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PhilRice Genebank: recent developments in managing and sharing the Philippine rice germplasm

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Abstract. The Genetic Resources Division (GRD) of PhilRice collects and conserves rice genetic resources to ensure the future generations of available seeds needed to build better rice plants in facing climate change and growing population. At present, GRD maintains the national collection of rice genetic resources with 7,129 accessions. To effectively manage the germplasm collection, the search for, development of, and implementation of the best conservation strategies and innovation in technology have been the utmost priority of the GRD. Thus, georeference data such as latitude, longitude and elevation of germplasm origin during collecting mission were recorded using a handheld global positioning system (GPS) receiver. The e-Seedfile software was developed to provide virtual access of the reference collection for regenerated germplasm seed verification and valid type confirmation for new and old germplasm collection. Barcoding, on the other hand, facilitated accurate inventory of seed stocks, making the distribution and regeneration of germplasm more efficient. Moreover, paperless data collection using android application was implemented for immediate data validation and accurate data downloading from tablets to workstations, making it an ideal tool for germplasm characterization. Furthermore, the current database system was upgraded and adjusted to adopt the use of digital object identifier (DOI) through registration to the global information system (GLIS) on Plant Genetic Resources for Food and Agriculture (PGRFA). The DOI allows the use of material to be tracked, thus meeting the legal obligations of the SMTA and monitor the impact of genebank collections in utilization in research and breeding programs. These innovative technologies are of great importance to expand the toolbox for the management and conservation of the germplasm collection that will help enhance the long-term conservation of rice diversity and easy access to germplasm and germplasm-related information.

Keywords: barcoding, characterization, DOI, e-seedfile, GPS, rice.

1. Introduction

The Philippines lies in one of the eight centres of crop origin and diversity recognized by the great Russian conservationist Nikolai Vavilov [1]. Philippines hotspot is also identified as one of the world's biologically most productive countries [2]. It is an archipelago characterized by a wide variation in climate, eco-geography, and farming systems, which sustain and promote an extensive diversity of rice.

Rice has a high phenotypic diversity results from a long history of domestication, driven by human demographic expansion, and by sympatry of the cultivated rice ecotypes with their wild relatives [3]. The abounding diversity of rice in the Philippines is a national heritage that must be highly valued. These rice genetic resources possess a vast wealth of desirable genes that serve as one of the most important raw material in meeting the current and future needs of rice improvement as well as production programs. These are the building blocks for the development of new varieties to address sustainable development and meet the needs of the continuously growing population. Their efficient conservation and use are critical to safeguarding food and nutrition security, now and in the future.

Maintaining biodiversity for food and agriculture is a national responsibility and cooperative efforts are needed to halt genetic erosion. Its preservation is of paramount importance as these germplasms are the repository of useful genes for plant scientists to solve future problems. However, despite being ecologically rich, Philippines ranks among the top countries with the largest number of threatened species with extinction [4]. The threat of losing these valuable materials makes conservation efforts increasingly more urgent and essential. Thus, the need to conserve essential rice germplasms has been addressed by PhilRice with the establishment of the genebank.

Genebank enabled the conservation of plant genetic materials *ex situ* to ensure seed availability and survival. PhilRice established a rice genebank that will address the conservation of these rice genetic resources for the future generation. Germplasm conservation activities involved manual processing of dried materials for permanent packaging and medium-term storage. Processing included manual sorting to remove all inert and damaged materials as well as separate mixtures. Identities will be cross-referenced against the original seed and panicle files. A properly managed and well characterized germplasm collection will attract an intensified utilization by breeders, leading to more diverse and better-designed varieties which are the critical technology in high productivity rice farming. As more information is available about the germplasm, the more comprehensive selection and diversity of materials can be made available for use in the breeding program and for future generations to appreciate.

Plant genetic resource conservation merits far greater attention than it is now receiving. Recent years have seen increasing efforts to improve both *in situ* and *ex situ* conservation methods, which in theory would foster dynamic conservation of plant species and populations [5,6]. Rice genetic resources conservation is crucial to ensure germplasm sources for further crop breeding [7]. Innovations in technology are expected to expand the toolbox for the management and conservation of the collection that will help enhance the long-term preservation of biodiversity and the easy access of germplasm and germplasm-related information [8]. Thus, to effectively manage the germplasm collection, the search for, development of, and implementation of the best conservation strategies and innovation in technology have been the utmost priority of the genebanks.

