

Conservation of rice genetic resources: the role of the International Rice Genebank at IRRI

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Abstract

Rice genetic resources, comprising landrace varieties, modern and obsolete varieties, genetic stocks, breeding lines, and the wild rices, are the basis of world food security. The International Rice Genebank at the International Rice Research Institute in the Philippines conserves the largest and most diverse collection of rice germplasm. The facilities of the genebank ensure the long-term preservation of this important diversity. In field research, factors that affect long-term viability of rice seeds have been identified, leading to the introduction of modified practices for germplasm multiplication and regeneration. 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. The application of molecular markers is changing perspectives on germplasm management. International policies affecting access to and use of rice germplasm are discussed.

Introduction

Rice is the world's most important staple food crop, and the rice genetic resources stored in the International Rice Genebank (IRG) at the International Rice Research Institute (IRRI), Los Baños, Philippines, represent the largest and most diverse collection of rice in any genebank [2, 9]. In fact the collection comprises about 20% of all rice germplasm samples conserved worldwide, donated from more than 110 countries. The collection of more than 80 000 registered samples is made up of landrace varieties nurtured by farmers for generations, modern and obsolete rice varieties, some breeding lines and special genetic stocks, the 21 wild species in the genus *Oryza* [28, 30], and related genera in the tribe Oryzeae (Table 1). In the future, transgenic rices that even incorporate alien DNA should be considered part of the rice gene pool, but they are not yet part of the IRG collection.

Most samples in the collection are landrace varieties of *O. sativa*. Ecological differentiation involving cycles of hybridization, differentiation, and selection was enhanced when ancestral forms of *O. sativa* were carried by farmers and traders to higher latitudes,

higher elevations, dryland sites, seasonal deep-water areas, and tidal swamps [3]. Two major eco-geographic races differentiated as a result of isolation and selection; *indica* and *japonica* [21]. The differentiation also involved morphological and serological characters as well as intervarietal fertility [12]. Selections made to suit cultural and socioreligious traditions added diversity, especially in grain size, shape, and color, and endosperm properties. Today thousands of varieties are grown in more than 100 countries [17].

Genetic erosion and germplasm collection

Although there can be no doubt that high-yielding varieties are needed to meet the food demands of ever-increasing human populations, there is a price to pay in terms of loss of genetic diversity or genetic erosion. In many areas, high-yielding modern varieties were adopted by farmers and the cultivation of the landrace varieties declined. The wild species are threatened with extinction through changes in land use, extension of agriculture into marginal areas, and deforestation.

Table 1. *Oryza* species and other genera in the collection of the International Rice Genebank at IRRI.

Species complex	Species	Genome ¹	Accession (n)
<i>O. sativa</i> complex	<i>O. glaberrima</i>	AA	1 255
	<i>O. barthii</i>	AA	224
	<i>O. longistaminata</i>	AA	134
	<i>O. sativa</i>	AA	76 614
	<i>O. nivara</i>	AA	468
	<i>O. rufipogon</i>	AA	712
	<i>O. meridionalis</i>	AA	43
	<i>O. glumaepatula</i>	AA	37
<i>O. ridleyi</i> complex	<i>O. longiglumis</i>	4x	6
	<i>O. ridleyi</i>	4x	17
<i>O. meyeriana</i> complex	<i>O. granulata</i>	2x	22
	<i>O. meyeriana</i>	2x	8
<i>O. officinalis</i> complex	<i>O. officinalis</i>	CC	247
	<i>O. minuta</i>	BBCC	65
	<i>O. eichingeri</i> ²	CC	23
	<i>O. rhizomatis</i>	CC	19
	<i>O. punctata</i>	BB, BBCC	54
	<i>O. latifolia</i>	CCDD	37
	<i>O. alta</i>	CCDD	10
	<i>O. grandiglumis</i>	CCDD	10
<i>O. australiensis</i>	EE	25	
<i>Oryza</i> species not assigned to any complex	<i>O. schlechteri</i>	4x	1
	<i>O. brachyantha</i>	FF	17
Hybrids			587
Other genera in the Oryzaeae			
	<i>Chikusichloa aquatica</i>		1
	<i>Hygroriza aristata</i>		4
	<i>Leersia hexandra</i>		1
	<i>L. perrieri</i>		1
	<i>L. tisseranti</i>		3
	<i>Porteresia coarctata</i>		1
	<i>Rhynchoriza subulata</i>		1
Total number of registered accessions			80 647

