74 TRVs were planted for seed increase at PhilRice Batac Experiment Station, however, only 64 TRVs were harvested.
- A catalogue of the varieties characterized in 2012 was developed. Photos of TRVs, together with their agromorphological characteristics and grain quality were submitted to PhilRice CES for laying out. A total of 64 TRVs (34 TRVs from CAR) were included in the catalogue. The grain quality of the TRVs was determined in collaboration with PhilRice Los Baños.

### Germplasm conservation and evaluation of traditional rice varieties in Northeast Luzon

ATIO Rebong and JV Galapon

Northeast Luzon (NE) has a vast collection of rice germplasm containing a diverse source of important and desirable traits. Evaluation of collected traditional varieties from Region 2 and CAR for abiotic stress tolerance and incorporating them in our rice breeding efforts would greatly benefit our rice farmers.

- In 2015 dry season, 28 traditional rice varieties were planted. Entries included 10 from Ifugao, 7 from Kalinga, 8 from Mt. Province, 1 from Quirino, 1 from Isabela and 1 from Benguet (Table 6).
- Important agro-morphological traits were evaluated for all the entries from vegetative, reproductive to mature and postharvest phase. Number of productive tillers, plant height, filled and unfilled grains, maturity and yield of entries are shown in Table 7.
- A total of five entries from 22 acquired TRV samples passed the germination test, rejuvenation of these materials was done

but still most did not germinate.

- In 2015 wet season, 15 traditional rice varieties were planted on station. Entries included four from Ifugao, Kalinga and from Mt. Province, two from Quirino, and one from Nueva Vizcaya.
- Twenty traditional varieties last season still bundled in panicles were submitted to PhilRice Isabela's newly opened rice museum as display.
- Last October, two varieties from Kalinga and the Mt. Province which are Chong-ak and Ominio were acquired. These materials will be seed increased and characterized next season.

**Table 6.** Collection of traditional rice seeds from provinces in NortheastLuzon planted in 2015DS.

No	Entry name	Place of origin
1	MInaangan	Ifugao
2	Ugnah	Ifugao
3	Palawan white	Ifugao
4	Lacoop with awn	Ifugao
5	Ingudpor	Ifugao
6	Palawan	Ifugao
7	Lapog Blatinaw	Ifugao
8	Gilgilang	Ifugao
9	Balatinao	Ifugao
10	Gobierno	Ifugao
11	Pingkitan	Ifugao
12	Pinilisa	Isabela
13	Kintoman	Benguet
14	Kenyo	Quirino
15	Ulikan red	Kalinga
16	Dumalengan	Kalinga
17	Mimis	Kalinga
18	Maylo	Kalinga
19	Uskil	Kalinga
20	Binol-layao	Kalinga
21	Amore	Mt. Province
22	Sariping	Mt. Province
23	Pinili	Mt. Province
24	Manmantha	Mt. Province
25	Binontoc	Mt. Province
26	Balatinaw	Mt. Province
27	Balatinao	Mt. Province
28	Waray	Mt. Province

Table 7. Yield	component	data encoc	led for en	ntries estal	blished o	during
2015DS.						

dex	Entry name	Plant beight	Prod. Tillers	Filled	Unfilled	Maturity	Total amount seed
		(cm)	(No.)	(No.)	(No.)	(Days)	produced
1	Binontoc	165.00	10	161	56	138	5844
2	Balatinaw	137.00	16	123	30	152	4177
3	Balatinao	150.00	21	74	38	152	3667
4	Kintoman	141.60	12	80	53	127	3443
5	Pinilisa	129.60	14	95	46	123	2098
6	Dumalengan	159.40	15	95	22	127	4285
7	Binol-layao	178.40	12	140	19	123	6112
8	Maylo	142.80	13	57	63	127	4720
9	Lapog Balatinaw Amore (red	150.60	11	226	69	138	5679
0	awn)	131.60	10	139	17	139	2576
1	Minaangan	172.40	21	69	61	139	5749
2	Ulikan Red	158.20	24	152	54	130	7309
3	Mimis	140.20	11	120	28	140	2506
4	Gobierno	147.00	13	103	28	123	5366
5	Sariping	157.00	11	100	26	125	7831
6	Ingudpor	174.00	11	126	18	145	2065
7	Gilgilang	147.40	23	105	32	139	4699
8	Palawan White	154.00	13	170	67	138	7692
9	Palawan	171.00	11	246	34	139	6523
20	Ugnah	184.40	10	179	104	145	5221
	Lacoop with						
21	awn	159.60	12	70	61	152	2567
22	Uskil	200.40	11	133	47	141	2370
23	Kenyo	131.20	9	114	19	130	4443
24	Unoy sugo	149.80	11	124	29	132	0
?5	Pinili	143.60	11	159	23	125	3108
26	Pingkitan	153.20	14	243	69	141	9633
27	Manmantha	148.00	12	129	22	152	3590
28	Waray	160.40	11	129	39	141	7892

### II. Evaluation of PhilRice Germplasm Collection for Biotic and Abiotic Stresses in Irrigated Lowland and Grain Quality

Project Leader: IM Niones

PhilRice Genebank conserves a diverse pool of rice germplasm with 15,257 collections from different parts of the country, particularly from the upland and rainfed areas. These germplasm possess desirable genes and traits that serve as building blocks for the development of improved and new rice varieties. They may have inherent genes for key traits such as high yield, good eating quality, pest and disease resistance, and tolerance to abiotic stresses. Identifying promising rice germplasm with useful traits is an important pre-breeding activity in rice improvement. The significant value of these genetic resources, however, depends on the available information about these accessions. To date, available information on morphological and agronomic traits, reaction to biotic and abiotic stresses as well as grain guality characteristics are less sufficient. Rice germplasm therefore, must be efficiently harnessed and properly evaluated in order to identify potential genetic donors for direct use and as parents in breeding program. This project aimed to establish resistance of the rice germplasm to abiotic stresses, disease and insect pests. Also, generating grain quality descriptors of the PhilRice germplasm collection and efficiently providing quality data through a computerized database system.

- Twenty accessions were resistant and 109 accessions were intermediate to BPH, while 112 accessions showed intermediate reactions to GLH under screen house condition.
- 212 accessions were found resistant and 68 accessions having intermediate reactions to blast disease. All germplasm accessions were susceptible to rice tungro virus.
- 121 accessions profiled for complete grain quality. One accession had good brown rice yield.
- In terms of total milled rice yield, 120 accessions were grade 3. Thirty-nine (39) rice accessions had grade 1 head rice yield, 71 were grade 2, and 10 were grade 3.
- Based on alkali spreading value (ASV), 12 rice accessions had high GT, 79 accessions had intermediate GT, and 22 accessions had low GT, while AC assay using ammonium buffer, 108 were nonwaxy and 13 were waxy rices.
- Twelve germplasms were selected with potential drought tolerance Mimis (PRRI003299), Ibarraki (PRRI006928),

Cuevas (PRRI003147), Kinaruray 1 (PRRI003034), Inapostol (PRRI003163), Malagkit 1 (PRRI003046), Incantado (PRRI003166) and Ortoc (PRRI002867) genetic material showed higher shoot biomass and yield to different gradient and intensities of water deficit stress. These genotypes exhibited less reduction in yield under moderately to severe drought stress.

- Ortoc (+21.3%) and Mimis (+71.8%) genotypes showed higher shoot biomass under different gradient and intensities of water deficit stress.
- Kinaruray 1 (PRRI003034), Dumali (PRRI002912) and Pilit Tapul (Coll 14221) showed a significant increase in grain yield due to high percentage of filled grains.
- Five rice germplasm accessions identified with tolerance to soil zinc deficiency with good to excellent phenotypic performance these are: Minaangan (Coll. No. 5480), Unknown (Coll. No. 10895-A), Burik (Coll. No. 11161), Dinurado (Coll. No. 11125) and Red Tonner (Coll. No. 11296).
- PR25792-B-10-4 genotype showed intermediate reaction to RTV at 60 Days after Transplanting.
- 25 rice blast resistant Philippine traditional varieties were evaluated for eight rice blast differential isolates.

### Evaluation of PhilRice germplasm collection for biotic stresses JP Rillon, GDC Santiago, and MSV Duca

Rice germplasm possess useful genes for key traits such as resistance to insect pests and diseases. These rice germplasm needs to be continuously screened for resistance to blast, bacterial leaf blight (BLB), sheath blight (ShB), rice tungro virus (RTV), green leafhoppers (GLH), brown planthoppers (BPH) and stemborer. In severe infections of blast, coalescence lesions cause dried leaves and whole plants are stunted and killed. Rice tungro causes yellow orange leaf, stunted growth, reduced number of tiller and incomplete panicle insertion. Bacterial leaf blight water-soaked lesion start near the leaf tip and margin, extends downward, enlarge and turn yellow to gray and the affected parts die. Sheath blight lesion gradually extends to upper leaf sheath and leaf blades and coalesce causing lodging because of leaf sheath death. Green leafhoppers and brown planthoppers are insect pests of important concern because they spread viral diseases in the rice fields. Both nymphs and adults infest the rice crop at all stages of plant growth. As a result of feeding at the base of the tillers, plants turn yellowish and dry up rapidly. Stemborer injured the rice stem causing deadheart during vegetative stage and whiteheads during reproductive stage. Stability of resistant accessions is dependent on many factors such as the environment and cultural management used. Resistance may breakdown at any point or it may remain the same. Resistant accessions will be used as parent materials for new crosses of potential rice varieties.

