9 Managing Genetic Resources and Biotechnology at IRRI's Rice Genebank

Michael T. Jackson

Abstract

Ex situ conservation in genebanks is a safe and cost-efficient method of preserving the genetic diversity of crops and their wild relatives, particularly for species whose seeds can tolerate desiccation and storage at low temperature. Biotechnology is particularly useful for in vitro culture, cryopreservation, and disease elimination in vegetatively propagated crops. The use of molecular markers to study genetic diversity, identify duplicate accessions, and increase utilization by more efficient screening of germplasm are recent developments. Management of biotechnology in a genebank depends on the value of biotechnology over other approaches, the cost of investment, the trade-offs in not using biotechnology, and the resource allocation prioritization over all genebank activities and operations. The principal applications of biotechnology in the International Rice Genebank of the International Rice Research Institute (IRRI) are in vitro culture of seedlings and the study of genetic diversity using a range of molecular markers. Investment in biotechnology was made only after conservation operations per se had been upgraded.

Introduction

For thousands of years, farmers worldwide have been cultivating many different crops. The combined effects of adaptation to different environments, the breakdown of reproductive isolation between domesticated species and their wild relatives, and selection by farmers over many generations led to a multiplicity of varieties, each with particular traits valued by the communities that developed them. These are the genetic resources of the agricultural crops that sustain the world's growing population, and the genetic building blocks for more productive crop varieties (Ford-Lloyd and Jackson 1986). They are the source of traits to transfer to commercial varieties through conventional breeding techniques or through genetic transformation.

In a broad sense, the genetic resources of a crop include not only the varieties developed by farmers in indigenous farming systems and maintained by them for generations (often referred to as traditional, landrace, or farmers' varieties) and the related wild species, but also modern commercial varieties, obsolete varieties, breeding lines, and genetic stocks. However, genebanks usually give priority to the conservation of the landrace varieties and wild species. Ex situ conservation is a safe and cost-effective method

 Managing Agricultural Biotechnology: Addressing Research Program Needs and Policy Implications (ed. J.I. Cohen)

of preserving the genetic diversity of crops and their wild relatives, particularly for species whose "orthodox" seeds can tolerate desiccation and storage at low temperature. The long-term safety and integrity of genetic resources—seeds, living plants, cuttings, tissue cultures—are its primary goals.

An additional advantage of ex situ conservation in genebanks is the easy access to germplasm by breeders and researchers who wish to use these sources of genetic diversity in crop improvement programs, or to understand their reaction to biotic and abiotic stresses such as pests and diseases or drought, for example. Increasingly, the molecular basis of traits is being studied, which should facilitate their transfer to commercial varieties through genetic engineering.

Plant genetic resources are among the most vulnerable of all nonrenewable natural resources—once lost, they are lost forever. That is why, for several decades, there have been concerted international efforts to collect and conserve plant genetic resources for food and agriculture in genebanks worldwide. These efforts culminated in June 1996 during the Fourth International Technical Conference on Plant Genetic Resources in the adoption by 150 countries of a Global Plan of Action for the Conservation and Sustainable Utilization of Plant Genetic Resources for Food and Agriculture (FAO 1996). The framework of the plan is to ensure the long-term preservation of genetic resources at national, regional, and international levels, as well as the necessary actions to facilitate use of these valuable resources for the benefit of all humans. Biotechnology is recognized as an important component of implementing the global plan.

Biotechnology and ex situ conservation

A genebank has several functions, including (1) collection and acquisition of germplasm, (2) the long-term conservation of germplasm, including multiplication and regeneration in whatever is the most convenient and accessible form (such as seeds, in vitro cultures, and living plants), (3) germplasm characterization and evaluation, (4) data management, (5) germplasm exchange, and (6) promotion of germplasm use to enhance crop productivity. There are many different applications of biotechnology that are useful in this respect, but the relative importance of different techniques depends on the particular characteristics of a specific crop and its wild relatives (Callow et al. 1997). For example, in vitro culture of explants is essential for plant species that produce so-called "recalcitrant" seeds that cannot be stored at low moisture content and low temperature. Long-term cryopreservation of vegetative propagules or culture in slow-growth culture media are biotechnology options that must be explored for species difficult to conserve as seeds. Furthermore, tissue culture methods are widely applied for elimination of systemic diseases such as viruses. Engelmann (1997) provides a comprehensive review of in vitro conservation methods.

