Development and utilization of the PhilRice Genebank

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Germplasm materials are the building blocks for the construction of improved rice varieties. However, as farmers turn to few modern varieties, extant germplasm undergoes a narrowing of diversity. There is therefore a great need to conserve the rice biodiversity found in nature and also those developed through plant breeding. *Ex-situ* conservation in genebanks provides a safe storage system for the germplasm. It is also important to have a systematic and sufficient characterization of these materials. In selecting plants for breeding, information on their morpho-agronomic traits, grain quality, and reaction to pests and diseases are primary considerations. As more information is available about the germplasm, the wider selection and diversity of materials can be utilized for varietal improvement. In PhilRice Genebank, rice germplasm are collected, conserved, and evaluated to support plant breeding programs. Emphasis is on conserving Philippine traditional varieties to safeguard this natural heritage. The Genebank holds around 11,000 collections of landrace varieties, foreign introductions, hybrid parental lines, and wild rices that can be used in developing new varieties for famers or directly utilized by other clienteles. From its establishment, the Genebank has undertaken many steps to improve rice genebanking procedures and provide requestors high quality seeds with optimum viability.

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INTRODUCTION

The Philippines is an archipelago characterized by a wide variation in climate, eco-geography and farming systems, which sustain and promote an extensive diversity of rice. Traditional rice varieties abound representing a rich genetic resource. In support of breeding, the rice germplasm materials have been collected from around the country, conserved and evaluated in the Philippine Rice Research Institute (PhilRice), and exchanged

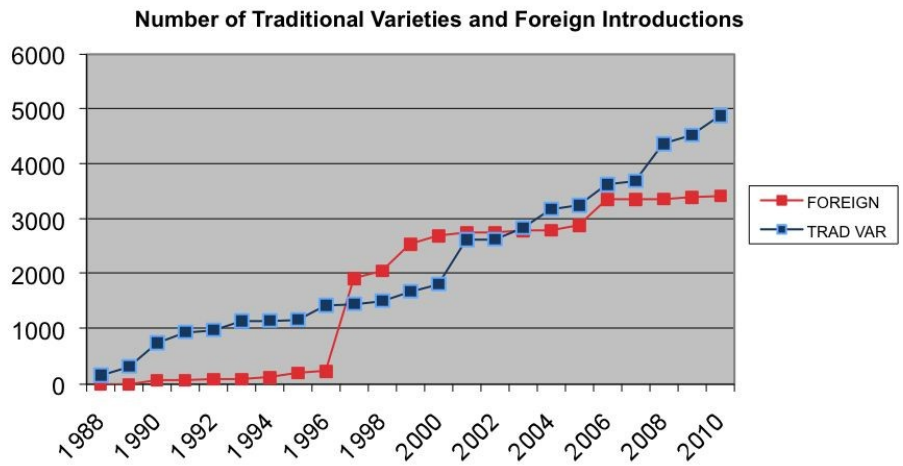

Figure 1. Yearly accumulation of Philippine traditional varieties and foreign introductions in the PhilRice GeneBank (1988-2010).

to local and foreign users.

The safekeeping of Philippine rice germplasm started in the 1900s through the independent efforts of rice breeders affiliated with the Bureau of Plant Industry and the College of Agriculture of the University of the Philippines. The separate collections were consolidated and deposited at the International Rice Research Institute (IRRI) in the early 1960s. Upon the establishment of PhilRice in 1985, the Philippine collection in IRRI was duplicated in the new PhilRice Genebank, and more materials were added over the years through partnerships and collecting activities. Purposeful collecting in recent years was prompted by serious threats to rice diversity in the country, such as the spread of modern rice varieties and rapid loss of natural habitat due to urbanization. With its vast network around the country, PhilRice is also envisioned to serve as a conduit for the local utilization of the rice germplasm. This paper provides an account of the growth, conservation, characterization and use of the PhilRice Genebank collection, and discusses strategies taken by PhilRice to ensure beneficial exchange in the light of Convention on Biological Diversity (CBD) and Trade-Related Aspects of Intellectual Property Rights (TRIPS)-instigated regulatory regimes.