2. Review of related literature

In the effort to preserve biodiversity, there has been a concerted world effort to explore, collect, conserve and document the genetic diversity of this important germplasm before they are lost forever. Genebanks around the world play a vital role in the conservation, availability and use of a wide range of plant genetic resources, with the overall aim of long-term conservation. They help bridge the past and the future by ensuring the continued availability of genetic resources for research and improved seed delivery for a sustainable and resilient agricultural system. Well managed genebanks safeguard genetic diversity and make the materials available as a source of variation for plant breeding and selection [9]. Genebanks as a concrete example of *ex situ* conservation ensure that stored materials are readily accessible, can be well characterized and documented and are relatively safe from external threat [10]. This method of conservation cannot, though, provide the opportunity for a wild relative to continue the evolutionary process that a species undergoes in its natural environment. However, it keeps germplasm safe when plants are destroyed in their natural habitat and they have the advantage, for the user, of making material from widely scattered localities available in one place ready for use.

Conserving and increasing the sustainable use of plant genetic resources is necessary for achieving food security and addressing nutritional requirements of present and future generations. Sustainable conservation of these plant genetic resources depends on the effective and efficient management of genebanks through the application of standards and procedures that ensure the continued survival and availability of plant genetic resources. Therefore, it is vital to conserve the diversity of plant genetic resources so that it is available to the global community [9].

In order to increase the efficiency and effectiveness of germplasm conservation efforts to discover, conserve, and use new qualities in plant genetic resources. Technological advances and developments on germplasm repositories and seed banks must be generated to foster innovation platforms [11]. Availability of detailed documentation of passport, phenotypic, and genetic data increases the value of all genebank accessions. Inclusion of georeferenced sources, habitats, and sampling data in collection databases facilitate interpretation of genetic data for genebank accessions [12]. Furthermore, genebanks often conserve multiple samples that have the same cultivar name because they are genetically distinct. Regardless of the cultivar name, genebanks attempt to maintain the genetic composition of accessions unchanged from sample to sample and from generation to generation. Where the sample originally received is genetically heterogeneous, the genebank may choose to split it into homogeneous groups to be conserved as independent accessions, or keep it as a heterogeneous population for conservation as a single accession [13]. While accession identifiers are assigned to enable each accession to be identified uniquely, and rigorous quality standards are followed to ensure that samples of the accession remain true to type [9]. These advancements and innovations will contribute in the generation of a complete and detailed picture of global rice diversity in the future, thus, definitely play a vital role in the conservation, management and utilization of rice genetic resources.

One hundred and nine non-redundant accessions were selected from the available KKN in LNG as a whole collection model (Table 1). In order to construct our whole collection model genetic data set, a random individual for each non-redundant accession was selected and associated with 24 highly informative genomes spread SSR data. Molecular characterization procedures have been published elsewhere [14].

2.1. Assembly of rice germplasm

Conservation of plant genetic resources, guarantees the ability of seeds to survive in storage which has been successfully applied by genebanks worldwide for the preservation and exchange of crop genetic resources threatened by genetic vulnerability [15]. Exploration and collection comprise a problematic and challenging phase of genetic conservation. A systematic approach to germplasm collection program is necessary to ensure that the maximum range of diversity of germplasm will be collected in a cost and time efficient manner. As a rule, germplasm collecting is undertaken for two purposes for conservation and utilization. Rice improvement programs rely on the vast gene pool represented in genebanks for the source of genes and novel alleles needed to build better rice.

PhilRice through Genetic Resources Division (GRD) collects and conserve rice genetic resources. Collecting activities prioritized the underrepresented provinces and tribal area and stored at PhilRice's genebank. To expand existing collections, field collections in priority locations all over the country are performed and donations from individuals, organizations and institutions are actively encouraged. Georeference data such as latitude, longitude and elevation of germplasm origin during collecting mission were recorded using a handheld global positioning system (GPS) receiver. To date, PhilRice Genebank currently holds 16,233 collections and 7,129 of which are assigned as accessions, identifying them as unique among the registered collections (Table 1).