¹ The basic chromosome number is 12.

² A diploid species, in which 4x cytotypes have been identified, for which the genomic constitution has yet to be determined.

The problems of genetic erosion are severe, but international efforts to conserve rice genetic resources, in which IRRI has taken a leading role, have led to the establishment of several gene banks in Asia. These

joint efforts between national, regional, and international organizations ensure the long-term preservation of the biodiversity of the rice gene pool. Much germplasm exploration for rice was completed by the early

1990s. Since 1994, IRRI has coordinated a project funded by the Swiss Agency for Development and Cooperation, involving 14 countries in Asia, Madagascar, several countries in eastern, central and southern Africa, and South America, which aims at completing the collection of cultivated and wild rices by the year 2000.

The collecting activities are closely linked to conservation and use, and in this respect, it is extremely fortuitous that rices are predominantly inbreeding. Outcrossing has been estimated at <2%, although the actual figure is not known and almost certainly depends on environmental conditions during flowering. Farmers throughout Asia usually maintain the identity of each rice variety, and enlisting their help to identify different varieties is an effective way of collecting germplasm. Using this method, more than 2000 samples of *O. sativa* were collected during the second half of 1995 from the southern provinces of the Lao People's Democratic Republic (PDR). It is estimated that about 60% of these samples are unique varieties. Maintaining the germplasm samples as purelines also facilitates their multiplication or regeneration, conservation in the genebank, and ultimately the use by rice research workers worldwide.

Conservation strategies for rice

For many plant species, *ex situ* conservation of seeds is safe and cost-efficient [11], provided proper attention is paid to seed drying and storage conditions. Fortunately, rice seeds exhibit orthodox storage behavior, and can be dried to a low moisture content of ca. 6%, and stored at -20°C , retaining their viability for decades, if not longer. Germplasm in the gene bank is readily available for use by breeders and other researchers. As a complement to static conservation in gene banks, there are several initiatives worldwide that are studying the on-farm or *in situ* conservation of crop varieties, including one at IRRI on rice [1].

The International Rice Genebank

The long-term preservation of rice genetic resources is the principal aim of the IRG. Formerly known as the International Rice Germplasm Center, the gene bank has operated since 1977, although genetic conservation activities started in the early 1960s, just after the Institute was founded. It meets all the approved or preferred

international genebank standards adopted in 1994 by the FAO Commission on Genetic Resources for Food and Agriculture [10].

For several countries, including Sri Lanka, Cambodia, Lao PDR, and the Philippines, the germplasm conserved in the IRG represents a more or less complete duplicate of their national collections. For other countries, such as India and the People's Republic of China, only part of their national collections are duplicated at IRRI. Nevertheless, the IRG has provided an important safety net for national conservation efforts. On several occasions, it has been possible to restore rice germplasm that had been lost in national gene banks with accessions already conserved at IRRI. Such germplasm restoration has had a significant impact on national conservation efforts [15, 16, 17].