CENTER OF ORIGIN AND DIVERSITY

The Philippines lies in one of the eight centers of crop origin and diversity recognized by the great Russian conservationist Nikolai Vavilov (1951). Rice is believed to have originated in China and Southeast Asia where tremendous diversity emerged especially in the tropical countries (Harlan 1971). Hamilton (2006) of IRRI estimates the number of total rice varieties worldwide at 500,000, one-fifth of which is now conserved at IRRI. There could be around 10,000-15,000 rice varieties in the Philippines, 4,889 of which are now represented in the PhilRice Genebank. The high level of rice diversity is deduced from the wide geographical and climatic variation in the country.

GERMPLASM COLLECTION

The PhilRice Genebank's initial collection comprised about 300 traditional rice varieties repatriated from IRRI in 1985. These materials can be traced to the original germplasm used in the early 1900s by Filipino plant breeders in the Bureau of Plant Industry and the University of the Philippines College of Agriculture for breeding, which were deposited in IRRI's Genebank in 1962. The PhilRice collection tripled in size when 604 materials from the Cordilleras were added through the efforts of Professor Harold Conklin of Yale University as part of his anthropological research among the Cordillera tribes in the Ifugao Province in the early 1990s. Additional 88 native varieties from the cool elevated areas were also collected from 2002 to 2004 mainly from Banaue and Hungduan, Ifugao by a team of PhilRice staff who were involved in the rice-based farming system project in the highlands. Most of the varieties from the highlands are grown at 1,500 to 2000 meters above sea level (masl).

PhilRice, in partnership with the Swiss Development Cooperation and IRRI, initiated formal collecting to safeguard rice diversity in the Philippines from 1995-1997. This collaboration yielded 458 traditional rice varieties and 8 samples of wild species that are now conserved at PhilRice and IRRI. However, due to limited funds, many germplasm collecting trips that followed were conducted as ride-on activities with other research projects of PhilRice staff or collaborators. One of the active collaborators of PhilRice is the Plant Genetic Resources Conservation Unit of the University of the Philippines at Los Baños, which shared 74 materials with the PhilRice Genebank. From 1988 to 2010, the PhilRice collection grew on average by 212 traditional varieties every year, coming from 59 of the 80 provinces in the country (Figure 1). In 2006, a farmer in Aurora, who grows and sells traditional varieties, handed 31 traditional varieties, 3 modern, and one wild species to the PhilRice collecting mission team (Figures 2a and 2b).

The Genebank collection further expanded through collecting efforts forged with members of the National Rice Seed Network (SeedNet) and community seed banks (CSB) like the one in Mindanao (Figure 2c). SeedNet and CSB are networks of accredited seed growers and farmers, respectively, who are trained in quality seed production. SeedNet members particularly from Mindanao focus on producing traditional or upland rice varieties suitable to their areas. A total of 85 germplasm materials were obtained from Muslims in Cotabato and Lanao del Sur in 2008. CSBs deposited 30 varieties at

Figure 2. A. Collecting mission in Aurora; B. Sample of traditional variety from the province; C. Collecting mission in Lanao del Sur.

PhilRice for long-term storage.

In Northern Philippines, 67 varieties were obtained in 2010, of which 32 were from the Benguet State University collection. Farther north, 43 varieties were collected from the Batanes group of islands at 0-5 masl.

Opportunistic collecting by PhilRice staff, while on travel around the country for other purposes such as conducting pest and disease monitoring, technology promotion, and other activities, added hundreds of materials to the Genebank. Other provincial agricultural colleges and development centers have also contributed to the growth of the collection through the efforts of graduate students and as generous gifts of seeds from their existing collections.

Foreign materials were incorporated in the local rice gene pool as early as the 1910 to 1930 when local breeders acquired varieties from various countries, namely Australia, Burma, China, Egypt, India, Indonesia, Malaysia, Japan, Persia, Spain, Thailand, and the US. Scores of new varieties were developed from crosses with Thai, Vietnamese, Malaysian, and Indonesian varieties. In 1997, PhilRice received more than 2,000 hybrid parental lines from China under partnerships with several agricultural universities involved in hybrid rice R&D. The Memorandum of Agreement with China provided for joint ownership of commercial hybrids that will be derived from the Chinese lines. The total foreign materials in the collection are now 3,394 (Figure 1).