Table 1. Current status of materials conserved inside PhilRice Genebank.

Biological status	Accessions	Collections	Total
Philippine TRVs	3,943	2,955	6,898
Breeding/research materials	1,615	3,313	4,928
Improved cultivars	631	739	1,370
Unspecified germplasm	494	1,024	1,518
Foreign TRVs	430	787	1,217
Wild rice	16	28	44
Farmers' lines		*258*	258
Total	7,129	8,869	16,233

2.2. Germplasm conservation

Conservation of rice germplasm is a continuous process. The PhilRice Genebank follows the international standards on various processes which includes registration of incoming (new) germplasm collections, preparation of seed files (for seed verification/identity), seed cleaning, viability test (germination rate), slow drying of seeds to achieve 6% seed moisture content (MC) and packaging in standard foil packets for storage in medium-term (active collections) and duplicated in long-term (base collection) storage facilities of PhilRice Genebank.

Regeneration of genebank collections is necessary due to decreasing seed viability as well as diminishing amount of seeds over time through active distribution. Seed multiplication on the other hand, is the best way to revitalize stocks to maintain the genetic integrity of germplasm collection. To keep these valuable materials alive, regeneration has to be undergone to maintain their viability and genetic integrity.

As part of the PhilRice Genebank continuous improvement, germplasm conservation procedures and facilities are still being improved. The facilities of the genebank ensure the long-term preservation of this critical rice diversity. In 2017, PhilRice constructs the facility for genetic resources conservation and management of rice germplasm and acquire the needed equipment for its full operation (Figure 1). Construction of genetic resources building the facility will house upright freezers (-20°C) as storage facilities for *ex situ* germplasm conservation of rice genetic resources. Laboratories for seed health and quality testing, and molecular laboratory for DNA fingerprinting and storage of DNA bank of all rice germplasm collections will also include in the new facility. It is located in PhilRice CES, Maligaya, Munoz, Nueva Ecija. This facility in PhilRice CES was selected since it is the central repository or rice germplasm in the Philippines.

Figure 1. The newly constructed PhilRice Genebank facility.

2.3. Germplasm inventory
Managing genebanks involves repeatedly identifying the samples, or accessions, to track them and to update the information describing them after various processes like pathogen testing, cleaning, multiplication, characterization, evaluation, seed storage and inventory, and distribution. This process is both time-consuming and prone to human error if it is done using conventional hand registration with paper records [16]. To ensure that the conserved germplasm are same as the original collection, seed identity was 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, etc.) were also noted.

To minimize labelling and handling mistakes during seed production process, a computer-generated barcode labels (accession number, lot number and cropping season) for field tags and seed production process. Barcoded labels printed on polyester paper using thermal printer have been tested for readability and durability under field and screen house conditions for an expanded application [17]. Furthermore, color coded vacuum sealed foil was used for the precise inventory of seed stocks, making the more efficient inventory, conservation, distribution and regeneration of germplasm. Blue code for wild rice, red for germplasm with restricted access, yellow for base collections and white for active collections (Figure 2).

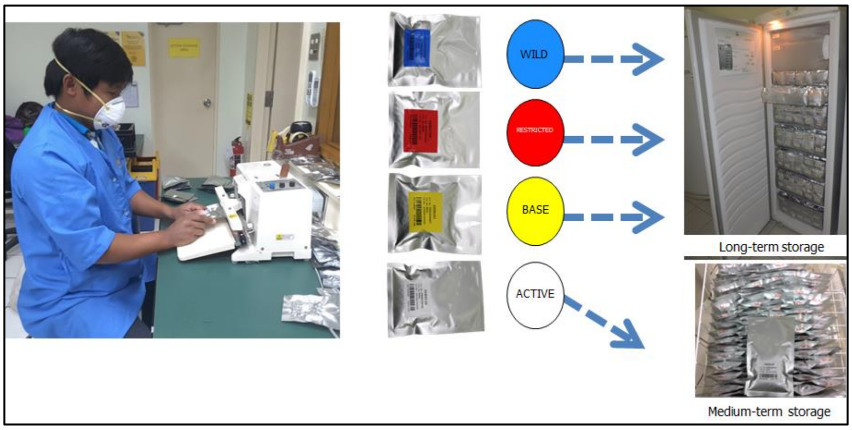

Figure 2. Color-coded classification of foil packets.