### Highlights:

- Insect resistance: out of 353 accessions, 20 accessions were resistant, 109 were intermediate and 223 were susceptible to BPH. Similarly, 112 were intermediate and 236 showed susceptible reactions to GLH under screen house condition (Table 8).
- Disease resistance: out of 353 germplasm accessions, 212 accessions were resistant while 68 were intermediate reactions were susceptible to the blast disease. All germplasm accessions were susceptible to rice tungro virus (Table 8).

		No. of Accessions			
Reaction	Blast	Rice Tungro Virus	Brown Planthopper	Green Leafhopper	
Resistant	38	0	0	0	
Moderately Resistant*	-	-	-	-	
Intermediate	33	0	32	34	
Moderately Susceptible*	-	-	-	-	
Susceptible	10	100	68	65	

**Table 8.** Summary table of reactions of PhilRice germplasm accessions to major diseases and insect pest 2015 DS.

\* for insect pest only

### Evaluation of PhilRice germplasm collection for grain quality

RC de Leon, APP Tuaño, MC Ferrer, MIC Calayugan, and LM Perez

PhilRice Genebank is the country's repository of rice and non-rice genetic resources besides IRRI Genebank. It currently holds around 7, 129 rice accessions composed of wild rices, traditional varieties, elite breeding lines, and interesting foreign cultivars. The value of these rice genetic resources depends on the available information about these accessions. To date, information on morphological and agronomic traits, reaction to biotic and abiotic stresses, as well as grain quality characteristics, is lacking. Grain quality in particular, dictates consumer acceptability and marketability of rice, hence considered an important component in the rice breeding program. This continuing study aims to generate grain quality data of the PhilRice germplasm collection and efficiently providing grain quality data through a computerized database system handled by the PhilRice Genetic Resources Division (GRD).

- 500 accessions analyzed for AC and GT; 121 accessions profiled for complete GQ such as milling potentials, grain size and shape, amylose content (AC), gelatinization temperature (GT), and Instron hardness of freshly cooked and staled rice.
- Milling potentials were analyzed from 150 g of rough rice. Of the 121 accessions, only 1 had good brown rice yield. In terms of total milled rice yield, 120 accessions were grade 3. Meanwhile, 39 rice accessions had grade 1 head rice yield, 71 were grade 2, and 10 were grade 3.
- Majority of the rice samples were medium in length and intermediate in shape.
- GT type was determined using the alkali spreading value (ASV; Little et al. 1958, Cereal Chemistry 35:11-126). Based on ASV, 12 rice samples had high GT, 79 had intermediate GT, and 22 had low GT.
- AC assay using ammonium buffer (Juliano et al. 2012, Cereal Foods World 57:14-19) was employed. Of the 121 samples, 108 were non-waxy and 13 were waxy rice (Table 9).

Property	Non-waxy ac	cessions	Waxy ac	Waxy accessions	
	Range	Mean	Range	Mean	
Brown rice, %	46.3-111.4	57.5	54.9-58.9	57.4	
Total milled rice, %	43.1-56.2	51.5	48.3-54.1	52	
Head rice, %	33.1-53.5	45.4	44.1-53.3	49.1	
Grain length, mm	3.8-7.4	5.9	5-6.5	57	
Grain shape, length/width	1.7-3.4	2.4	1.7-2.4	2.1	
Chalky grains, %	1.0-98	34	74-100	94	
Apparent amylose content, %	2.04-25.47	19	0.72-1.82	1	
Alkali spreading value	3.0-7.0	5	3.3-7.0	6	
Instron hardness, kg cm <sup>2</sup>	.85-3.44	2	0.85-1.28	1	
	n=	108	n=13		

**Table 9.** Ranges and mean values of grain quality properties of PhilRice genebank accessions harvested from the GRD regeneration plots, 2014WS.

### Evaluation of PhilRice germplasm collections to abiotic stress

JM Niones, MCN Julaton, and RR Suralta

The single line-source sprinkler system (LSS) was conceptualized and introduced to impose and create a continuous variable water application across a research field plot (Hanks et al., 1976; Bauder et al., 1975; Willardson et al., 1987; Hanks et al., 1980). The LSS configuration provides a linearly decreasing irrigation application rate perpendicular to the sprinkler line, thus has been utilized to study crop response to variable amounts of irrigations and to different soil moisture intensities. This study aimed to evaluate and screened PhilRice germplasm collections under different intensities of drought stress using the line source sprinkler system.

On hundred two germplasm accessions were transplanted and conducted in the watertight experimental bed with line source sprinkler system under a rain-out shelter. Each line was planted at 20cm between rows and 45cm between hills in augmented design, in which IR64 and KDML 105 served as control genotypes. Fertilizer rate was 120-60-60 kg NPK/ ha applied in two splits (basal and maximum tillering stage). Supplemental fertilization of 10 kg/ha ammonium sulfate was done at 50DAT to correct sulfur deficiency. Draining of water started at 14DAT after the plants have recovered. Soil moisture sensors were placed at both sides of the seedbed at varying distance from line source sprinkler (10cm, 40cm, 80cm, 120cm, 160cm), respectively. Re-irrigation was done when the soil moisture at 80cm distance was below 30%VMC. Each entry was final sampled and terminated 3 weeks after heading with reference to the hill planted near the line source (10cm). Agronomic data (plant height, number of tillers and biomass) and root data (number of nodal roots (NRN), Total Root Length (TRL), Total nodal root length (TNRL), Total Lateral Root Length (TLRL), Root Dry Weight (RDW) were gathered.

- 102 germplasms were evaluated using the line source sprinkler system (LSS). Twelve germplasms were selected with potential drought tolerance at 10% soil moisture content based on biomass weight (Table 10, Figure 3).
- Duryat (Coll 14278), Maliket (Coll 14256), Mating (Coll 14268), Palawan (Coll 14240) and Tubod (Coll 14073) showed less reduction in shoot biomass under moderate (≥22% SMC) to severe (≤12%, SMC) drought stress. Shoot biomass reduction among these genotypes ranged from 22.4-33.7% under severe drought stress as compared to IR64 and KDML105 (Figure 4).
- Mimis (PRRI003299), Ibarraki (PRRI006928), Cuevas (PRRI003147), Kinaruray 1 (PRRI003034), Inapostol (PRRI003163), Malagkit 1 (PRRI003046), Incantado (PRRI003166) and Ortoc (PRRI002867) germplasms showed better adaptation due to the increase in shoot biomass under different intensities of soil moisture.
- The genotypes Ortoc (+21.3%) and Mimis (+71.8%) showed higher shoot biomass under different gradient and intensities of water deficit stress.
- Kinaruray 1 (PRRI003034), Dumali (PRRI002912) and Pilit Tapul (Coll 14221) showed a significant increase in grain yield due to high percentage of filled grains.
- Inapostol (PRRI003163), Dinominga (PRRI000280), Macaraniag (PRRI005644) and KDML105 were the photoperiod sensitive genotypes.
- Soil moisture content on the experiment decreased as the distance increase far from line (water) source. The soil moisture content showed a 27.9% nearest (10 to 30 cm) to 11.3% farthest (130 cm) in the water source (Figure 5).
- Root scanning for 2015 DS and WS samples is still in progress.

Accession/	Accession Name	Season
Collection Number	Accession Name	Jeason
14258	3 BUWAN	2015DS
14270	42 MA	2015DS
14257	75 DAYS	2015DS
14259	AZUCENA(IGP-219)	2015DS
14264	BALIANGLAD	2015DS
14215	BIHOD(ARAKAN)	2015DS
14271	BINNIT	2015DS
14269	BINURA	2015DS
14245	BONKITAN/PINKITAN	2015DS
14277	DENORADO	2015DS
14076	DUMANGKAL	2015DS
14278	DURYAT	2015DS
14273	EMOLAYAN	2015DS
14216	GAPON GAPON(ARAKAN)	2015DS
14275	GILINGAN	2015DS
14252	GOBYERNO	2015DS
14239	GUMAYAD	2015DS
14242	IMBOLLAH	2015DS
14238	INNAWI(TINAWON)DONA'AL	2015DS
13919	INUMAY(IGP14-209)	2015DS
13921	INUMAY(IGP14-211)	2015DS
14083	IR10A183	2015DS
14082	ISMAGOL	2015DS
14077	ISMAGOL	2015DS
14074	KABUONG	2015DS
13923	KALIPAPA(IGP14-213)	2015DS
14260	KATIBUS(IGP-220)	2015DS
14265	KINORON	2015DS
13924	KWA-KWA(IGP14-214)	2015DS
14244	LOCO W/ AWN	2015DS
14237	LOCOOP	2015DS
14080	MALIGAYA	2015DS
14256	MALIKET	2015DS
14071	MARAGAYA	2015DS
14075	MARAGAYA(WHITE)	2015DS
14268	MATING	2015DS
14276	MILAGROSA	2015DS
14279	MILAGROSA	2015DS
14241	MINA-ANGAN	2015DS
14240	PALAWAN	2015DS
13922	PILIT(IGP14-212)	2015DS
14266	PINARUMPONG	2015DS
14253	PINILI	2015DS

**Table 10.** List of germplasm evaluated under different intensities of drought stress using the Line Source Sprinkler (LSS) system in 2015.