The facilities of the IRG include:

- An Active Collection for medium-term storage and distribution of rice germplasm, maintained at $+2^{\circ}\text{C}$, 927 m^3 , capacity for ca. 110 000 samples, each ca. 500 g in sealed laminated aluminium foil packets.
- A Base Collection for long-term (50 → 100 years) conservation, at -20°C , 164 m^3 , capacity for about 108 000 samples, each with two vacuum-sealed aluminium cans, ca. 60 g each.
- Two screenhouses with a combined area of more than 4000 m^2 . One is used for the cultivation of accessions with low seed viability or few seeds. The other one is used exclusively for the cultivation of wild rices, in pots or special seed beds, according to Philippines quarantine regulations.
- A seed drying room at 15°C and 15% RH, where seeds equilibrate to ca. 6% moisture content. It has a capacity for about 9000 1-kg samples.
- A seed testing and germplasm characterization laboratory.
- A data management laboratory. In 1997, the International Rice Genebank Collection Information System (IRGCIS) will be connected to the System-wide Information Network for Genetic Resources (SINGER), on the World Wide Web.
- A conservation support laboratory for tissue culture of samples with low seed viability or few seeds, and for cytological and biosystematic studies of the collection.
- A molecular marker laboratory, for studies of isozymes, random-amplified polymorphic DNA (RAPD), and other markers.
- Access to more than 10 ha of field space on the IRRI Central Research Farm, with assured irriga-

tion facilities for the multiplication or regeneration and field characterization of conserved germplasm.

The germplasm collection is held 'in trust' by IRRI under the auspices of FAO in an International Network of *Ex-Situ* Collections. This is a complex legal concept, under which IRRI does not claim ownership of the collection, but has a responsibility to preserve the germplasm in a safe manner. Duplicate storage of the IRG collection is carried out at the National Seed Storage Laboratory (NSSL), Fort Collins, Colorado, USA, and about 75% of the collection is currently stored there under 'blackbox' conditions. Duplicate storage of African rices is shared between IRRI, the International Institute of Tropical Agriculture (IITA) in Nigeria, and the West Africa Rice Development Association (WARDA) in Côte d'Ivoire.

Conservation methods

Considerable attention has been paid to postharvest management standards of orthodox seeds [10]. What has received less attention are effects of the multiplication or regeneration conditions in the field on seed quality and potential longevity. In the IRG, all cultivated rice germplasm accessions are multiplied or regenerated for long-term conservation in the field on IRRI's Central Research Farm in Los Baños (14°13' N, 121°15' E) between the beginning of November and May. A significant number of accessions in the collection are temperate-adapted *japonica* varieties, or photoperiod-sensitive varieties.

In recent research, the environmental factors which affect seed quality, and therefore potential longevity in storage, of the different types of rice have been identified [18, 19, 20]. Following on from research carried out under controlled conditions at the University of Reading, UK [6, 7], the field research in Los Baños included more rice varieties and a range of environmental conditions. Changes in seed quality during the ripening stage were studied in 16 rice cultivars representing the two main types of *O. sativa*, *indica*, *japonica* (including *javanica* or tropical *japonica*), and one cultivar of *O. glaberrima* [18]. Also, changes in seed quality during development and maturation in three *japonica* cultivars and one *indica* cultivar planted on three different dates between October and early January were also studied [19].

This research has permitted the introduction of new germplasm regeneration procedures, and seed production during this period has several important advant-

ages. First of all, during the dry season in Los Baños, which lasts from December to May, there is a much lower incidence of pests and diseases. Fortunately, there are no known seed-borne viruses of rice, but diseases like rice tungro disease, rice blast, or leaf blight can seriously debilitate or even kill susceptible plants. Insect pests eat the leaves or cause other damage to plants; others transmit diseases. The end result is the same: a strongly reduced harvest from germplasm plots, and seed quality compromised. Second, solar radiation during the day is high, but water for irrigation is not limited since the IRRI farm has access to several artesian wells. During the dry season when plants are maturing, generally there are no tropical depressions or typhoons, which not only lead to protracted periods of heavy rainfall, but also considerable damage to plants in the field. Third, and perhaps most important, are the effects on flowering, grain filling, and seed quality. Photoperiod-sensitive accessions planted at the beginning of November receive a short-day stimulus at the end of December to initiate flowering. Furthermore, flowering and grain filling occur when the nights are coolest in December and January, which has a positive effect on seed quality [6, 7, 18, 19, 20]. The end result of these changes to seed regeneration practices has been an increase in initial seed viability. Even varieties such as *japonica* rices which are difficult to cultivate under Los Baños conditions respond well, and seeds with an initial viability around 100%, as high as the *indica* rices, can be harvested. Postharvest selection of healthy seeds, careful and slow drying, and pre-storage seed health testing all contribute to the enhanced quality of rice seeds in the IRG.