The e-Seedfile software was developed to provide virtual access to the reference collection for seed verification of the regenerated germplasm and valid type confirmation of the new and old germplasm collection. While panicle file was also initiated to provide a virtual reference to the accession.

2.4. Germplasm characterization
Assessment of agro-morphological diversity among rice germplasm is an essential endeavour in any genetic resources management and crop improvement. Discovery of desirable traits from traditional rice varieties (TRVs) and incorporating these traits in rice breeding efforts would greatly benefit rice farmers to mitigate the effect and better manage rice production in changing environmental conditions. This study provides information on the morphological characterization of TRVs used to establish each accession’s genetic identity, to identifying varieties with desirable traits for direct utilization and potential donors for crop improvement and assess the extent of genetic diversity of the collections.

Many software packages are available for assessing phenotypic diversity parameters that increased the efficiency of germplasm curators and, plant breeders to speed up the crop improvement [18]. Recently, paperless data collection using an android-based application called FieldLabV2.9 developed by IRRI is being implemented for immediate data validation and accurate data downloading from tablet PC to the central database of GRD. Efficient paperless data collection using android technology results in immediate data validation and accurate data downloading from tablets to workstations, making it an ideal tool in germplasm characterization [17].

2.5. Germplasm distribution and information management

The complex processes of managing rice germplasm collection at PhilRice Genebank are supported by its in-house documentation system called Germplasm Management System (GEMS). This serves as a central repository of integrated rice germplasm information that provides links to the different genebank operations, from registration, characterization, evaluation and seed inventory to seed distribution to end-users thus increase the efficiency in doing its decision-support that helps genebank managers.

There are also datasheets, record books and forms since it is not always possible to record/upload data directly into the system. These datasheets can be used for the chain of procedures performed by different genebank staff in their respective tasks. These are also correctly filed, bound and archived for future references and back-up files. Recently, GEMS was upgraded into advanced version 'GEMS v2.0' that involves redesigning of the system architecture and adding new functions in synchronization with the current documentation needs of the Genebank. All germplasm data uploaded in the database were also checked and validated.

One of the constraints in the utilization of germplasm by breeders, researchers and farmers is the access. At present, the development of its web-based version is being done for broader dissemination of information to breeders, researchers and other stakeholders.

2.6. Germplasm exchange

Genebank collections around the world hold the raw genetic materials needed to breed new and better plant type to feed the growing population. Many holders of these plant genetic resources have their documentation and management system. However, there is no standardized or shared method for assigning unique identifiers relative to the accessions. The digital object identifiers (DOIs) has been implemented as an agreed method for the assignation of global identifiers to standardize the method of providing accessions' permanent unique identifiers. The use of DOI allows the materials to be tracked as these genetic resources are being shared, duplicated, and used among institutions. Through DOI, the impact of genebank collections in utilization in research and breeding programs are monitored, including its conformance to the legal obligations stated in the SMTA. Recently, a multicountry construction of a test platform for the development and allocation of unique identifiers of rice germplasm was implemented in Asia. This initiative was organized by the Indonesian Center for Agricultural Biotechnology and Genetic Resources Research and Development (ICABIOGRAD) in collaboration with the secretariat of the International Treaty on Plant Genetic Resources for Food and Agriculture (ITPGRFA) and the International Rice Research Institute (IRRI), in participation of genebank curators from Bhutan, India, Indonesia, Malaysia, Philippines and Zambia.

2.7. PhilRice germplasms with DOI

There are variations in characters observed in 101 registered cultivars (Table 2). Maturity days ranged from 99 to 193 DAS, where in Taal 2 was found to be early maturing while Panesopek was late maturing. Plant height had an average of 151.50 cm. The 100 seed weight ranged from 1.3 g to 3.3 g, where in Iniba-bain and Gundang were observed to have the heaviest grain weight (3.3 g). The rice collections that exhibited the longest panicle (>35 cm) is Bandera.