**Table 10.** List of germplasm evaluated under different intensities of drought stress using the Line Source Sprinkler (LSS) system in 2015. Con't.

·····	c bource sprinkler (LSS) system in Zo	D. Cont.
14255	RED RICE	2015DS
14274	SINAGINTING	2015DS
14272	SINANDUYO(MALAGKIT)	2015DS
14267	ТІРАК	2015DS
14261	TOMINDOG	2015DS
14073	TUBOD	2015DS
13920	UNKNOWN(IGP14-210)	2015DS
PRRI002422	AMBOL	2015WS
PRRI000669	AZUCENA	2015WS
PRRI002445	BAGSANG (FIAGSANG)	2015WS
PRRI002463	BALIBOD	2015WS
14215	BIHOD	2015WS
PRRI000660	BINATO / LUBANG (BINATO WHITE)	2015WS
PRRI003087	BINIRAO	2015WS
12836	BLACK RICE	2015WS
PRRI003147	CUEVAS	2015WS
PRRI000280	DINOMINGA	2015WS
PRRI003007	DIORON	2015WS
PRRI002912	DUMALI	2015WS
PRRI003553	GANTLE-NUO	2015WS
PRRI003013	GINILINGAN PUTI	2015WS
PRRI003014	GINORGOR	2015WS
PRRI006928	IBARRAKI	2015WS
PRRI003768	ILOCOS (90-DAY VARIETY)	2015WS
PRRI003160	IMMARAMANG	2015WS
PRRI003022	INAPORAONON	2015WS
PRRI003163	INAPOSTOL	2015WS
PRRI003166	INCANTADO	2015WS
PRRI003178	KANINANG	2015WS
12400	KAPAKLA	2015WS
PRRI003181	KASAKAW	2015WS
PRRI003184	KINAMPOPOY	2015WS
PRRI002936	KINANDA	2015WS
PRRI002916	KINANDANG INUZO	2015WS
PRRI002903	KINANDANG ITIM	2015WS
PRRI003663	KINANDIT	2015WS
PRRI003034	KINARURAY 1	2015WS
PRRI003700	LAWANG	2015WS
PRRI003712	LINUGMOK (MALAGKIT)	2015WS
PRRI003392	LUBANG	2015WS
PRRI005644	MACARANIAG	2015WS
PRRI003046	MALAGKIT 1	2015WS
PRRI003739	MALANDI	2015WS
PRRI003738	MALANDI	2015WS

**Table 10.** List of germplasm evaluated under different intensities of drought stress using the Line Source Sprinkler (LSS) system in 2015. Con't.

PRRI003299	MIMIS	2015WS
PRRI000321	MINONDOC	2015WS
PRRI003823	MUNAN	2015WS
PRRI003230	NAGPUNIT	2015WS
PRRI002867	ORTOC	2015WS
14221	PILIT TAPUL	2015WS
14223	PILT ECOGAN/PILIT CARABAO	2015WS
PRRI003069	PINILI	2015WS
13777	POKPOKLO	2015WS
12394	SALWI	2015WS
14226	SIMABATES	2015WS
14274	SINAGINTING	2015WS
135	TOKLING	2015WS
14217	TRES MARIAS(ARAKAN)	2015WS
PRRI003312	UYAK	2015WS
	IR64 (check 1)	
	KDML105 (check 2)	



Figure 3. Soil moisture dynamics in the line source sprinkler system 2015 DS and WS.



Figure 4. Shoot dry weight of 102 germplasm materials under different intensities of soil moisture in 2015.



Figure 5. Shoot dry weight of selected germplasm evaluated under different intensities of soil moisture in 2015.

## Evaluation of rice germplasm for zinc deficiency tolerance in Caraga Region

HA Jimenez, JB Culiao, and LM Perez

The biotic and abiotic stresses are important factors that limit rice production in Caraga region. Low solar radiation, flooding and soil-zinc deficiency are common problem frequently occurred in the region. Zinc (Zn) deficiency was first diagnosed in rice (Oryza sativa) on calcareous soils of northern India (Yoshida and Tanaka, 1969). It is currently a widespread micronutrient deficiency in Caraga Region causing low rice yields on the farmer's field. Zinc deficiency can be corrected by adding Zn compounds to the soil or plant, but the high cost associated with applying Zn fertilizers in sufficient quantities to overcome Zn deficiency places considerable burden on resource-poor farmers and it has therefore been suggested that breeding efforts should be intensified to improve the tolerance to Zn deficiency in rice cultivars (Quijano-Guerta et al., 2002; Singh et al., 2003).

Using tolerant varieties is an efficient, economical and sustainable management strategy to mitigate these problems. Genetic variability in tolerance to stresses exists which can be explained by various physiological mechanisms underlying certain adaptations to unfavorable conditions. These will serve as bases in varietal selection and development of improved rice varieties.

- A total of 305 traditional varieties and 4 check varieties (NSIC Rc122, IR64, IR10A183 and NSIC Rc240) were screened and evaluated under without zinc oxide and zinc sulphate treatment application.
  - Five rice germplasm accessions identified with tolerance to soil zinc deficiency with good to excellent phenotypic performance, these are: Minaangan (Coll. No. 5480), Unknown (Coll. No. 10895-A), Burik (Coll. No. 11161), Dinurado (Coll. No. 11125) and Red Tonner (Coll. No. 11296).
- 10 rice germplasm accessions identified with moderate resistance to zinc deficiency comparable to IR10A183 (a tolerant genotype). The following accessions namely: Oklan Minaangan (Coll. No. 5513), Landis (IRGC 44541) (Coll. No. 6198), Unknown (Coll. No. 10904), Diket (PRISA-KLG 7) (Coll. No.11229), Dinorado (Coll. No. 11126), Golden Pilit (PMES 0866) (Coll. No. 7809), Intan (Red) (PRISA-KLG 11) (Coll. No. 11232), Manabang (Coll. No. 11108), Oltan (Red) (Coll. No. 11176) and Onoy (Unoy) (PRISA-KLG 23) (Coll. No. 11235).



Figure 6. Set-up on evaluation of rice germplasm for Zinc deficiency tolerance in Caraga Region.

## Evaluation of rice germplasm for rice tungro resistance at PhilRice Midsayap

GD Baleras, JM Niones, SJ Labarosa, JLD Genilla, and LM Perez

Disease infection and pest infestations contribute to low and unstable yield of rice farmers in Mindanao region. Crop diversity has a vital role to play in helping the upland farmers improve their livelihood. Rice tungro virus (RTV) is the most economically important viral disease of rice in the Philippines. It affects the plants at any growth stages. Currently, varieties ARC 11554, Utri merah, Utri Rajapan, Habiganj DW8 and some wild rice relatives are being used as resistant donors for RTV breeding program at PhilRice, but little attention has given in searching new sources of resistance from our PhilRice germplasm. This study focused on screening of PhilRice germplasm collection against RTV. PhilRice germplasm collections were screened in batches. In 2015, 1883 different rice germplasm accessions were evaluated for field resistance against tungro disease. Diseasse rating of germplasm was done at 30, 45 and 60 DAT. Selection was done 60 DAT based on its tungro disease rating and phenotypic acceptability.

#### 30 Rice R&D Highlights 2015

- Out of the 1883 entries screened for RTV field resistance; 90 accessions showed resistance, 31 accessions intermediate and 937 accessions susceptible at 30 DAT. At 45 DAT 4 were resistant, 12 intermediate and 977 susceptible while at 60 DAT only PR25792-B-10-4 breeding line showed intermediate reaction to RTV and all other entries showed to be susceptible (Figures 7 and 8).
- There are 11 entries that showed good phenotypic acceptability beyond infection (Table 11).

**Table 11.** Eleven germplasm accessions showed good phenotypic acceptability and RTD rating.

Entry Number	Accession Number	Cultivar Name	RTD Rating
401	PRRI000583	TCCP266-1-3B-13-1-3	SUSCEPTIBLE
597	PRRI0001471	PR23823-1-1-2	SUSCEPTIBLE
589	PRRI0001472	PR25769-B-9-3	SUSCEPTIBLE
599	PRRI0001473	PR25792-B-10-4	INTERMEDIATE
600	PRRI0001474	PR25792-B-4-2	SUSCEPTIBLE
601	PRRI0001480	PR25792-B-6-2	SUSCEPTIBLE
609	PRRI0001520	IR38	SUSCEPTIBLE
802	PRRI0002731	BATANG ANAI	SUSCEPTIBLE
804	PRRI0002758	KHAO DAWK MALI 105	SUSCEPTIBLE
988	PRRI0003209	MALIKET	SUSCEPTIBLE
990	PRRI0003212	MALIKET	SUSCEPTIBLE
999	PRRI0003223	MILAGROSA	SUSCEPTIBLE



**Figure 7.** Field screening of germplasm collection at 30 DAT (A) and 60 DAT (B) against rice tungro disease.



**Figure 8.** Frequency distribution of reaction of germplasm collection at 30, 45, 60 days after transplanting (DAT) of rice tungro disease in 2015 at PhilRice Midsayap.