The PhilRice Genebank now holds 5402 (45.6%) traditional varieties, 922 (7.8%) breeding/research materials, 46 (0.4%) irradiated mutants, 3064 (25.9%) hybrid parental lines, 78 (0.7%) interspecific crosses, 20 (0.2%) anther culture derived, 59 (0.5%) wild rices, 666 (5.6%) advanced/improved cultivars, and 1592 (13.4%) unknown varieties for a total of 11,849 entries (Figure 3). Verified unique are 6,405 accessions.

GERMPLASM CONSERVATION

All incoming seeds are registered in the Genebank with important passport data such as variety name, place or country of origin, collection site, ecosystem, and special trait, if any. The seeds are cleaned manually, a few are set aside for seed file or panicle file if available, and tested for germination. Seeds with high viability and adequate amount are packed for cold storage; otherwise, seed increase is undertaken.

Initial characterization in terms of adaptability and resistance to pest and diseases under normal conditions is accomplished in the observational nursery, which also serves as seed increase for weak and few incoming seeds. Foreign materials are planted in the screenhouse nursery to prevent spread of seedborne diseases, if any. The harvest is verified against the seed and panicle files, cleaned, and slow-dried under silica gel. Seeds with 7-8% moisture content are vacuum-packed in aluminum foil for medium-term storage in the cold room with a temperature of 5-10°C and relative humidity of 40%. The facility could preserve the viability of seeds for 25 to 40 years. In 2005, PhilRice acquired ten freezers to extend the seed life up to 100 years. Each freezer can accommodate 1,000 accessions. Seeds are ultra-dried to 5-6% moisture for long-term storage in the freezers (Figure 4).

Regular germination tests are carried out for each accession every four years to check their viability. Seeds falling under 85% germination are rejuvenated to replace the seed stock with highly viable seeds.

CHARACTERIZATION, EVALUATION, AND DOCUMENTATION

An extensive field and laboratory characterization of the germplasm materials is undertaken on 58 agro-morphological parameters, which include leaf, stem, panicle, and seed structures. In the evaluation phase, the materials are scored for reaction to 7 bacterial and 17 viral diseases, 11 insects, and 9 abiotic stresses. Grain quality is also assessed by measuring 27 parameters, including shape and actual eating properties.

An in-house developed computerized database system is used to manage the large volume of data on the inventory and trait information of the collection. In the system, quick search buttons are provided for easy access to data on seeds with popular traits such as aroma and resistance (Figure 5).

UTILIZATION OF RICE GERM-PLASM

Germplasm materials provide the building blocks of new varieties. The breeders are therefore notified about accessions that have excellent traits useful in the breeding program. Selected local varieties and foreign materials from both PhilRice and IRRI Genebanks are crossed, followed by selection for outstanding offspring. Yield tests are usually conducted in 36 sites around the country and consistent high yielders with good eating quality and pest and disease resistance are released as new varieties.

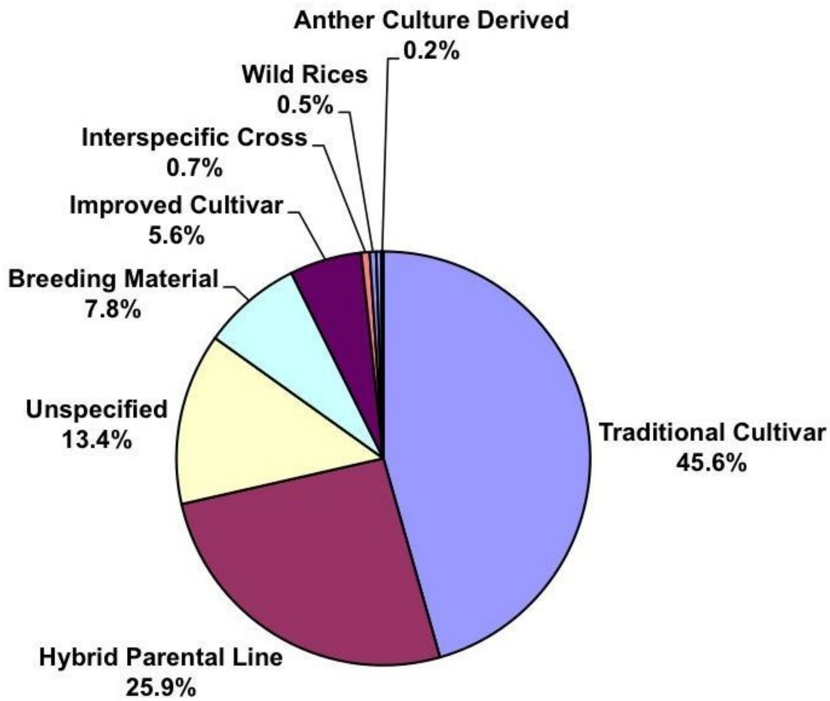

Figure 3. Proportion of the types of materials deposited in the Genebank.