In terms of qualitative traits, some of the traditional Philippine varieties has glabrous leaves (Table 3). Glabrous leaves rice was usually fed to ruminants. For the DOI registered germplasm, Sinatyar and Taal 2 have glabrous leaves. On the other hand, extreme lodging resistance were observed on Ligantong, Bimmotiti and Kintuman (Calipago).

Table 2. Descriptive statistics of the 101 registered cultivars based on 12 quantitative traits.

Descriptor	Mean		SE	Sd	Min	Max
Maturity	141.24	±	2.0	20.6	99	193
Grain width	2.63	±	0.1	0.5	2.00	4.00
Grain length	8.13	±	0.1	1.0	5.00	10.00
Culm diameter	4.86	±	0.1	0.6	4.00	6.00
Culm length	128.80	±	3.4	23.6	67.00	162.00
Culm number	14	±	0.4	2.6	10	20
Grain weight	2.45	±	0.1	0.4	1.3	3.3
Leaf blade length	63.08	±	2.0	13.8	37	99
Leaf blade width	1.44	±	0.0	0.3	0.8	2
Ligule length	20	±	0.6	4.4	10	30
Panicle length	25.28	±	0.4	3.2	18	35
Plant height	151.50	±	4.4	30.8	88	187

Table 3. Varieties with desirable qualitative attributes.

Descriptor	Descriptor state	Name of cultivar
Leaf pubescence	Glabrous	Sinatyar, Taal 2
Culm lodging resistance	Very strong	Ligantong, Bimmotiti, Kintuman (Calipago)

Some of the registered varieties are resistant to a specific disease. Reactions to the blast were resistant in Hinamog, Nagpili, Bandera, Casungsong, Caboyo, Sologanon, Binolawan, Paliod, Korasisi and Kalisokasaya. Meanwhile, Doctor, Guinabioka and Kintuman (Calipago) showed resistance to bacterial leaf blight. On the otherhand, Kalagnon was resistant to sheath blight. There are several available germplasm accessions that could offer resistance against major rice diseases, which could be considered as option for selection of parent materials for rice breeders to produce rice lines/varieties that would perform well against problematic rice diseases. It is highly recommended to continue exploring the potential of available rice lines/varieties against major rice diseases that would further expand the option for breeders in selecting parent materials and also to monitor the stability of resistance of the earlier tested accessions.

3. Concluding remarks and future perspective

There are significant constraints in the genebank operations that undeniably affect its capacity to manage rice germplasm. The present storage conditions in PhilRice are far from ideal. During germplasm storage, unstable power supply and cold room malfunction adversely affect the quality and viability of stored seeds. Desired temperature and corresponding relative humidity levels are unattained regarding international genebank standards. Continual upgrading and expansion of laboratory and storage facilities will be pursued to enhance the capacity of the genebank to store high-quality seeds for long period. To maximize storage life, maintain genetic integrity and ensure availability of high-quality seed and information of the collection, PhilRice Genebank continuously upgrading its facilities and optimizing its protocols.

An essential aspect of genebank management is to secure duplicates of germplasm for safety back-up to mitigate the risk of its partial or total loss caused by natural or human-made catastrophes. Although a portion of PhilRice Genebank accession were duplicated in IRRI Genebank, there is a need to send (if not all), at least the core collections to ensure materials are safely duplicated in other locations so that if a collection suffers a loss, germplasm can be restored from duplicate sets.

Lots of things are yet to be done, increasing inter-disciplinary approaches to facilitate fast, reliable and accurate conservation and management. PhilRice Genebank is also on the move to explore increased collaboration with various institutions in the field of bioinformatics, DNA/RNA sequencing, and protein profiling. Innovations in technology are expected to expand the toolbox for the management and conservation of the collection that will help enhance the long-term preservation of biodiversity and the easy access of germplasm and germplasm-related information. These advancements and innovations will contribute in the generation of a complete and detailed picture of global rice diversity in the future, thus, definitely play a vital role in the conservation, management and utilization of rice genetic resources.

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