### Characterization of new sources of disease resistance genes in PhilRice Genebank accessions

JT Niones and JA Poblete

Diseases are one of the significant factors limiting rice production causing a conservative 5% annual yield loss (Zhang et al. 2009). Among the 70 diseases caused by fungi, bacteria, viruses or nematode recorded on rice, rice blast (Magnaporthe grisea), bacterial leaf blight (Xanthomonas oryzae pv. oryzae) and sheath blight (Rhizoctonia solani) are the most serious constraints on high productivity. Local and indigenous traditional varieties are usual sources of genes useful in improving rice with resistance against various important rice diseases. However, before our plant breeders can use and exploit these identified disease resistant accession lines as potential source of resistance genes, there is a foremost need to establish the nature and mechanism of resistance of these rice accessions. Identifying the mechanism of disease resistance facilitates manipulation, effective transfer of genes to popular varieties and eventual deployment of disease -resistant cultivars. Thus, this means greater opportunity of using and exploiting these lines for breeding and genetic improvement of our rice varieties for resistance to rice diseases. Moreover, with the growing attention given on issues of intellectual property rights particularly on ownership of rice germplasm under development, characterizing and identifying our own materials for potential source of disease resistance genes will mean protecting these heirloom plant materials from inappropriate, misuse and unauthorized exploitation. The number of plant accessions to be subjected to phenotypic and molecular characterization of host resistance will largely depends on the field evaluation results of PhilRice germplasm collection for biotic stresses.

#### **Highlights:**

.

Evaluated the resistance spectrum of 25 rice blast resistant Philippine traditional varieties against eight rice blast differential isolates.

### **III. Genetic Resources Research**

Project Leader: XGI Caguiat

The Genetic Resources Research was created to provide basic and advance information on molecular data, current diversity status and aid in accelerating trait discovery from the germplasm accessions conserved at PhilRice Genebank. This project aims to support characterization and evaluation data leading to promote utilization. Over the years, the low utilization of conserved germplasm is due to the lack of useful information sought by breeders, chemists and other stakeholders. Developing a sytem that can provide quick information what is stored at genebank will facilitate the genebank curator and researcher in germpalsm management and enhanced gene pool utilization, respectively.

The studies under this project focuses on development of multiple founder lines , exploration of landraces for CMS diversification, utilization of next-generation sequencing for in silico gene discovery, and survey of ethnobotanical studies on rice with medicinal use.

Future plans include broad range diversity analysis using various types of marker systems and different bioinformatics softwares. Another promising prospect for this project is the development of core collection that represent current diversity of the entire collection, biotic stress resistance, abiotic stress tolerant and superior grain and eating quality traits.

### MAGIC (Multi-parent Advanced Generation Inter Crosses) in PhilRice Genebank

LM Perez, MIC Calayugan, TE Mananghaya, VG Dalusong, RP Mallari, R Baybado

In the development of rice cultivars adapted to specific environmental conditions, genetic resources are important in providing appropriate parental donors that can be sources of important traits or genes for high yield, resistance to pests and diseases, and superior grain quality. Numerous studies were conducted exploring favorable alleles using mostly bi-parental mapping populations in genetic and association studies. However, the natural richness of alleles or genetic variations existing in our germplasm has not been exhaustively explored yet to the breeders' advantage. This study will explore the method of MAGIC to exhaust natural allelic variants that can be sources of novel quantitative trait loci (QTL) for traits like yield, disease resistance, tolerance to abiotic stresses like drought, submergence, and salinity, as well as grain quality.

MAGIC is an experimental method in which genetic markers are linked to quantitative trait loci (QTL) (IRRI, 2011). It was introduced

by Mott et al. (2000) in mice as an extension to the advanced intercross (AIC) procedure of Darvasi and Soller (1995). MAGIC populations are established by several rounds of intercrossing multiple founder lines and the resulting populations are, hence, genetically diverse, essential for the detection of multiple QTLs at the same time. The focus of the study is to determine potential founder lines in the rice germplasms conserved at PhilRice genebank. Selection of founder lines to be included in the MAGIC population will be done with the breeders and in consideration with unique agronomic traits suitable to the need of rice farmers in the Philippines. Phenotyping as well as molecular characterization of the founder lines will be explored by establishing the traits and methodologies as well as appropriate genetic marker systems for the molecular analysis.

- Azucena showed intermediate resistant reaction to rice blast disease (Table 12).
- 12 two way crosses generated to be used in four way cross (Table 13).
- 8 four way crosses were generated for advanced generation (Table 14).
- 253 SSR markers surveyed and 149 showed polymorphism to be used in molecular characterization of founder lines (Table 15).

Code	Variety Name	Reaction
MGC-5	AZUCENA	Intermediate
MGC-6	GOBERNO PUTI	Susceptible
MGC-8	BUWA	Susceptible
MGC-9	MONDAY	Susceptible
MGC-10	BINIGNAY	Susceptible
MGC-13	PSB RC3	Susceptible
MGC-14	TAPOL WHITE	Susceptible
MGC-15	KALANGIKING	Susceptible

Table 12. Blast resistance evaluation of eight MAGIC founder lines.

Cross Combination	No. of F1 plants generate
ASUCENA/ BUWA	4
ASUCENA/ MONDAY	12
GOBERNO PUTI/ MONDAY	15
BUWA/ MONDAY	8
GOBERNO PUTI/ BINIGNAY	21
BUWA/ BINIGNAY	28
ASUCENA/ PSB Rc3	5
GOBERNO PUTI/ PSB Rc3	26
ASUCENA/ KALINGKING	24
BUWA/ KALINGKING	6
PSB Rc-B51-B23/ KALINGKING	23

 Table 13. Two-way cross combinations generated.

 Table 14. List of four-way crosses to be established for 8 way crosses.

Female	Male
MGC-14/MGC13 F1-4	MGC-15/MGC-5 F1-6
MGC-14/MGC-8 F1-3	MGC-14/MGC-6 F1-4
MGC-14/MGC-6 F1-1	MGC-14/MGC-8 F1-1
MGC-15/MGC-8 F1-1	MGc-15/MGC-5 F1-21
MGC-15/MGC-13 F1-9	MGC13/MGC-6 F1-10
MGC-15/MGC-13 F1-1	MGC-15/MGC-10 F1-10
MGC-15/MGC-10 F1-12	MGC-15/MGC-13 F1-6
MGC-15/MGC-10 F1-6	MGC-10/MGC-6 F1-1

Crosses	No. of polymorphic SSR markers	Crosses	No. of polymorphic SSR markers
MGC5xMGC6	5	MGC8xMGC10	9
MGC5xMGC8	8	MGC8xMGC13	21
MGC5xMGC9	5	MGC8xMGC14	9
MGC5xMGC10	8	MGC8xMGC15	10
MGC5xMGC13	18	MGC9xMGC10	28
MGC5xMGC14	8	MGC9xMGC13	62
MGC5xMGC15	7	MGC9xMGC14	24
MGC6xMGC8	7	MGC9xMGC15	16
MGC6xMGC9	5	MGC10xMGC13	63
MGC6xMGC10	8	MGC10xMGC14	24
MGC6xMGC13	20	MGC10xMGC15	10
MGC6xMGC14	6	MGC13xMGC14	64
MGC6xMGC15	6	MGC13xMGC15	36
MGC8xMGC9	8	MGC14xMGC15	16

Table 15. Polymorphism survey of parents using 253 SSR markers.

### Exploration of landraces for CMS diversification

IG Pacada, CF Libayao, LM Perez, and TM Masajo

Wild abortive (WA) type of cytoplasm has been used extensively in identifying and developing new maintainer lines and breeding rice F1 hybrids. To date, most of PhilRice maintainer lines converted into male sterile has WA cytosterility source. However, single source of male sterile cytoplasm maybe disastrous in case of sudden outbreak of pest and diseases specifically if the susceptibility is associated with a CMS-inducing factor. Furthermore, cytoplasmic influences yield and agronomic characters, hence, diversification of cytoplasmic source play an important role in improving crop productivity. This study aimed to develop new sterile cytoplasm source using nucleus substitution approach.

### **Highlights:**

Potential combinations were found between Primitive indica x Modern Varieties (Inter-varietal crosses), and Javanica x Modern Varieties (Inter-sub specific crosses). These were evaluated based on the degree of pollen sterility/fertility of generated F1. However, the big difference in maturity between the identified traditional and modern varieties hinder the generation of BC1F1 progenies. • From nine inter-varietal and 16 inter-sub specific crosses, only one cross from inter-sub specific was detected to possess sterility.