Recent developments in the area of molecular biology hold the promise of more efficient management and study and exploitation of genetic resources in ways that could not be imagined only a few years ago. These include molecular technologies to assess and monitor biodiversity, facilitate critical decisions on what should be conserved, or increase utilization through more efficient screening of germplasm (Barlow and Tzotsos 1995). In addition, molecular markers will certainly be used to define core collections within genebanks (Gepts 1995). It is perhaps in this molecular area of biotechnology more than any other that critical management decisions must be taken. Applications of molecular

biology are certainly in vogue, but that does not mean that many aspects are appropriate yet for all genebanks.

Managing biotechnology for ex situ conservation

Even though biotechnology has been used effectively for many years to ensure the safe conservation of plant genetic resources in genebanks, several management questions should be addressed before investing heavily in biotechnology:

- Does biotechnology enhance the access to and the management, conservation, and use of genetic resources?
- What alternatives to biotechnology can be used?
- What are the resource implications—human, equipment, or budget—to sustain applications of biotechnology in a genebank?
- What are the trade-offs for not investing in biotechnology?
- Will investment in biotechnology affect resource allocation to other areas of genetic conservation essential for the long-term security of a germplasm collection?

Conservation priorities should shape the strategy for adopting and using biotechnology rather than finding a use for biotechnology under any circumstances. The needs of a genebank with only base collection responsibilities may be different from one that has both active and base collections, distributes germplasm to users, or has a program of germplasm research. Most genetic conservation programs operate with limited financial support. The prioritization of resource allocation across all activities and operations is an essential step to integrate biotechnology successfully into the overall work plan of the genebank. Quite often, different biotechnology tools will be adopted because they are in vogue rather than contributing specifically to the more efficient conservation or exploitation of germplasm. For example, there is the commonly held perception that molecular biology, and particularly molecular markers, will automatically facilitate the development of a core collection or that such markers will help identify traits and their exploitation. Refining these techniques for successful and routine use with diverse germplasm takes time and considerable investment.

Managing biotechnology and rice genetic resources at IRRI

Located in Los Baños, the Philippines, the International Rice Research Institute (IRRI) holds in trust the world's largest and most genetically diverse collection of rice genetic resources in its International Rice Genebank, which is managed as part of the institute's Genetic Resources Center (Jackson 1997; Jackson et al. 1997). In 1994 the collection was placed under the auspices of the Food and Agriculture Organization of the United Nations (FAO) in the International Network of Ex Situ Collections. Under the agreement with FAO, a material transfer agreement is used to facilitate access to and use of the conserved germplasm. This prohibits IRRI or any other recipient from seeking intellectual property rights (IPR) on the germplasm directly.

The genebank currently maintains a collection of more than 102,700 samples of Asian rice *Oryza sativa* (95%), West African rice *O. glaberrima* (1.5%), and all 21 wild species (3.5%). Since 1991 the infrastructure and operations of the genebank have been upgraded, for example by adding a seed drying room. All these changes were aimed at meeting

international genebank standards (FAO/IPGRI 1994), while at the same time increasing the quality of conserved germplasm (defined in terms of seed viability and potential storage longevity). **Our first priority was to ensure the long-term conservation of this strategically important germplasm collection.** This has been achieved by exploiting the seed production environment in Los Baños to achieve maximum seed longevity in storage for all the diverse rice accessions (Ellis et al. 1993; Kameswara Rao and Jackson 1996a, 1996b, 1996c, 1997).

The decision to use biotechnology in various forms for managing and studying the rice germplasm collection was not taken lightly. Our assessment was guided by the need to ensure the safety of the germplasm *per se* and to employ new molecular technologies that would facilitate better understanding of the underlying genetic structure of the collection. IRRI has made considerable investment in biotechnology to support its rice improvement activities, especially through transgenesis and marker-assisted selection. In assessing the molecular marker systems available, we had to determine what level of investment would be appropriate for genetic resource purposes in terms of the overall recurrent costs and infrastructure development in the Genetic Resources Center, as well as safety considerations. Additionally, we felt that rather than using state-of-the-art technology that our partners in the national agricultural research systems (NARS) would not be able to adopt, we should use biotechnology approaches that they might feasibly develop in the foreseeable future.

