JARQ 39 (1), 231 – 237 (2004) http://www.jircas.affrc.go.jp

REVIEW

Plant Genetic Resources in Japan: Platforms and Destinations to Conserve and Utilize Plant Genetic Diversity

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Abstract

Since 1985, the Genebank project has been implemented as a national program to collect, characterize, evaluate, rejuvenate, conserve and use plant, microorganism and animal genetic resources for food and agriculture. NIAS has functioned as a center-bank of the project in cooperation with other agricultural public and private sectors. This national network on genetic resources for food and agriculture currently preserves approximately 233,000 accessions of crop germplasm and wild relatives of about 1,450 species involving 45,000 accessions of clonal crops. Among them 205,000 accessions are conserved as base collection, while 129,000 accessions, as active collection, are distributed for research purposes under the regulation of MTA between NIAS Genebank and recipients worldwide. We have undertaken international collaboration focusing on exploration for collecting genetic resources under the regulation of MOU or MOA with collaborating parties and on *in situ* conservation and on farm management of plant genetic resources in several Asian countries. Research highlights on conservation and utilization of plant genetic resources will be discussed in this report.

Discipline: Genetic resources
Additional key words: cryopreservation, *ex situ* conservation, Genebank, genomics-based germplasm enhancement, *in situ* conservation

Introduction

Concerning genetic resources for food and agriculture, the Ministry of Agriculture, Forestry and Fisheries (MAFF) of Japan has implemented the Genebank project on plants, microorganisms, and animals since 1985. The National Institute of Agrobiological Sciences (NIAS) has functioned as a center-bank in cooperation with other agricultural research institutions as sub-banks, and is responsible for coordinating project planning and implementation. The project was originally planned to collect, characterize, evaluate, conserve and utilize genetic resources of agricultural plants, microorganisms, and domesticated animals. The DNA Bank was also established based at the NIAS in 1993 to fulfill new project requirements. In addition to the Genebank, the Rice Genome Resources Center commenced operation in April, 2003 to provide plant genetic materials and DNA clones produced by the Rice Genome Research Program.

On the other hand, the Government of Japan decided a basic policy for research and development on life science in 1997, stressing the importance and necessity of conservation, utilization, and information networks of genetic resources for successful research and development in the future. A working party of the Science Council, the Ministry of Education, Culture, Sports, Science and Technology (MEXT) plays an important role in coordinating the domestic committee concerned with technological infrastructure for academic activities on genetic resources. A national committee for overall genetic resources has been discussed and preparations made, but it has not yet been established.

Genebank project in Japan

The Genebank project currently has conserved approximately 233,000 accessions of plant genetic resources of about 1,450 species involving 40,000 of rice, 60,000 of wheat and barley, 16,000 of food legumes,

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Received 4 March 2005; accepted 14 April 2005.

26,000 of vegetables and 10,000 of fruit trees (Table 1). A total of about 45,000 accessions of vegetatively propagated crops have been also conserved. The Genebank project has conserved 205,000 accessions for long-term storage (base collection) and 129,000 accessions for distribution to users as active collection. About 7,000–8,000 accessions have been annually distributed for research purposes (Table 2) under the regulation of MTA (Material Transfer Agreement) between NIAS Genebank and recipients. Passport data and evaluation data can be accessed through the website, http//www.gene.affrc.go.jp.

Japan has a rich source of plant genetic resources. However, as with any country we are always looking for new sources of biotic and abiotic stress resistance/tolerance, sources contributing to human health and nutrition, and other traits to improve plant genetic resources for food and agriculture. The international exchange of germplasm is a key to mining new resources and genes. In recent years we have developed various new mechanisms that take into account the different countries concerns over farmers' rights and intellectual property rights. Collaboration with countries that have highly restrictive policies on germplasm exchange has been depressed.

Table 1. Number of accessions of plant genetic resources preserved in the Genebank project (31 December 2004)

Plant group	Total no. of accessions*	
Rice	41,729	(30,912)
Wheat & barley	61,187	(37,254)
Legumes	16,352	(12,358)
Root & tuber Crops	8,423	(4,148)
Cereals & industrial Crops	18,519	(10,279)
Forage	32,403	(15,919)
Fruit trees	9,782	(4,669)
Vegetables	26,067	(10,413)
Ornamental flowers	5,853	(490)
Tea	7,483	(1,350)
Mulberry	1,502	(875)
Tropical & subtropical Plants	417	(15)
Other	3,438	(383)
Total	233,155	(129,065)

*: Total number of accessions (base and working collections).
Figure in parenthesis indicates number of active collections.