### Complete genomic DNA sequencing of selected Philippine traditional varieties for in silico gene discovery

XGI Caguiat, MVG Embate, VG Dalusong, RP Mallari and LM Perez

Genomic DNA sequencing is a biotechnology tool for discovering genes coding for traits including resistance to pests and diseases, tolerance to abiotic stresses, grain quality. With the revolution of molecular tools and fastpaced evolution of DNA analysis technology, it becomes a common measure for gene discovery in plants especially rice.

Philippine traditional rice varieties currently conserved in PhilRice Genebank have immense genetic diversity and potential novel genes for rice genetic improvement. With the advent of intellectual property rights and ownership of rice particularly germplasm under development, there is a need to discover local sources of genes or traits for breeding and genetic improvement of rice varieties for resistance to pests and disease, abiotic stress resistance, and good grain quality. This study will generate genomic sequence information of selected Philippine traditional varieties and identification of potential novel genes using in silico gene discovery. Molecular analysis and phenotyping of rice germplasm helps in identification of novel genes. Trainings and memorandum of agreement with collaborators will be useful in handling data analysis. The discovery of genes and potential source of germplasm in local and indigenous traditional rice varieties will mean opportunity for commercialization of rice science advancement in the Philippines.

- In 2015 dry season (DS), four Philippine traditional rice varieties were selected based on morpho-agronomic traits and evaluation for resistance or tolerance to biotic and abiotic stresses.
- FastQC (version 0.11.3) results of three Philippine traditional rice varieties were obtained from PGC-Core facility for bioinformatics at UP-Diliman, Quezon City. FastQC provided modular set of analysis to assess or checks on quality control of raw sequencing data obtained from high sequencing pipelines. The figures below showed the sequence quality results of three Philippine traditional rice varieties. The y-axis in the graph showed quality scores. The green color in the y-axis represents as very good in quality calls, the orange color as reasonable

quality calls and the red as poor in quality calls.

Accession Number	Coll. No.	Cultivar Name	Donor Name	Province	Important Traits	Plant Height (cm)	Maturity
PRRI000319	2302	MINDA	IRRI	llocos Norte	R and MR to rice to rice blast and bacterial blight	202.8	159
PRRI000327	2310	PAICOT (GLUT)	IRRI	Pangasinan	R and MR to rice to rice blast and bacterial blight	122.22	107
PRRI006749	5978	DINORAD O	Gener Villanue va	Cotabato	Low/ no visible sign of sensitivity to stemborer	184	128
PRRI000098	2081	DUKAB	IRRI	Metro Manila	Salinity resistant	200.2	144

**Table 16.** Germplasm information of four tentatively selected Philippinetraditional rice varieties from PhilRice Genebank.

**Table 17.** Preliminary results of per base sequence quality control of three Philippine traditional rice varieties.

Accession Number	Coll. No.	Cultivar Name	Total Sequences	Sequences Flagged as Poor Quality	Sequence Length	% GC
PRRI001014	1367	MALAY 2	31899078	0	50-385	40
PRRI004381	5218	BINATO	50985446	0	50-101	40
	12808	ARABON	31757957	0	50-387	41



Figure 9. Per base sequence quality control checks of Malay 2 traditional rice variety.



Figure 10. Per base sequence quality control checks of Binato traditional rice variety.



Figure 11. Per base sequence quality control checks of Arabon traditional rice variety.

### Investigating the bound form of 2-acetyl-1-pyrroline (2-AP) in Philippine aromatic rices in the PhilRice Gene Bank

APP Tuaño, TE Mananghaya, MVG Embate, ATP dela Cruz, RP Mallari, LM Perez and BO Juliano

Aromatic rice with superior eating quality commands higher price in the market and has been increasingly demanded for by niche markets abroad. The popular Thai Jasmine rice "Khao Dawk Mali 105" and Pakistan "Basmati" rice both have been recognized worldwide for their distinct aroma, appearance and eating quality and continue to be highly priced in the international market. The Philippines has released a few aromatic varieties (e.g., NSIC Rc128, Rc148 and Rc218SR) but has not yet popularized its own version of aromatic cultivars with high export potential.

The 2-Acetyl-1-pyrroline (2AP) has been identified as the most potent compound causing the popcorn-like aroma of cooked rice. Significantly higher amounts of 2AP were found in aromatic rice varieties. The gene for fragrance in rice has been identified and associated with high 2-AP levels. Molecular markers have been developed to aid the selection for the fragrance (fgr) gene in aromatic rice breeding and efforts to combine various alleles of the fgr gene (expressing different or equal levels of 2AP) have been made to come up with "super-aromatic" rice. In 2005, the presence of bound and free forms of 2AP on a nearly 1:1 ratio has been reported by Japanese rice scientists and showed that the free form of 2AP volatilizes easily on storage while the bound form has been suspected to have higher retention. The bound form of 2AP is of greater interest to breeders and consumers as this will give a lingering and persistent aroma and flavor to cooked rice. This study intended to identify and characterize Philippine aromatic traditional varieties with high amounts of bound 2AP and novel alleles of the fragrance gene. Once a genotype with high amount of bound 2AP has been identified and characterized, genetic improvement and studies on the effects of environment, cultural and post-harvest management may follow.

- Molecular analysis of the DA export rices (2015DS crop) using the Bradbury marker for fragrance (fgr) gene has been completed. Of the 41 accessions (24 variety names), only 14 had the fgr allele, 19 were non-fragrant while the rest did not amplify and needs verification. Phenotypic qualitative aroma using KOH resulted in 9 aromatic samples regardless with fgr allele presence.
- Selected scented rice accessions (2015DS crop) from the PhilRice gene bank were also genotyped using the same marker and the distribution of samples with fgr allele, non-fgr

allele and heterozygous is as follows: 27, 14, 2, respectively. Among the NSIC released modern aromatic rices, only NSIC Rc148, Rc218 and Rc342 had the fgr allele, though all had aroma using the KOH method, except for Rc128 and Rc344. Surprisingly, waxy rice varieties Malagkit Sungsong, Improved Malagkit Sungsong 2 and UPLRi-1 gave non-fgr results.

- Optimization of the Shi and Shao markers for the other fgr alleles is nearly complete. These markers will also be adopted for the same set of materials prior to selection of samples for bound 2-AP studies using gas chromatography.
- Philippine released aromatic rices (named as Mabango 1 to 5: NSIC Rc128, Rc148, Rc218, Rc342, Rc344; and Mestizo 1) from 2014DS crop were subjected to preliminary storage treatments and will be analyzed for total and bound 2-AP determination using gas chromatography. Molecular analysis of these samples showed that only Rc148, Rc218 and Rc342 had the fgr allele.

## Ethnoguided survey and collection of Philippine medicinal traditional rice varieties

RMF Cabanting and LM Perez

The need to discover and conserve natural sources of medicine and other high value products is becoming increasingly important. Ethnoguided approaches involving ethnobotany and ethnomedicine have been reported as significant tools for elucidating the roles of plants in basic health care system of many societies. In the Philippines, rice has always been a strong pivot in the culture and traditions of Filipinos and agriculture based research. However, the ethnomedicinal attributes of rice especially those of traditional varieties remain scarce and underexplored. To date, no baseline catalogue describing the rice varieties used for ethnomedical treatment exits. This study aims to collect and conserve traditional rice varieties and gather information from local communities concerning their use of rice in folk medicine. Ethnoguided survey was held using semi-structured interviews with knowledgeable locals as our key informants.

- Close coordination between local government units and key informants in five provinces namely: Zamboanga del Norte, Zamboanga del Sur, North Cotabato, Palawan and Agusan del Norte was established from January- June 2015.
- Letters of intent for the upcoming activity on August-

September 2015 were sent to respective key individuals and government units. Planning and coordination of itinerary for the verification and documentation was also conducted among agricultural technicians and key informants.

### **IV. Optimization of Germplasm Conservation Procedures**

Project Leader: IG Pacada

Characterization and evaluation of germplasm is linked to utilization. Low utilization of conserved germplasm is due to lack of documentation and inadequate description of the collections. Developing a system that can provide quick information on what is conserved in the genebank will facilitate the genebank curator and researcher in germplasm management and enhanced gene pool utilization, respectively.

### Development of reference collection digital database and find-match software

IG Pacada, MC Ferrer, MD Duldualo, and LM Perez

Traditionally, reference collection or seed file (duplicate of what is conserved at genebank), is located in an organized box. However, conserving, documenting, evaluating and securing long-term maintenance of reference collection is not simple. The development of virtual seed file provides back up and long term preservation. In addition, this can be coupled by software with the capability of digitizing available seed file and storing its grain characteristic information. This study aimed to create digital database of all available seed file, and develop germplasm query system for quick retrieval information base on seed file grain dimension.

- Modified the developed graphical user interface (GUI) for reference collection digital database wherein additional of four important information will be seen: accession number, accession name, Collection number and Batch ID. The scanned and saved images have its corresponding Collection Number information (Figure 12).
- The germplasm query (find-and-match system) will be based on the rice grain length, width and shape. Development of algorithm for grain size detection is in progress.