The value of conserved germplasm

The rice varieties nurtured by farmers for generations have an inherent genetic value because of their adaptation to different farming conditions and resistance to pests and diseases. Knowledge of these traits, their genetic and molecular control, and stability under different conditions enhances the value of conserved germplasm.

Over three decades, the germplasm collection at IRRI has been systematically characterized for a range of morphological and agronomic traits that facilitate conservation, as well as selection of suitable phenotypes by breeders (Table 2). Thousands of individual rice accessions have been evaluated for their resistance to or tolerance for a wide range of pests, diseases,

and abiotic stresses, such as brown planthopper, rice blast and bacterial leaf blight, and adaptation to cold temperatures or saline soils [17].

The use of landraces and wild species in rice breeding has had an enormous impact on rice productivity in many countries. For example, one accession of the wild species *O. nivara* (IRGC 101508) was used to introduce resistance to grassy stunt virus into cultivated rice, which led to the release of IR36. This variety also had 15 landrace varieties in its pedigree [24], and at one time was planted on more than 11 million ha, making it the world's most widely cultivated cereal crop variety [27]. Now, hybrids between *O. sativa* and many wild species have been achieved through the use of various biotechnological tools [22].

The economic value of the rice germplasm collection for rice improvement has also been assessed [8]. It is clear that over the past 15 years there has been a significant increase of the use of landraces in rice breeding. Nevertheless, relative to the large number of rice accessions conserved at IRRI and in other gene banks, the use of conserved germplasm for breeding is really rather limited. What has had real significance are the contributions to rice science through the many studies of landrace varieties and wild species concerning their reaction to pests and diseases, the nature of biochemical pathways, and molecular basis of resistance, for instance, which guide more strategically the utilization of germplasm accessions in rice breeding.

Molecular approaches to study rice genetic resources

In the IRG, a range of biochemical and molecular approaches is used to study the diversity of conserved germplasm. Classification on the basis of isozyme patterns [12] has great utility for conservation as well as use of rice germplasm, particularly since it clearly differentiates indica and japonica rices, between which there are fertility barriers. Such information does facilitate the use of rice varieties when morphological identification is ambiguous.

Several molecular marker systems have been used to study the diversity and evolution of *Oryza* [25, 26]. In collaboration with the University of Birmingham, UK, the IRG is using RAPD markers to understand the patterns of diversity in *O. sativa* landraces [32], as well as for identification of duplicate accessions in the IRG collection [34]. Probably there are many duplicate accessions, but varietal name alone cannot be taken as

certainty of duplicate status. Molecular markers such as RAPD do offer an opportunity to identify duplicate accessions, but there are still drawbacks. What level of similarity is needed to identify accessions as duplicates, and how many primers must be used? It is unrealistic to undertake a molecular analysis of all gene bank accessions, but for pairs or groups of accessions where duplicate status is suspected, the application of molecular marker technology adds another dimension to the study of genetic diversity in germplasm collections. The collaboration between IRRI and the University of Birmingham also showed that RAPD markers can have predictive value for quantitative traits in germplasm accessions grown in the field [33]. This is an interesting development that opens a new approach to study diversity in the IRRI collection, and other germplasm collections. Such predictive capacity of RAPD markers has also been demonstrated in beets (Brian Ford-Lloyd, personal communication).