About 340 accessions are requested from the Genebank each year. A major request in 2006 was made for the genetic analysis of tungro resistance by association mapping in diverse germplasm materials. About 2,000 varieties were issued for DNA fingerprinting in 2007. The largest batch comprised 649 traditional varieties distributed in 2008 for genetic studies by PhilRice researchers. Starting in 1996, PhilRice has issued a Materials Transfer Agreement (MTA) for seed distribution. For distribution overseas, the seeds are also accompanied by a phytosanitary clearance.

A great majority of users are PhilRice personnel (75%), followed by state colleges and universities, and private institutions (Figure 6). A few countries obtained materials from PhilRice Genebank and, altogether, 33 traditional and modern varieties were provided to Australia, Brazil, China, Cuba, and Suriname. The seeds requested by clients were intended mostly for breeding and genetics studies (52%), and to a less extent for demonstration and photo documentation purposes, thesis/student research, physiological studies, and stress tolerance screening (Figure 7).

COLLECTING AND EXCHANGE REGULATIONS

Being a developing country with one of the highest levels of biodiversity, the Philippines immediately acceded to the CBD in 1992, which enshrined national ownership of biodiversity. While collecting by local and foreign entities before CBD was unrestricted, three laws were enacted as a result of CBD to regulate collecting in the country. Executive Order 247 on “Prescribing Guidelines and Establishing a Regulatory Framework for the Prospecting of Biological and Genetic Resources, their By-products and Derivatives, for Scientific and Commercial Purposes" issued in 1995 laid down several requirements for bioprospecting: 1) a permit from five government agencies – Department of Environment and Natural Resources, Department of Agriculture, Department of Science and Technology, Department of Health, and Department of Foreign Affairs; 2) prior informed consent (PIC) from local communities; 3) an agreement for both commercial and academic research applications; and 4) a 60-day wait period. In 1998, the Indigenous Peoples Rights Act took effect allowing indigenous communities to enforce their own customary laws regarding collecting, which may either reinforce or totally disregard EO 247. The overbearing conditions, however, effectively stifled collecting. Only one of 13 commercial and one of 20 academic research collecting applications were approved.

As a remedy, the Wildlife Act was enacted in 2001, with the implementing rules and regulations embodied in the Joint Departments of Environment and Natural Resources (DENR) and Agriculture (DA), Palawan Council for Sustainable Development (PCSD), and the National Commission on Indigenous People (NCIP) Administrative Order No. 1 finalized

Figure 4. Conservation of rice germplam. A. Seed cleaning of newly harvested germplasm materials for storage; B. Seed viability testing using the standard germination method; C. Long-term storage of foiled accessions in freezers; D. Medium-term storage of rice germplasm in the cold room.

in 2005, centralizing the granting of permit to the Department of Environment and Natural Resources and removing the 60-day wait. In addition, the Act required foreign academic collectors to have local partners, declaration of the Philippines as country of origin, disclosure of collecting permit in case of patent applications for the collected sample and/or derivatives, and payment of fees for commercial bioprospecting. Despite these regulations, the rice germplasm collection of PhilRice continued to grow from opportunistic collecting and through the endeavors of graduate students under gratuitous permits from municipal and village agricultural officers. New collection sites, fortunately, were not under the control of indigenous peoples.