						Phil	Rice	GRD Manag	gement Syste	m				 ×
	0 - Seed	Files												^
Sa	ve Refresh No Options	y Seed Load Image	Scan Save Image To	Image De File In Tasks	elete From hage Excel	War Sca	m Uj	p 1:1 Image 1 Matching	N SURF/Flann Classifier Classification	Segment Background				
Sei	ed File Manage	ment ×					-							*
SI	red Files			1		*								
	Accessio *	Accession Na	Collectio	Batch Id	Has Image			1984						
		KAUNAYAN	13640	2014		1		- 12						
	4686	INIBA-BAIN	1998					<u> </u>						
	PRR1000015	INIBA-BAIN			1			. =						
	PRRI000001	HINAMOG	1984		1			0 =						
	PRR/000002	MANGASA	1985		1									
	PRRI000003	MALINIS 1	1986		1			N2 =			-	-		
	PRRI000004	WAGLON	1987		1			×.=						
	PRRI000005	ERUIAN	1988		1									
	PRRI000006	NAGPILI	1989		1	0		8-1						
	PRRI000007	KALAGNON	1990		1			<u> </u>	V .	- V				
	PRRI000008	AGLIPAY	1991		1			4						
	PRRI000009	ARGEL	422 / 1992		V			0						
	PRR/000010	BINONDOC	1993		1				6					
	PRR/000011	BINUHANGIN	1994		$\checkmark$			Q1 =	-		-		100	
	PRR/000012	CAPITAL	1995		$\checkmark$			•						
	PRR/000013	DOCTOR	1996		$\checkmark$			0						
	PRRI000014	GARAYGAY	1997		1			2						
	PRRI000016	KATUHAN	1999		~	•								
L				0	Count = 13614	•								

Figure 12. Modified GUI for reference collection database development.

### Classification of rice germplasm into ecotypes (Japonica/Indica)

MC Ferrer, MIC Calayugan, and LM Perez

Exploitation of new germplasm played important role in rice breeding. This is due to its diverse background and its potential source for generating novel adapted allele combinations which can be used for genetic improvement by various breeding programs. Significant prerequisite of utilizing germplasm for improvement is to accurately identify their ecotypes whether they are belong indica and japonica group. In this way, it provides baseline information for a breeder to design appropriate breeding approach when they utilize them. Among the physiological and morphological characteristics, the grain shape and grain phenol reaction have been widely used as conventional tools for classifying rice varieties into Japonica and Indica types.

- Caryopsis length and caryopsis width of 2864 accessions were gathered. Results showed that 7 are round, 204 semi-round, 838 spindle-shaped, 687 half spindle-shaped and 1105 are long spindle-shaped.
- Data on phenol reaction were uploaded to the data base. From the 5144 accessions tested, result revealed that there are 3880 Indica, 1182 Intermediate and 82 Japonica

### V. Conservation of Genetic Resources in the Rice Environment

Project Leader: JT Niones

Recognizing the impact, importance and potential utilization of microbial, invertebrates and plant resources from the rice environment, PhilRice has been studying, evaluating and promoting the use of beneficial microbes ranging from fungi, bacteria and actinomycetes either as potential biological control agent of specific rice pest or as plant growth promoter. Pure isolates of rice pathogens are regularly cultured and utilized in the evaluation of rice plant breeding materials for disease resistance. Moreover, PhilRice has recently renewed its interest in Azolla technology and other N-fixing systems in support to the organic agriculture program.

Along with the increasing collection of these beneficial microbial and non- microbial resources at PhilRice, is the pressing concern to provide a reliable and safe preservation and storage protocol for these genetic resources. Physiological or genetic damage to economically important strains could potentially result in considerable loss of investment in a research and product development program.

The project aims to: (1) develop conservation and preservation strategies for beneficial microbes, invertebrates and plant resources from the rice environment to ensure their physiological and genomic integrity and quality, for research and development, and public utilization purposes; (2) to establish management system that facilitates record-keeping, utilization, distribution and exchange of these genetic resources.

#### Conservation and management of azolla species

CLC Mondejar and GO San Valentin

When the National Azolla Action Program (NAAP) was implemented in 1982, selected azolla varieties were distributed in different regions of the country. These varieties of azolla were identified to be suitable in the area of dispersal. Nowadays, azolla thrives in rice community in small population only and some plants can be found in irrigation canals instead on rice paddies. Only few farmers are aware of the importance of azolla in rice farming and sustain the utilization of azolla.

The PhilRice has the capability to continue R&D activities on azolla and other N-fixing systems in support to the organic agriculture program of the Department of Agriculture (DA). As a leader of rice R&D in the country, PhilRice initiated the recovery of the original accessions and continue the selection and hybridization to produce superior strains. The researches on azolla in PhilRice have been focusing mainly on how these plant would become a stable part of rice farming system. PhilRice makes the azolla technology available by propagating fresh biomass and other inoculum for distribution to farmers.

The re-establishment of azolla technology by PhilRice requires the development of living azolla reference collections. The reference collection will serve as a source of inoculum for (1) continuous production of azolla and (2) basic and applied researches on azolla. The main goal of the establishment of azolla germplasm collections in PhilRice is to conserve the Philippine azolla indigenous species and accessions widely adapted in the Philippine condition for utilization.

- Two methods are used in conserving the azolla in PhilRice Los Baños. These are the in vitro method and soil-and-water medium. Both of these methods use the vegetative means of propagation. The in vitro method uses the protocol of Watanabe et al. 1992 and nutrient solution as the medium (Figure 13). In soil-and-water medium, the azolla are grown in plastic trays filled with paddy soil with at least 2cm high water level (Figure 14).
- A system of accessing the different azolla strains in PhilRice azolla germplasm collections was made. Figure 15 shows the entry of new accessions in the germplasm while figure 16 shows the process of acquiring azolla from PhilRice.
- A method to characterize azolla based on growth rate was standardized. Azolla mexicana # 2024 or the UPLB Hybrid 1 was used as the test strain. Figure 17 shows the differences in the growth response of four azolla strains under Los Baños, Laguna condition.

No	Accession no.	Other Code	Donor	Date of Acquisition	Remarks
1	prri-az-001-001	PI 0072	IRRI	7/8/2014,	NAAP recommended strain
				8/19/2014	
2	prri-az-001-002	CA 3002	IRRI	7/8/2014,	NAAP recommended strain
				8/19/2014	
3	prri-az-001-003	CA 3005	IRRI	7/8/2014,	NAAP recommended strain
				8/19/2014	
4	prri-az-001-004	MI 4018	IRRI	7/8/2014,	NAAP recommended strain
				8/19/2014	
5	prri-az-001-005	ME 2024	IRRI	7/8/2014,	NAAP recommended strain
				8/19/2014	
6	prri-az-001-006	ME 2028	IRRI	7/8/2014,	NAAP recommended strain
				8/19/2014	
7	prri-az-001-007	PP 7001	IRRI	7/8/2014,	NAAP recommended strain
				8/19/2014	
8	prri-az-001-008	PP 7004	IRRI	7/8/2014,	NAAP recommended strain
				8/19/2014	
9	prri-az-001-010	PI 0005	IRRI	7/8/2014,	NAAP recommended strain
				8/19/2014	
10	prri-az-001-011	ME 2002	IRRI	7/8/2014,	NAAP recommended strain
				8/19/2014	
11	prri-az-001-023	PRRI 14-001	CLC Mondejar	8/4/2014	Brgy. Taytay, Majayjay, Laguna
			GO San		
			Valentin		
12	prri-az-001-024	PRRI 14-007	CLC Mondejar	8/4/2014	Brgy. Tinamnan, Lucban, Quezon
			GO San		
			Valentin		
13	prri-az-001-029	PRRI 14-013	CLC Mondejar	10/3/2014	Brgy. Basud, Polangui, Albay
			GO San		
			Valentin		
14	prri-az-001-030	PRRI 14-004	CLC Mondejar	10/3/2014	Brgy. 4, Tugawe-Site, Malilipot,
			GO San		Albay
			Valentin		
15	prri-az-001-031	PRRI 14-005	CLC Mondejar	10/3/2014	Brgy. Balabag, Milaor, Camarines
			GO San		Sur
			Valentin		
16	prri-az-001-032	PRRI 14-006	CLC Mondejar	10/3/2014	PhilRice Bicol Station, Batang,
			GO San		Ligao City
			Valentin		
17	prri-az-001-065	PRRI 15-009	TC Fernando	2015	Adams, Camarines Norte
					(mexicana)
18	prri-az-001-066	PRRI 15-010	TC Fernando	2015	Adams, Camarines Norte (pinnata)
19	prri-az-001-067	PRRI 15-007	CLC Mondejar	2015	Brgy. 4, Paete, Laguna
20	prri-az-001-068	PRRI 15-008	CLC Mondejar	2015	Talavera St., Pakil, Laguna

**Table 18.** List of azolla accession available at PhilRice Azolla GermplasmCollection as of December 31, 2015 for distribution.



Figure 13. In vitro conservation of azolla.



Figure 14. Conservation in soil-and-water medium at PhilRice Azolla Nursery in PhilRice-Los Baños.



Figure 15. The flow in the entry of new accession to the azolla collection.



Figure 16. The flow in acquiring accessions from the azolla collection of PhilRice.



**Figure 17.** Growth rate of four azolla strains (ME 2024, ME 2027, ME 2030 and ME 2033) at linear phase of growth using the standard protocol in characterizing azolla.