We were fortunate to establish collaboration with the School of Biological Sciences at the University of Birmingham in the UK in 1993. With funding from the Department for International Development (DfID—formerly the Overseas Development Administration), a research project was initiated to study the diversity of rice germplasm using molecular markers. One of our staff was trained at Birmingham, which put us in a better position to decide what was needed in terms of molecular studies.

As relative costs of molecular techniques fell and their value for the study of germplasm collections was proven, it became clear that we should take the opportunity of adding these to the suite of characterization and evaluation approaches already being used in the genebank. Otherwise, we felt the genebank would be locked in "traditional" approaches and would not take advantage of new technologies in which others had already made the necessary research development investments. We could not hire new staff for this endeavor, but we redeployed existing staff, who were given additional, appropriate training. Biotechnology for genetic resources is supported from the annual budget of the Genetic Resources Center, provided by IRRI from its core budget. We are seeking additional donor support to expand our molecular studies.

By considering these factors and options for the use of biotechnology, we decided that the principal genebank applications of biotechnology would be in vitro culture of seedlings and the study of genetic diversity using a range of biochemical and molecular markers.

In vitro culture

In vitro culture is used to ensure the survival of seed lots with low viability. Seeds may have low viability when they are sent to our genebank for long-term conservation. Sometimes, only a few seeds are sent for conservation purposes. In a 1992 monitoring survey of the International Rice Genebank collection, the viability of about 300 samples (mainly japonica rices) fell into this category. Since such accessions must be multiplied to provide

the 500 g necessary for conservation in the active collection and the 120 g for the base collection, it is unwise to plant these seeds directly in the field.

In vitro culture involves germination of hulled seeds (i.e., with the lemma and palea removed) on nutrient agar containing Murashige and Skoog medium (Murashige and Skoog 1962), and a period of growth in culture solution in a phytotron (Yoshida et al. 1976) until vigorous plants are obtained. These plants can then be transplanted to a screenhouse and given more care than is possible in field plots.

Isozyme electrophoresis

A classification of *O. sativa* varieties into six groups based on the allelic variation at 21 polymorphic loci coding for 14 isozymes (Glaszmann 1987) is an important tool for rice germplasm management, although varieties can be separated quickly using only five loci for two isozyme systems (phosphoglucose isomerase and aminopeptidase). Groups I and VI correspond to the indica and japonica rices, respectively. Furthermore, the javanica rices are included in group VI with the japonica rices. Consequently, they have been renamed "tropical japonicas" and, based on this classification, were selected as germplasm for the development of the so-called "new plant type" (Khush 1995). The remaining groups II to V represent indica varieties found only in the Indian subcontinent, especially in the foothills of the Himalayas, like the floating *rayada* varieties of Bangladesh (group IV) and the *basmati* rices of northern India, Pakistan, and Nepal, prized for their aromatic flavor (group V). Since the crossability barriers between indica and japonica rices affect their utilization in rice breeding, correct identification of these varieties is extremely important. The development of isozyme classification provides an unequivocal biological framework for the use and analysis of diversity patterns of germplasm based on other molecular markers.

DNA markers

DNA markers such as RFLP, AFLP, RAPD, and SSR are routinely used for the management and evaluation of crop germplasm collections (Westman and Kresovich 1997) for three principal purposes. First, molecular markers may be used to answer so-called forensic questions such as whether two samples are genetically the same. Second, there are questions of location and diagnostics, where the objective is to determine the presence or location of a particular allele or nucleotide sequence, be that in all species and accessions in the genebank, or a population, particularly those related to desirable traits. Such questions are important for monitoring the genetic health and changes of a genebank sample over time and as a consequence of various regeneration procedures. Finally, Westman and Kresovich (1997) highlight the questions of relatedness, of genetic diversity *per se*, and how diversity is distributed in individuals, populations, and species. Such information is useful, perhaps necessary, for adequately targeting areas for germplasm collecting, or designing in situ programs, complementary to ex situ conservation in genebanks. Until relatively recently, such techniques were beyond the means of most genetic conservation programs and may remain for some time to come beyond the immediate resource allocation of many.

In the International Rice Genebank we began using molecular markers after several different approaches had been validated in the joint project with the University of Birmingham (Ford-Lloyd et al. 1997). This collaboration permitted the genebank to take

advantage of expertise elsewhere to evaluate various protocols for rice germplasm while facilities for molecular biology were developed and personnel trained.