Table 2. Number of plant genetic resources used to each purpose distributed from the Genebank project

Purpose	1994	1995	1996	1997	1998	1999	2000	2001	2002	2003	Total
Characterization	1,679	736	808	1,562	3,528	3,287	3,297	18,641	5,256	6,115	44,909
Screening	1,650	2,322	2,082	2,621	1,706	1,758	2,650	2,927	1,108	4,430	23,254
Analysis of diversity	452	451	523	579	82	73	108	219	322	159	2,968
Molecular research	402	370	217	198	268	1,147	527	2,314	242	180	5,865
Breeding	388	155	695	373	408	274	466	690	277	332	4,058
Physiological research	1,436	784	593	905	597	64	147	618	227	890	6,261
Direct application	22	403	80	50	18	5	28	5	14	23	648
Bio-assay for disease	298	292	709	185	307	86	101	106	95	53	2,232
Stress tolerant assay	388	734	118	269	372	295	33	266	419	48	2,942
Exchange	13	2	28	4	12	64	11	14	0	0	148
Other	1	4	47	21	26	48	36	29	89	62	363
Total	6,729	6,253	5,900	6,767	7,324	7,101	7,404	25,829	8,049	12,292	93,648

International joint exploration and field survey for *ex situ* and *in situ* conservation of genetic resources

The Genebank project has annually undertaken overseas exploration missions for collecting plant germplasm in collaboration with participating countries[1] (Table 3). Prior to the exploration, NIAS Genebank and the counterpart organizations have to discuss and settle some agreement about the implementation of the exploration plan and on the treatment of the genetic resources to be collected: Memorandum of Understanding (MOU), Memorandum of Agreement (MOA) and/or approval of work plan. After a cooperative exploration, the plant materials collected in each country are always divided into two sets: one set is transferred to Japan based on the Material Transfer Agreement (MTA) between Japan and the country, and the other set is conserved in the country of origin. This duplicated *ex situ* conservation of genetic resources is beneficial for users in both countries, and has been practiced since the beginning of the Genebank project long before the CBD. Such joint exploration can provide plant genetic resources, and serve as a good opportunity to initiate and enhance further development of research cooperation. Domestic explorations for collecting unique plant genetic resources have also been conducted within Japan[1]. In 2004, a collaborative exploration with USDA/ARS was carried out to collect wild forms of small fruit trees indigenous to Hokkaido.

Particularly in the conservation of wild species, it is often difficult to preserve their diversity *ex situ* within

Table 3. A list of overseas exploration missions dispatched by the Genebank project since 1985

Fiscal year	Region	Target crops
1985	Taiwan	Fruit trees
	Italy, Hungary & Yugoslavia	Wheat barley & wild relatives
	Papua New Guinea	Sweetpotato & sugarcane
	Argentine Rep.	Groundnut & maize
	Thailand	Soybean
1986	Morocco, Portugal & Spain	Grasses
	Thailand	Rice & vegetables
	Peru & Ecuador	Wild *Solanum* & *Ipomoea*
	Tanzania	Millets, sesame & sorghum
1987	Nigeria	Vegetables
	Nepal	Food legumes
	Morocco & Syria	Wheat & barley
	Italy, Greece & Israel	Fruit trees
1988	Nepal	*Rhododendron* & *Lilium*
	India	Sugarcane, tea & mulberry
	Argentine Rep.	Alfalfa
	Indonesia	Rice
1989	Algeria	Wheat & barley
	Malaysia & Thailand	Sweetpotato
	France & Italy	Wine grape
	Greece	Wild *Tulipa* & *Allium*
1990	Morocco & Spain	Wild *Beta*
	Finland & Sweden	*Trifolium* & timothy
	Thailand	Cultivated and wild rice
	Malaysia	Food legumes
1991	USSR	Cold temperate grasses
	Poland	Vegetables
	Sri Lanka & Thailand	Millets
	Uruguay & Chile	Sweetpotato & potato

Table 3. A list of overseas exploration missions dispatched by the Genebank project since 1985 *(continued)*