### Conservation and management of biocontrol agents

GF Estoy Jr. and BM Tabudlong

The use of biocontrol agents provide an alternative pest control measure to reduce pest population below damaging level. It has shown promise in managing the rice pests' problem (PhilRice, 1995). By their specific nature, it is likely to cause less harm if any to beneficial organisms than chemical pesticides. During outbreak of pests, there is a great demand of biological control agents (BCA's) production of adequate quantities of high quality inoculums.

Conservation biological control is a strategy that seeks to integrate beneficial insects back into crop systems for natural pest control. This strategy is based upon ongoing research that now demonstrates a link between the conservation of natural habitat and reduced pest problems on farms. Conservation strategies for BCA's are necessary in order to use them and optimize its efficacy, stability, safety and ease of application.

The study aims to develop management strategies to conserve, preserve BCA's and evaluate the efficacy of these conservation strategies.

- Ten isolates of Beauveria bassiana (Table 19), 10 isolates of Metarhizium anisopliae (Table 20) and one isolate of Paecilomyces sp. are being maintained in potato dextrose agar. Pure cultures of these entomopathogens were overlaid with mineral oil prior to their storage inside a refrigerator.
- The viability of Beauveria bassiana, Metarhizium anisopliae and Paecilomyces sp. preserved using mineral oil was evaluated after four and six months in storage inside a refrigerator. Upon culture revival, mycelia of the fungal isolates grew well in culture medium and thus remained viable (Table 21).
- The virulence of the preserved cultures of entomopathogens was evaluated against major rice pests. Strains of B. bassiana, 6 months in storage in mineral oil, were pathogenic to rice bug adult with a mortality ranging from 43% to 100% at 10 days post treatment (Table 22). On the other hand, strains of M. anisopliae caused 60 to 83% mortality on a population of white stemborer larvae, 56 to 93% against rice bug and 70 to 86% mortality on rice black bug (Table 23).

No.	Code Name	Insect Host	Place Collected
1	Bb.#01	Sweet potato weevil	ViSCA, Baybay, Leyte
2	Bb.#02	Coleoptera	ViSCA, Baybay, Leyte
3	Bb.#21	Rice bug	Mahanub, Gigakit, Surigao Del Norte
4	Bb.#22	Rice Bug	Prosperidad, Agusan Del Sur
5	Bb.#27	Rice bug	Trento, Agusan Del Sur
6	Bb.#33	Undetermined larvae	RTRomualdez, Agusan Del Norte
7	Bb.#41	Coccinellid beetle	Alipao, Alegria, Surigao Del Norte
8	Bb.#42	Coccinellid beetle	Gigakit, Surigao Del Norte
9	Bb.#49	Rice bug	RTRomualdez, Agusan Del Norte
10	Bb.#52	Rice bug	RTRomualdez, Agusan Del Norte

**Table 19.** List of different strains of *Beauveria bassiana* in potato dextrose agar slants.

Table 20. List of different strains of Metarhizium	anisopliae in	potato	dextrose
agar slants.			

No.	Code	Insect Host	Place Collected
	Name		
1	Ma.#01	Sweet potato	Tiaong, Quezon
		weevil	
2	Ma.#3	Rice black bug	Capatungan, Trento, ADS
3	Ma.#5	Rice black bug	Bual , North Cotabato
4	Ma.#6	Rice black bug	Basilisa, RTR, ADN
5	Ma.#15	Rice black bug	Ponyente, Gigakit, SDN
6	Ma.#16	Rice black bug	Esperanza, Prosperidad,
			ADS
7	Ma.#17	Rice black bug	Kitcharao, ADN
8	Ma.#19	Rice black bug	San Andres, Bunawan,
			ADS
9	Ma.#20	Rice black bug	San Francisco, ADS
10	Ma.#116	Rice black bug	Basilisa, RTR, ADN

Fungal biocontrol	Code	Effectivity of Fungal Growth	
Agents	Name	After 4	After 6
		months	months
Beauveriabassiana	Bb.#42	Viable	Viable
	Bb.#001	Viable	Viable
	Bb.#33	Viable	Viable
	Bb.#208	Viable	Viable
Metarhizium anisopliae	Ma.#19	Viable	Viable
Paecilomyces sp.	Pf.#9	Viable	Viable

**Table 21.** Preservation of *B. bassiana, M. anisopliae* and *Paecilomyces sp.* isolates in mineral oil.

**Table 22**. Mortality (%) of the rice bug at 7 days post treatment with different isolates of B. bassiana under laboratory condition.

Fungal	Rice Bug adult		
Isolates	Mortality (%)	LT50(days)	
Bb.27	93.33 a	4.8	
Bb.33	100.00 a	4.9	
Bb.49	60.00 bc	5.8	
Bb.22	66.67 bc	5.5	
Bb.52	50.00 c	7.5	
Bb.21	80.00 ab	6.2	
Bb.42	50.00 c	7.5	
Bb.02	53.33 bc	7.2	
Bb.01	43.33 c	*	
Control	0		

**Table 23.** Mortality (%) of 2-day old white stemborer larvae, rice bug and rice black bug, 7 days post treatment with different isolates of M. anisopliae under laboratory condition.

Fungal	Mortality (%)		
Isolates	WSB	RB	RBB
Ma.SanFrancisco	80.00 ns	60.00 b	86.67 ns
Ma.Surigao	83.33	73.33 ab	76.67
Ma.Bunawan	86.67	56.67 b	70.00
Ma.Esperanza	63.33	63.33 b	80.00
Ma.Buenavista	66.67	66.67 ab	83.33
Ma.Capatungan	80.00	70.00 ab	80.00
Ma.Gigakit	73.33	83.33 ab	83.33
Ma.Sogod	60.00	56.67 b	76.67
Ma.Tiaong	80.00	93.33 a	86.67
Control	0	0	0



Figure 18. Preservation of fungal biological control agents in oil form.

### Conservation and management of microbial agents

JT Niones and FR Sandoval

The role and impact of microorganisms on agronomically important crops depend on their interaction with their host plant. Negative interaction of microorganisms with their host resulted to diseased plants and significant crop loss while positive host- microorganism interaction can improve crop nutrition and the ability of crops to resist biotic and abiotic stress. With the increasing awareness of the undesirable human and environmental effects of the use of inorganic fertilizers, herbicides and pesticides, PhilRice thorugh its R&D programs had long recognized that beneficial microbes provide an alternative strategy to combat limiting soil nutrient and the destructive effects of weeds and pests on crops.

Maintaining and preserving fungal cultures are not only essential on systematics and biodiversity studies but also in ensuring the quality of microbial agents especially for commercialization and public utilization purposes. Preservation methods of potentially important isolates for agrobiological applications have to be optimized early in the development process of a product so as to avoid potential economic and scientific loss in the event of deterioration of a production strain.

The study aims to preserve biological important microbial isolates such as plant growth promoters and biological control agents against rice pathogens developed through PhilRice- funded projects. Moreover, virulent isolates of major rice pathogens are also being preserved and maintained at the laboratory.

- One year in different storage conditions, two strains of a biocontrol agent, *Trichoderma sp*, and one isolate of a plant growth promoting bacteria, *Streptomyces mutabilis*, were evaluated in terms of culture viability, virulence and stability of their biologically important physiological traits.
- At one year in storage in different culture preservation conditions, fungal spores of *Trichoderma sp.* remained viable and maintained its inhibitory activity against sheath blight pathogen, *Rhizoctonia solani*. Viability and virulence of fungal cultures did not differ among different storage conditions (Figure 18). However, periodically subcultured Trichoderma sp. has smaller mycelial colony size than those cultures stored in filter paper, mineral oil and 10% glycerol (Figure 19).
- At one year in storage in carbonized rice hull (CRH) and a soil-based carrier, cells of S. mutabilis remained viable and

colony count is comparable with the periodic subcultured samples. (Figure 20). However, colony count is lower when S. mutabilis (either in CRH or soil-based carrier) is stored inside a refrigerator than left at room temperature. IAA production and ACC- deaminase activity of S. mutabilis were maintained regardless of the storage condition. (Figure 21 and Figure 22).

For our collection of rice pathogens, differential isolates of rice blast (*Pyricularia oryzae*) are being maintained in filter paper and stored at -20°C. Differential isolates of bacterial leaf blight pathogen, *Xanthomonas oryzae*, are stored in Microbank<sup>™</sup> and in Wakimoto culture medium covered with 10% skim milk. Sclerotial bodies of sheath blight pathogen, *Rhizoctonia solani*, are stored in microtubes and placed inside a refrigerator. Cultures of bakanae pathogen, *Fusarium moniliforme* are maintained in potato dextrose agar slants and stored inside the refrigerator.



Filter paper

10% glycerol

mineral oil

Periodic subculture

**Figure 19.** Dual culture assay between sheath blight pathogen, Rhizoctonia solani (right side of the plate) and *Trichoderma sp.* (left side of the plate) that has been subjected to different storage conditions. The microtubes containing fungal spores in filter paper, in 10% glycerol and in mineral oil were all stored in -80°C freezer for one year. The control has been regularly sub-cultured in PDA medium every 1 to 2 months.