The agreement on TRIPS established on January 1, 1995 addresses issues related to intellectual property protection of plant varieties, requiring protection either by patents or by a *sui generis* effective system or by a combination of both. While the CBD doused off international sharing of biological species, TRIPS placed restrictions on the access to protected plant varieties. PhilRice also became more cautious with the distribution of its germplasm materials and started requiring a Materials Transfer Agreement (MTA) since 1996. The MTA provides that seeds for research are unrestricted but user cannot claim any proprietary rights. Moreover, the commercial production of traditional varieties will need an agreement with PhilRice. To reverse the unintended consequence of CBD and TRIPS, and instead promote international sharing, the International Treaty on Plant Genetic Resources on Food and Agriculture (ITPGRFA) was ratified in 2001. The ITPGRFA advocated for unrestricted access to biodiversity of crops, called for financial resources and technical assistance for less developed countries, and promoted farmers rights over traditional varieties.

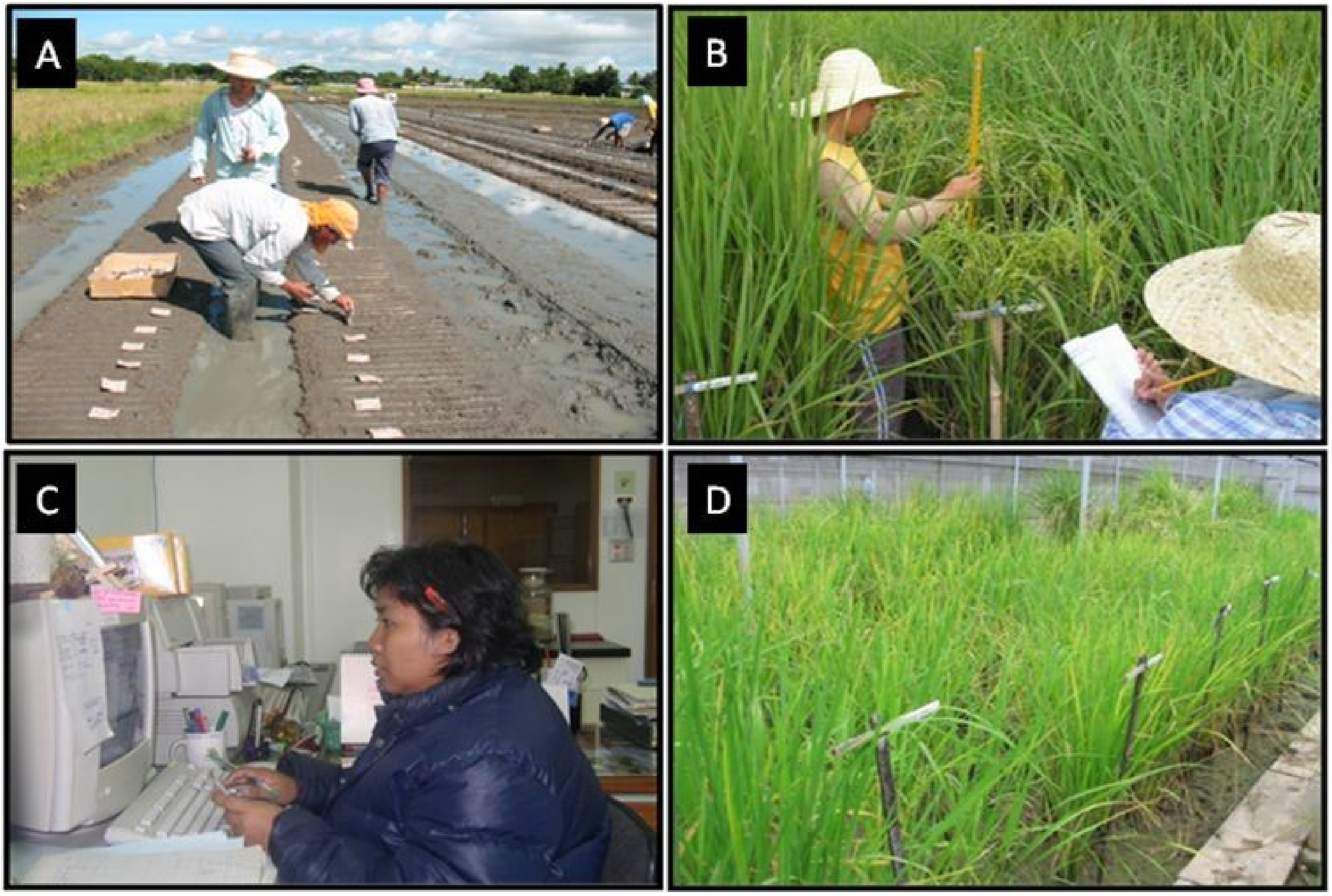

Figure 5. Characterization, evaluation, and documentation of rice germplasm. A. Sowing of accessions with low viability and seed stock in the field; B. Characterization of agro-morphological traits using standard descriptors for rice; C. Documentation of acquired data using the Germplasm Management System (GEMS) Database; D. Seed increase and rejuvenation of accessions in the screenhouse.