The use of molecular markers may also assist in the development of a core collection for rice – a subset of the whole collection that represents the complete diversity of the genus *Oryza*. Core collections are being established in other crops [13] to promote their evaluation and use. Some criteria for a rice core collection have been published [29], but in reality there is no need to promote use of the IRG collection given the amount of characterization and evaluation already carried out, and the use that has been made of the germplasm collection in breeding. However, a core collection could open different management options for duplicate conservation at several sites instead of just NSSL [31].

The advantages of DNA banks have been described for molecular diversity studies [14], but the prospect of using such banks for the preservation of genes encoding biologically active proteins has yet to be realized [4]. In this respect, the use of DNA markers to study the genome structure of rice and other cereals in the Triticeae is sufficient justification for the establishment of a DNA bank given the wide-reaching implications of possible gene homology across species [5, 23]. IRRI has no detailed plans at present for such a DNA bank, apart from molecular probes, plasmids and the like, although for rice and other crops, the reality of DNA banks must be achieved sooner rather than later given the rapid progress in technology.

Table 2. The number of *O. sativa* accessions in the International Rice Genebank Collection evaluated at IRRI for their reaction to insect pests and diseases by 1993 (modified from [16]).

Stress	<i>O. sativa</i> accessions screened	
		% resistant
<i>Insect pests</i>		
Brown planthopper biotype 1	44 335	15.4
Brown planthopper biotype 2	10 053	1.9
Brown planthopper biotype 3	13 021	1.8
Green leafhopper	50 137	2.8
Rice whorl maggot	22 949	3.0
White-backed planthopper	52 042	1.7
Zigzag leafhopper	2 756	10.1
Rice leaffolder	8 115	0.6
Yellow stem borer	15 656	3.8
Striped stem borer	6 881	<0.02
<i>Disease</i>		
Blast	36 634	26.2
Sheath blight	23 088	9.3
Bacterial blight	49 752	11.1
Rice tungro disease	15 795	3.5

Policy and political issues

In December 1993 the Convention on Biological Diversity came into force. Since then concern has grown over ownership of and access to plant genetic resources. Much of this concern has arisen because of the rapid advances in biotechnology, especially genetic engineering, and the widespread belief that germplasm might be misappropriated under various forms of intellectual property protection. The moves that one or two companies have made to patent genes, and the ongoing debate in many countries over the ethics of patenting of genes or life forms, have exacerbated this problem.

Under the Convention on Biological Diversity, germplasm collected before the convention came into force are not subject to its regulations, and this applies to most of the accessions in the IRG. Even for germplasm acquired after the Convention came into force, the gene bank continues to provide free access, because the donors of the germplasm have indicated that germplasm exchange on request is permitted. Under IRRI's Policy on Intellectual Property Rights adopted in 1994, no intellectual property protection will be applied to germplasm in the gene bank. The international 'in trust' status was further enhanced in October 1994 when the collection was placed under the auspices of the Food and Agriculture Organization of the United Nations

in an International Network of *Ex Situ* Collections. A Material Transfer Agreement is used for the exchange of germplasm from the collection, under which recipients must also certify that they will not apply intellectual property rights to these germplasm accessions.

Today, the IRG is well poised to contribute to global biodiversity conservation efforts through the Global Plan of Action for the Conservation and Sustainable Utilization of Plant Genetic Resources for Food and Agriculture, adopted by the FAO intergovernmental conference, held in Leipzig in June 1996.

It is clear that there are many scientific opportunities for the management and sustainable use of rice genetic resources. In particular the developments of molecular biology are providing fascinating insights into the range and structure of genetic diversity in *O. sativa* and related species. Given the importance of this crop for world food security, the IRG collection represents a resource of unparalleled value. On the other hand, the considerable political challenges surrounding biodiversity are almost bound to generate some constraints in the short term until new regimes for exchange and use of germplasm are better defined. The role of the IRG is to ensure that the genetic diversity of rice that has been nurtured by farmers for generations in the past is available for the generations yet to come.

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