Initially, our emphasis was on RAPD to understand the diversity in rice landraces (Virk et al. 1995a) and the identification of duplicate accessions (Virk et al. 1995b), but more recently, we started using AFLP. We also established the association between RAPD markers and quantitative variation and were able to predict the performance of rice accessions in the field in Los Baños based on RAPD markers (Virk et al. 1996). We have also used RAPD for taxonomic studies of wild rices, particularly the South American species *O. glumaepatula* (Martin et al. 1997). The results correspond well with taxonomic studies of this species based on morphology (Juliano et al. 1998). AFLP analyses of rice germplasm seem more robust (Zhu et al. 1998) than those based on RAPD, but both correlate well with the isozyme groups referred to earlier. The advantage of both RAPD and AFLP markers is their broad distribution across the rice genome, based on data from a wide diversity of rice varieties. We have been able to use the isozyme classification to validate those based on RAPD and AFLP markers. This gives us confidence that it is not necessary to use only mapped markers, whose distribution and "information" content may be a reflection only of the genetic distance of the original parents of a mapping population (Virk et al. 1999). Choice of markers is important because their position in the genome does affect the analysis of diversity patterns (Parsons et al. 1997).

Conclusions and management implications

The first priority of a genebank is to ensure the long-term conservation of germplasm with which it has been entrusted. Investments in biotechnology must be made at a level that is consistent with the overall budget and mandate of the genebank and that can be sustained. The development of a core collection using molecular markers is often cited as one activity that many genebanks should initiate. **It is essential that the basic elements of a strong conservation program are in place before taking decisions on developing capability within a genebank to use biotechnology.** Otherwise, the added benefits that biotechnology can bring may not be realized, or germplasm may not be readily available if it has not received proper care in the genebank. We believe that this cautious approach is appropriate for many genebanks where resources are limited.

In the future, molecular analysis of germplasm collections will permit more efficient utilization of wild species in rice breeding (Tanksley and McCouch 1997), and the synteny between cereal genomes (Devos and Gale 1997) presents opportunities to exploit molecular data from one species to search for traits in another. But these are not approaches for a single genebank alone. They will require strong collaboration between different genebanks and molecular biologists worldwide. The investment and resource implications are too great for any one institute alone. Nevertheless, it is necessary to grasp such opportunities in ways that are innovative and that do not compromise the principal purpose of germplasm conservation.

References

Barlow, B. and G.T. Tzotsos. 1995. Biotechnology. *In* Global Biodiversity Assessment, edited by V H. Heywood and K. Gardner. Cambridge: Cambridge University Press.

Callow, J.A., B.V. Ford-Lloyd and H. J. Newbury. 1997. Overview. *In* Biotechnology and Plant Genetic Resources: Conservation and Use, edited by J. A. Callow, B. V. Ford-Lloyd and H. J. Newbury. Wallingford: CAB International.

Devos, K.M. and M. D. Gale. 1997. Comparative genetics in the grasses. *Plant Molecular Biology* 35: 3-15.

Ellis, R.H., T.D. Hong and M.T. Jackson. 1993. Seed production environment, time of harvest, and the potential longevity of seeds of three cultivars of rice (*Oryza sativa* L.). *Annals of Botany* 72: 583-590.

Engelmann, F. 1997. *In vitro* conservation methods. *In* Biotechnology and Plant Genetic Resources: Conservation and Use, edited by J.A. Callow, B.V. Ford-Lloyd and H.J. Newbury. Wallingford: CAB International.

FAO. 1996. Global Plan of Action for the Conservation and Sustainable Utilization of Plant Genetic Resources for Food and Agriculture. Rome: Food and Agriculture Organization of the United Nations.

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

Ford-Lloyd, B. and M. Jackson. 1986. Plant Genetic Resources: An Introduction to their Conservation and Use. London: Edward Arnold.

Ford-Lloyd, B.V., M.T. Jackson and H.J. Newbury. 1997. Molecular markers and the management of genetic resources in seed genebanks: a case study of rice. *In* Biotechnology and Plant Genetic Resources: Conservation and Use, edited by J.A. Callow, B.V. Ford-Lloyd and H.J. Newbury. Wallingford: CAB International.

Gepts, P. 1995. Genetic markers and core collections. *In* Core Collections of Plant Genetic Resources, edited by T. Hodgkin, A.H.D. Brown, Th.J.L. van Hintum and E.A.V. Morales. Chichester: John Wiley and Sons.