The Philippine Plant Variety Protection Act took effect in 2002, providing breeders' rights as well as farmers' rights over modern varieties. Farmers have unrestricted breeding use of modern varieties and can engage in small-scale exchanges with fellow farmers. They can also sell their farm produce of a protected variety, except when the sale is for reproduction under a commercial marketing agreement. PhilRice adopted a new MTA starting 2004, which underscores its ownership of the materials, and sets a 25% distinction threshold for derived products to be allowed proprietary rights for developers.

The Standard MTA (SMTA) under the ITPGRFA was finalized in June 2006, which, in exchange for unrestricted access to germplasm, requires a royalty of 0.5 to1.1% of sales of derived product to be paid to the Multilateral System fund, to be used for financial and technical assistance to the country of origin. However, in August 2006, PhilRice issued Administrative Order 2006-10, confining the distribution of traditional varieties within the country, and requiring a licensing agreement for use of breeding lines.

On September 28, 2006, the Philippines ratified the ITPGR effectively then subscribing to the use of the SMTA. To facilitate the access and exchange of germplasm materials within PhilRice and with other institutions, PhilRice has created and institutionalized the guidelines for the acquisition, exchange, and distribution of germplasm materials. This is to further enhance the utilization of germplasm materials within and with other institutions for varietal development and other related research. These guidelines enhance the documentation of germplasm exchange through the use of the SMTA in compliance with the ITPGRFA. For strict implementation towards organized exchange of germplasm materials from Genebank and other PhilRice divisions, separate guidelines for breeders' and researchers' materials and those conserved by the Genebank were established.

Donation of materials by PhilRice breeders/researchers to farmers, local researchers, collaborators, and research institutes is covered by SMTA duly signed by the PhilRice

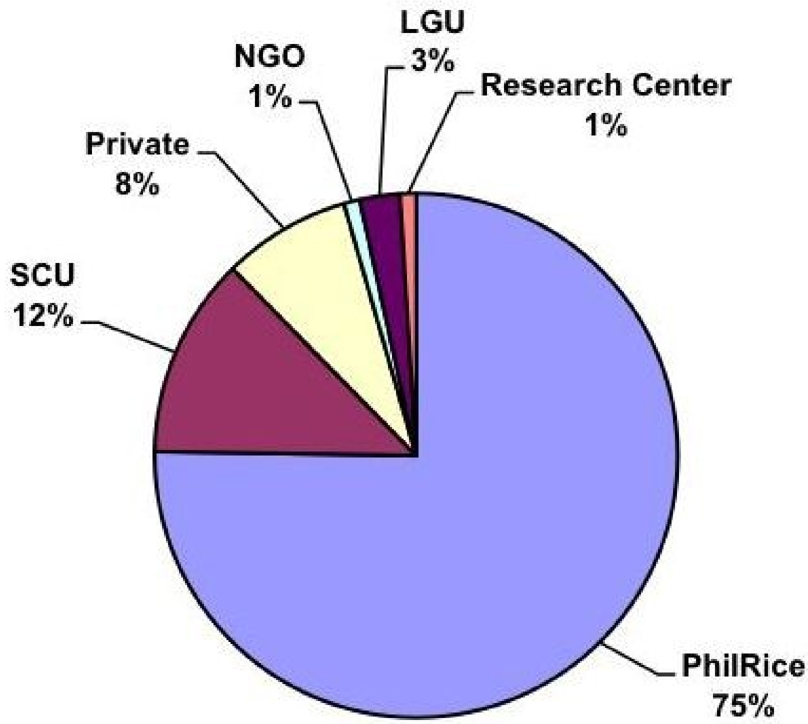

Figure 6. Breakdown of users of PhilRice Genebank materials (1994-2010).