Fiscal year	Region	Target crops
1992	India	Sesame
	Madagascar	Rice
	Mexico	Mulberry
	Brazil	Pineapple
1993	Ghana	Vegetables & legumes
	Vietnam	Rice
	Vietnam	Legumes
	Ecuador & Bolivia	Root crops
1994	Pakistan	Wheat & barley
	The Philippines	Sweetpotato
	Vietnam	Taro
	Kazakhstan & Uzbekistan	Wild *Tulipa* & *Allium*
1995	Bulgaria & Greece	Oat & *Medicago*
	Kenya	Wild *Cucurbitaceae* & *Solanaceae*
	Sri Lanka	Tea
	Paraguay & Bolivia	Food legumes
1996	Poland, Czech Rep. & Slovakia	Grasses
	Vietnam	Rice
	Kenya	*Sorghum*
1997	Kazakhstan	*Allium*
	Vietnam	Rice
	Vietnam	*Citrus* & loquat
	Thailand	Tropical fruits
1998	Greece	Wheat & barley
	Armenia & Georgia	Fruit trees
	Vietnam	Rice
	Vietnam	*Citrus*
1999	Greece	*Brassica*
	Thailand	Sugarcane
	Myanmar	Rice
	Spain	*Citrus*
2000	Myanmar	Rice
	Vietnam	Legumes
	Myanmar	Buckwheat, Job's tear & elephant foot yam
	Italy, France & Spain	Grasses
2001	Myanmar	Food legumes
	Myanmar	*Sorghum* & other grasses
2002	Australia	Wild rice
	Myanmar	Food legumes
	Vietnam	Tea
2003	Russia & Azerbaijan	Beet
	Turkey	Fruit trees
	Vietnam	Tea
	Pakistan	Mulberry

genebanks, therefore *in situ* conservation is needed with proper monitoring. The Genebank project has a special program to encourage researchers in developing countries to survey and monitor the genetic diversity of natural populations and landraces on farmers' fields (Table 4). This helps *in situ* conservation of wild relatives and on farm management of cultivated plants and also assists capacity-building of researchers in developing countries. *In situ* conservation research activities of the project on wild legumes and wild rice had been undertaken in cooperation with the Plant Genetic Resource Centre (PGRC) of Sri Lanka for three years since 2000. Japanese researchers joined missions for monitoring genetic structures of wild *Vigna* species and wild *Oryza* species there, while Sri Lankan researchers, who participated in the program, could learn various knowledge and techniques of DNA analysis. An *in situ* conservation project on *Fagopyrum* species sponsored by the International Plant Genetic Resources Institute (IPGRI) was conducted in Nepal from 1999 to 2001 in a similar manner. The genetic diversity study was done on a wild species, *F. cymosum* using RAPD analysis, in which a site was successfully identified to harbor the most feasible population for *in situ* conservation of the species. Indonesian and Korean researchers participated in a program on sweet potato and *Perilla* species in 2003, respectively. The project started a joint field survey on some fruit trees in Xinjian Uygur Province, China and on rice, legumes and sago in Papua New Guinea in 2004 (Table 4).

Research highlights in conservation and utilization of plant genetic resources

Future directions of genebank activities in relation to plant genetic resources are summarized as follows.

(1) For a genebank to be effective it is necessary to make full use of genomics to better understand and make germplasm more useful to plant breeders and other germplasm users. It is currently called "allele mining" and genomics-based germplasm enhancement.

(2) The core collection or genetically representative collections approach will be continued to pursue with those crops and wild relatives for which we have a comparative advantage in Japan.

(3) We have been conducting research on *in situ* conservation because the complexity of genetic resources can only partly be accomplished *ex situ*. Results of our research have illustrated that natural populations (of wild species) and farmers varieties are far more genetically diverse than we previously realized.

(4) Use of new technologies for genotyping traits, monitoring seed longevity, and enhancing cryopreservation will all be pursued.

(5) While new biotechnologies are of value for characterization and evaluation, new information technologies are equally an important area of progress in our genebank system. One aspect focused on is illustrated databases that are benefiting from huge advances in digital, hardware and software technologies.

1. Genome-based research focuses on sustainable utilization of plant genetic diversity

Advances in plant genomics give a great impact on discovery and use of cryptic genetic variation in plant genetic resources. As an example of what is currently called "allele mining", field resistance to rice blast in the Japanese upland rice cultivar has been analyzed[2]. Compared with true resistance based on the gene-for-gene concept, field resistance is characterized by a susceptible infection type and is controlled by multiple genes. Field resistance allows effective control of a parasite under natural conditions and is considered to be durable when exposed to new races of the pathogens. Most of Japanese traditional upland rice cultivars express a high level of field resistance. However, they also have several undesirable traits which are barriers for the use of

Table 4. A summarized list of cooperative field survey projects

Fiscal year	Region	Target crops
1996–1998	Chile	Tomato
1996–1997	The Philippines	Sweetpotato
1999–2001	Vietnam	Rice
2001–2003	Indonesia	Sweetpotato
2000–2002	Sri Lanka	Rice & legumes
2002–	Korea	Perilla
2004–	Xinjian Uygul Province, China	Apple, pear & stone fruits
2004–	Papua New Guinea	Rice, pulses & sago palm

Japanese upland rice. Using DNA markers mapped over 12 rice chromosomes, we detected three major QTL for field resistance to rice blast. The most effective QTL is located on chromosome 4 and the resistance controlled by alleles at this QTL is inherited by a single Mendelian factor, *pi21*. The *pi21* is much more effective on the expression of blast resistance compared with a dominant allele. Allele of upland rice (*pi21*) can beneficially enhance blast resistance of rice by DNA marker-assisted selection.