Glaszmann, J.C. 1987. Isozymes and classification of Asian rice varieties. *Theoretical and Applied Genetics* 74: 21-30.

Jackson, M.T. 1997. Conservation of rice genetic resources—the role of the International Rice Genebank at IRRI. *Plant Molecular Biology* 35: 61-67.

Jackson, M.T., G.C. Loresto, S.Appa Rao, M. Jones, E. Guimaraes and N.Q. Ng. 1997. Rice. *In* Biodiversity in Trust: Conservation and Use of Plant Genetic Resources in CGIAR Centres, edited by D. Fuccillo, L. Sears and P. Stapleton. Cambridge: Cambridge University Press.

Juliano, A.B., M.E.B. Naredo and M.T. Jackson. 1998. Taxonomic status of *Oryza glumaepatula* Steud. I. Comparative morphological studies of New World diploids and Asian AA genome species. *Genetic Resources and Crop Evolution* 45: 197-203.

Kameswara Rao, N. and M.T. Jackson. 1996a. Effect of sowing date and harvest time on longevity of rice seeds. *Seed Science Research* 7: 13-20.

Kameswara Rao, N. and M.T. Jackson. 1996b. Seed longevity of rice cultivars and strategies for their conservation in genebanks. *Annals of Botany* 77: 251-260.

Kameswara Rao, N. and M.T. Jackson. 1996c. Seed production environment and storage longevity of japonica rices (*Oryza sativa* L.). *Seed Science Research* 6: 17-21.

Kameswara Rao, N. and M.T. Jackson. 1997. Variation in seed longevity of rice cultivars belonging to different isozyme groups. *Genetic Resources and Crop Evolution* 44: 159-164.

Khush, G.S. 1995. Breaking the yield frontier of rice. *GeoJournal* 35: 329-332.

Martin, C., A. Juliano, H.J. Newbury, B.R. Lu, M.T. Jackson and B.V. Ford-Lloyd. 1997. The use of RAPD markers to facilitate the identification of *Oryza* species within a germplasm collection. *Genetic Resources and Crop Evolution* 44: 175-183.

Murashige, T. and F. Skoog. 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiologia Plantarum* 15: 473-497.

Parsons, B.J., H.J. Newbury, M.T. Jackson and B.V. Ford-Lloyd. 1997. Contrasting genetic diversity relationships are revealed in rice (*Oryza sativa* L.) using different marker types. *Molecular Breeding* 3: 115-125.

Tanksley. S.D. and S.R. McCouch. 1997. Seed banks and molecular maps: unlocking genetic potential from the wild. *Science* 277: 1063-1066.

Virk, P.S., B.V. Ford-Lloyd, M.T. Jackson and H.J. Newbury. 1995a. Use of RAPD for the study of diversity within plant germplasm collections. *Heredity* 74: 170-179.

Virk, PS., H.J. Newbury, M.T. Jackson and B.V. Ford-Lloyd. 1995b. The identification of duplicate accessions within a rice germplasm collection using RAPD analysis. *Theoretical and Applied Genetics* 90: 1049-1055.

Virk, P.S., B.V. Ford-Lloyd, M.T. Jackson, H. Pooni, T.P. Clemeno and H.J. Newbury. 1996. Predicting quantitative traits in rice using molecular markers and diverse germplasm. *Heredity* 76: 296-304.

Virk, P.S., H.J. Newbury, M.T. Jackson and B.V. Ford-Lloyd. 1999. Are mapped markers more useful for assessing genetic diversity? *Theoretical and Applied Genetics* (in press).

Westman, A.L. and S. Kresovich. 1997. Use of molecular marker techniques for description of plant genetic variation. *In* Biotechnology and Plant Genetic Resources: Conservation and Use, edited by J. A. Callow, B. V. Ford-Lloyd and H. J. Newbury. Wallingford: CAB International.

Yoshida, S., D.A. Forno, J.H. Cock and K.A. Gomez. 1976. Routine procedures for growing rice plants in culture solution. *In* Laboratory Manual for Physiological Studies of Rice. Manila: International Rice Research Institute.

Zhu, J., M.D. Gale, S. Quarrie, M.T. Jackson and G.J. Bryan. 1998. AFLP markers for the study of rice biodiversity. *Theoretical and Applied Genetics* 96: 602-611.