breeders/researchers and the division head of Plant Breeding and Biotechnology Division (PBBD), and the donation of materials by PhilRice breeders/researchers to international researchers, collaborators, and research institutes is covered by SMTA duly signed by the PhilRice Executive Director and the division head of PBBD. Requests for materials by PhilRice breeders/researchers from International Rice Research Institute-Genetic Resources Center (IRRI-GRC), as provider of the materials, are covered by IRRI's SMTA. IRRI-GRC prefers the shrink-wrap type of agreement, which does not require signing, but only a declaration of acceptance through email. Declaration is done by the head of the Germplasm and Seed Health Division (GSHD) as an authorized representative of PhilRice.

Requests for germplasm materials from PhilRice Genebank by farmers and PhilRice researchers are covered by a request form provided by the Genebank staff duly signed by the Genebank manager or Genebank staff. The requisitioner signs the declaration of acceptance included in the request form, which states the rights and obligations of the recipient. PhilRice breeders who would like to donate their advance breeding lines to PhilRice Genebank indicate the terms and conditions for the distribution (e.g., if the breeding lines should only be for PhilRice researchers and not for distribution to other institutes/researchers both local and abroad). Basic passport and agro-morphological characterization data must also accompany the materials. Requests for materials by other local researchers, collaborators, and institutes are covered by SMTA duly signed by the head of GSHD or Genebank manager, while requests for materials by international researchers, collaborators, and research institutes are covered by SMTA duly signed by the Executive Director and the head of GHSD.

MOLECULAR CHARACTERIZATION

In anticipation of proprietary claims and issues over the germplasm materials and derivatives, PhilRice has established a DNA fingerprinting system for varietal identification (Romero et al. 2002). A modified random amplified polymorphic DNA (RAPD) method using three SRILs Uniprimers has successfully distinguished 77 modern varieties. The fingerprinting is also useful in diversity analysis to ensure diverse germplasm in breeding programs, in association mapping of desirable traits for possible application in marker-aided selection, and in the removal of duplicate samples from the collection.

PRESENT PROBLEMS AND FUTURE PLANS

There are major constraints in the Genebank operations that undeniably affect its capacity to collect, manage, and distribute rice germplasm. Lack of trained manpower in Genebank-related disciplines and operations is a main factor that influences the overall quality of the genetic resource conservation work. At the present time, some personnel have minimal knowledge on genebanking and have not taken courses on plant genetic resources conservation.

Collecting missions are vulnerable to serious risks such as exposure to crime-related events, natural disasters, road hazards, and poor travel conditions. Certain operations in the Genebank subject personnel to extreme temperature changes during storage processes (stocking, retrieval, and inventory of samples in the cold chambers).

The present storage conditions in PhilRice are far from ideal. During germplasm storage, unstable power supply and cold room malfunction adversely affect quality and viability of stored seeds. Desired temperature and corresponding relative humidity levels are unattained with reference to international genebank standards (FAO/IPGRI, 1994), storage rooms are not properly lighted, and seeds are exposed to contaminating organisms and pests. General deterioration of facilities and equipment is observed and critical supplies are unavailable in the local market. Moreover, during seed registration, some materials with seed-borne pests and pathogens are occasionally admitted, putting the other stored materials at risk. To ensure efficient use of storage space and other resources, there is a need for rapid techniques in the identification and removal of redundancies in the collection.