2. Core collections of rice genetic resources

We initiated to prepare core collections of plant genetic resources to promote sustainable use of genetic resources. Since 2004 we have distributed two kinds of core collections of Asian cultivated rice, *Oryza sativa*. One of the core collections of rice, world rice core collection, consists of 66 representative varieties selected from each of 66 clusters on UPGMA dendrogram based on 571 alleles at 184 RFLP marker loci over 12 rice chromosomes. The other core collection comprises 35 Japanese local varieties selected based on the analysis using 247 alleles at 32 SSR marker loci. These core collections are very useful bio-resources to exploit existing natural variations in genetic resources and to detect chromosomal locations of new alleles using linkage disequilibrium mapping.

3. Cryopreservation of vegetatively propagated crops

Cryopreservation of plant materials has proven to be a potentially ideal method for long-term preservation, because it requires a minimum of space, labor, medium and maintenance. Our field collections are maintained using the duplicated conservation system in principle. However, there are exceptions. These include crops such as the mulberry, rush and taro, which are maintained without duplication. Cryopreservation should be considered as a backup to field collections to insure against loss[6]. In this, we mean the priority of collections to be cryopreserved should be given to the "at risk" lines that have an increased chance of being lost from a collection.

One way of achieving successful cryopreservation of woody plants is the use of dormant winter buds. We developed the protocol for cryopreservation of mulberry winter buds[4]. This method with slight changes has been successfully applied to winter buds of deciduous woody trees such as the apple, pear, blueberry, raspberry, and persimmon. At present, in our genebank, about 450 *Morus* germplasm accessions within several species have been cryopreserved in liquid nitrogen tanks.

Alternative new cryogenic procedures based on vitrification have been described. These dramatically increase applicability to a wide range of plant germplasm, especially in non-hardy materials and plants. For example, we developed a practical cryopreservation method of *in vitro* grown strawberry shoot tips by vitrification[5]. Using this protocol, we have already cryopreserved about 60 strawberry accessions. We have been also planning to start projects of practical cryopreservation of potato and rush by vitrification based protocols.

We intended that all genetic resources of vegetatively propagated crops stored using these cryopreservation methods should be backed up with field genebanks. The establishment of the national cryo-genebank in Japan is expected to be used for the germplasm of vegetatively propagated crops.

4. International collaborations on *in situ* conservation and on farm management of plant genetic resources

Our staffs have participated in IPGRI collaborative projects on diversity analysis of Asian *Vigna*, perilla and Job's tears. The other project funded by IPGRI focused on *in situ* conservation of wild relatives of buckwheat. The Genebank project has also performed collaborative programs on *in situ* conservation or on farm management of crop germplasm and their wild relatives in Sri Lanka, Vietnam[3], Indonesia, Papua New Guinea and Xinjiang Province in China. These collaborative programs involve joint exploration missions, invitation of researchers from collaborating countries and molecular diversity analysis in Japan and workshops with the same insight as the program. In addition, Japan is an active partner with FAO and IPGRI. One aspect of Japan's commitment to the FAO Global Plan of Action is funding for the project on formation of national information sharing mechanisms and methodologies of *in situ* conservation of Plant Genetic Resources (PGR) in several Asian countries.

In our Genebank project, a system that allows us to develop illustrated germplasm databases has been developed as a PGR research tool. The components of the system are digital camera, photographic microscope and linked computer. This will be a valuable component of our documentation system since it enables us to record, for example, habitats and the complex structure of flowers. We think that illustrated databases play a particularly valuable role in the conservation and documentation of wild relatives of crops. *In situ* conservation is of particular value for conserving wild relatives of crops since they are difficult to conserve *ex situ* and often are unadapted to *ex situ* conditions. However, to develop sound *in situ* conservation systems good databases are important. A key question regarding wild relatives of crops is how they can be properly identified. Careful study over several years has enabled our scientists to build up an illustrated database of wild relatives and cultigens of the genus *Vigna* that enables them to be readily identified.

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