Figure 21. Effect of different storage conditions on the population of Streptomyces mutabilis.



**Figure 22.** Effect of different storage conditions on the indole-3-acetic acid (IAA) production activity of Streptomyces mutabilis . To measure IAA production, test cultures were grown in arginine- glycerol- salt (AGS) broth supplemented wih tryptophan. After 7 days of incubation, the cultures were centrifuged and the IAA in supernatant was added with Fe-H2SO4 reagent. Pink to red color indicated positive reaction. Test tube in extreme left , in each picture panel, is AGS broth only (negative control).



**Figure 23.** Effect of different storage conditions on the 1-aminocyclopropane-1-carboxylate (ACC) deaminase activity of Streptomyces mutabilis . To test ACC-deaminase activity, the isolates were grown using the nitrogen-free Dworkin and Foster's salts minimal agar medium ( Dworkinand Foster, 1958). The plates were incubated at 28+/-2°C in the dark for 7 days. Growth and sporulation of the isolates are indicators of ACC utilization and production of ACC deaminase

### Abbreviations and acronymns

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

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

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

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

### List of Tables

	Page
<b>Table 1.</b> Summary of regenerated rice germplasm in 2015cropping seasons.	6
<b>Table 2.</b> Summary of processed rice accessions/ collections in2015 for conservation.	6
Table 3. Results of viability test conducted in 2015.	6
<b>Table 4.</b> Summary of characterized rice germplasm in 2015dry and wet season.	11
<b>Table 5.</b> List of germplasm collected in 2015 at PhilRice Midsayap.	13
<b>Table 6.</b> Collection of traditional rice seeds from provinces inNortheast Luzon planted in 2015DS.	16
<b>Table 7.</b> Yield component data encoded for entriesestablished during 2015DS.	17
<b>Table 8.</b> Summary table of reactions of PhilRice germplasmaccessions to major diseases and insect pest 2015 DS.	20
<b>Table 9.</b> Ranges and mean values of grain quality propertiesof PhilRice genebank accessions harvested from the GRDregeneration plots, 2014WS.	22
<b>Table 10.</b> List of germplasm evaluated under differentintensities of drought stress using the Line Source Sprinkler(LSS) system in 2015.	24
Table 11. Eleven germplasm accessions showed good           phenotypic acceptability and RTD rating.	30
<b>Table 12.</b> Blast resistance evaluation of eight MAGIC founderlines.	33
Table 13. Two-way cross combinations generated.	34
<b>Table 14.</b> List of four-way crosses to be established for 8 waycrosses.	34
<b>Table 15.</b> Polymorphism survey of parents using 253 SSRmarkers.	35
<b>Table 16.</b> Germplasm information of four tentatively selectedPhilippine traditional rice varieties from PhilRice Genebank.	37

### List of Tables

	Page
<b>Table 17.</b> Preliminary results of per base sequence quality           control of three Philippine traditional rice varieties.	37
<b>Table 18.</b> List of azolla accession available at PhilRiceAzolla Germplasm Collection as of December 31, 2015 fordistribution.	45
<b>Table 19.</b> List of different strains of <i>Beauveria bassiana</i> inpotato dextrose agar slants.	49
<b>Table 20.</b> List of different strains of Metarhizium anisopliae inpotato dextrose agar slants.	49
<b>Table 21.</b> Preservation of <i>B. bassiana, M. anisopliae</i> and <i>Paecilomyces sp.</i> isolates in mineral oil.	50
<b>Table 22.</b> Mortality (%) of the rice bug at 7 days posttreatment with different isolates of B. bassiana underlaboratory condition.	50
<b>Table 23.</b> Mortality (%) of 2-day old white stemborer larvae,rice bug and rice black bug, 7 days post treatment withdifferent isolates of M. anisopliae under laboratory condition.	51

### List of Figures

<b>Figure 1.</b> Number of acquired germplasm with corresponding sources of collection from January to December 2015.	4
<b>Figure 2.</b> Utilization of the germplasm seed requests received and processed in 2015.	8
<b>Figure 3.</b> Soil moisture dynamics in the line source sprinkler system 2015 DS and WS.	26
<b>Figure 4.</b> Shoot dry weight of 102 germplasm materials under different intensities of soil moisture in 2015.	27
<b>Figure 5.</b> Shoot dry weight of selected germplasm evaluated under different intensities of soil moisture in 2015.	27
<b>Figure 6.</b> Set-up on evaluation of rice germplasm for Zinc deficiency tolerance in Caraga Region.	29
<b>Figure 7.</b> Field screening of germplasm collection at 30 DAT (A) and 60 DAT (B) against rice tungro disease.a	30
<b>Figure 8.</b> Frequency distribution of reaction of germplasm collection at 30, 45, 60 days after transplanting (DAT) of rice tungro disease in 2015 at PhilRice Midsayap.	31
<b>Figure 9.</b> Per base sequence quality control checks of Malay 2 traditional rice variety.	37
<b>Figure 10.</b> Per base sequence quality control checks of Binato traditional rice variety.	38
Figure 11. Per base sequence quality control checks of Arabon traditional rice variety.	38
<b>Figure 12.</b> Modified GUI for reference collection database development.	42
Figure 13. In vitro conservation of azolla.	46
<b>Figure 14.</b> Conservation in soil-and-water medium at PhilRice Azolla Nursery in PhilRice-Los Baños.	46
Figure 15. The flow in the entry of new accession to the azolla collection.	46
<b>Figure 16.</b> The flow in acquiring accessions from the azolla collection of PhilRice.	47

### **List of Figures**

	Page
<b>Figure 17.</b> Growth rate of four azolla strains (ME 2024, ME 2027, ME 2030 and ME 2033) at linear phase of growth using the standard protocol in characterizing azolla.	47
<b>Figure 18.</b> Preservation of fungal biological control agents in oil form.	51
<b>Figure 19.</b> Dual culture assay between sheath blight pathogen, Rhizoctonia solani (right side of the plate) and <i>Trichoderma</i> <i>sp.</i> (left side of the plate) that has been subjected to different storage conditions. The microtubes containing fungal spores in filter paper, in 10% glycerol and in mineral oil were all stored in -80°C freezer for one year. The control has been regularly sub-cultured in PDA medium every 1 to 2 months.	53
<b>Figure 20.</b> Colony size of Trichoderma sp. after subjected to different storage conditions. Measurement was taken at 4 four days after placing spores of Trchoderma sp in fresh PDA medium. The microtubes containing fungal spores in filter paper, in 10% glycerol and in mineral oil were all stored in -80°C freezer for one year. The control has been regularly subcultured in PDA medium every 1-2 months.	54
<b>Figure 21.</b> Effect of different storage conditions on the population of Streptomyces mutabilis.	54
<b>Figure 22.</b> Effect of different storage conditions on the indole- 3-acetic acid (IAA) production activity of Streptomyces mutabilis . To measure IAA production, test cultures were grown in arginine- glycerol- salt (AGS) broth supplemented wih tryptophan. After 7 days of incubation, the cultures were centrifuged and the IAA in supernatant was added with Fe- H2SO4 reagent. Pink to red color indicated positive reaction. Test tube in extreme left , in each picture panel, is AGS broth only (negative control).	55
<b>Figure 23.</b> Effect of different storage conditions on the 1-aminocyclopropane-1-carboxylate (ACC) deaminase activity of Streptomyces mutabilis . To test ACC-deaminase activity, the isolates were grown using the nitrogen-free Dworkin and Foster's salts minimal agar medium ( Dworkinand Foster, 1958). The plates were incubated at $28+/-2^{\circ}$ C in the dark for 7 days. Growth and sporulation of the isolates are indicators of ACC utilization and production of ACC deaminase	55

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 PhilRice Agusan, Basilisa, RTRomualdez, 8611 Agusan del Norte • Tel: (85) 343-0778• Tel/Fax: 343-0768 • Email: agusan.station@philrice.gov.ph PhilRice Batac, MMSU Campus, Batac City, 2906 Ilocos Norte • Tel/Fax: (77) 670-1882; 670-1867 • Email: batac.station@philrice.gov.ph PhilRice Bicol, Batang, Ligao City, 4504 Albay • Cell:0905-7352078, 0918-9467439 • bicol.station@philrice.gov.ph PhilRice Isabela, Malasin, San Mateo, 3318 Isabela • Tel: (78) 0917-594-9285 or 0908-895-7796 • Email: isabela.station@philrice.gov.ph PhilRice Los Baños, UPLB Campus, Los Baños, 4030 Laguna • Tel: (49) 536-8620•501-1917 • Email: losbanos@philrice.gov.ph PhilRice Midsayap, Bual Norte, Midsayap, 9410 North Cotabato • Tel: (64) 229-8178 • Tel/Fax: 229-7242 • Email: midsayap.station@philrice.gov.ph PhilRice Field Office, CMU Campus, Maranag,8714 Bukidnon • Cell: 0917-615-8710 Liaison Office, 3rd Floor, ATI Bldg, Elliptical Road, Diliman, Quezon City • Tel/Fax:(20)920-5129, Cell:0920-9069052