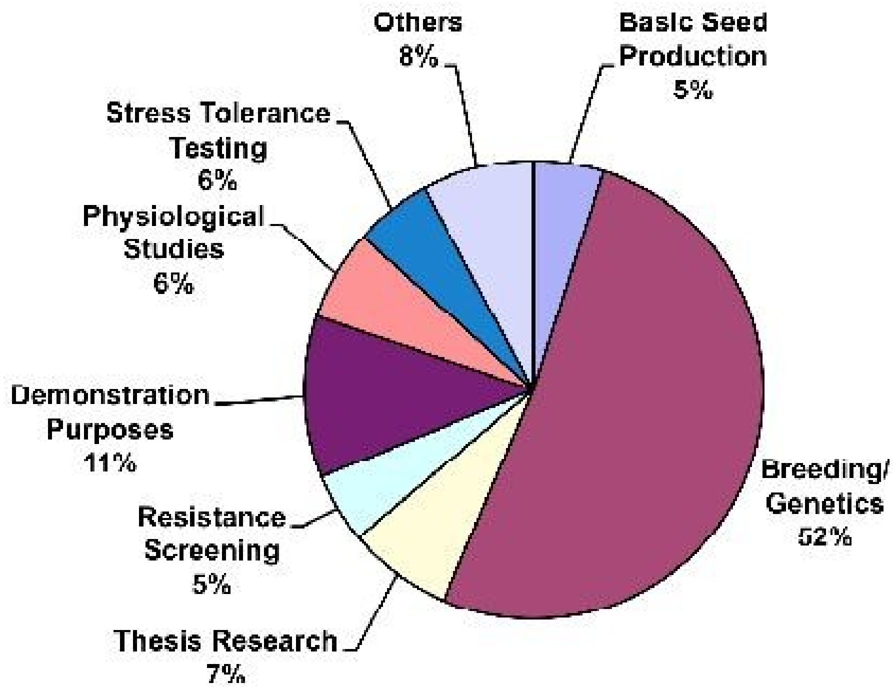

Figure 7. Breakdown of purposes for which PhilRice Genebank materials were used (1994-2010).

Several activities will be pursued to further build the capacity for rice collecting, management, and exchange. Genebank staff will be sent for regular training in plant genetic resources conservation as well as in related fields including seed physiology, genetics, taxonomy or crop/species identification, plant and seed pathology, engineering and maintenance, database management, and international regulations on germplasm exchange. Personnel will also be trained on drill operations and safety procedures to minimize the risk to stored germplasm, particularly the base collection. To broaden the collection, collecting expeditions will be sustained with priorities on underrepresented provinces and tribal areas. Schedule and itinerary of collection trips will be properly coordinated with Local Government Units (LGU) for optimal safety and results. A local well-versed collector who knows the local customs and dialect will be hired for the trips.

Continual upgrading and expansion of field, laboratory and storage facilities will be pursued to enhance the capacity of the Genebank to store pure and high quality seeds in the right amount. Protective equipment for personnel will be provided to avoid exposure to extreme temperature changes when entering the storage facility. Additionally, an alarm system for open doors, a lightning conductor rod, and a high temperature cut out for the cooling system will be installed as safety equipment. Hygro-thermographs that are connected to back-up power supply will be placed and storage rooms will be provided with multiple compressors and dehumidifiers that are programmed for alternate operation. An alternate refrigeration system will be installed and accessions will be multiplied, carefully processed, and sent to IRRI for back-up. Equipment or software for the establishment of a bar-coding system for genebank management will be purchased to further improve operations. A web interface for access to germplasm trait information by interested users and for seed request will also be constructed. The online database will contain useful germplasm data usually sought by plant breeders and other clienteles. Finally, the Genebank operations manual will be regularly revised to reflect continual modifications and improvements in the Genebank operations.

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CONFLICT OF INTEREST

None

CONTRIBUTION OF INDIVIDUAL AUTHORS

Gabriel Romero - Abstract, Introduction, Germplasm Collection, Collecting and Exchange Regulations, Molecular characterization, Problems and Future Plans

Evelyn Gergon - Introduction, Germplasm Collection, Collecting and Exchange Regulations

Ma. Cristina Newingham - Data Documentation and Germplasm Utilization

Celia Diaz - Characterization of Rice Germplasm

Marilyn Ferrer - Conservation of Rice Germplasm

Eric Nazareno - Abstract, Problems and Future Plans, Figures, Manuscript editing

REFERENCES

FAO/IPGRI. Genebank standards. Food and Agriculture Organization of the United Nations, International Plant Genetic Resources Institute, 1994:2-7.

Hamilton RS. Grain of truth: How many varieties are there? Rice Today 2006; 5:50.

Harlan JR. Agricultural origins: centers and non-centers. Science 1971; 174:468-474.

Romero GO, Anglacer AP, Falla MC. Polymerase chain reaction-based techniques for DNA fingerprinting of Philippine modern rice varieties. Philippine Agricul. Scientist 2002; 85:299-306.

Vavilov NI. The origin, variation, immunity and breeding of cultivated plants. Chronica Botanica. Waltham, Mass. 1951.