Breeding Science 60: 461–468 (2010)
doi:10.1270/jsbbs.60.461

Review

NBRP, National Bioresource Project of Japan and plant bioresource management

Nori Kurata*[1)], Hikaru Satoh[2)], Hidemi Kitano[3)], Yasuo Nagato[4)], Takashi Endo[5)], Kazuhiro Sato[6)], Ryo Akashi[7)], Hiroshi Ezura[8)], Makoto Kusaba[9)], Masatomo Kobayashi[10)], Eiji Nitasaka[11)], Fumie Kasai[12)], Yukiko Yamazaki[13)] and Atsushi Yoshimura[14)]

[1)] *Genetic Strains Research Center, National Institute of Genetics*, 1111 Yata, Mishima, Shizuoka 411-8540, Japan
[2)] *Institute of Genetic Resources, Faculty of Agriculture, Kyushu University*, 6-10-1 Hakozaki, Higashi, Fukuoka 812-8581, Japan
[3)] *Bioscience and Biotechnology Center, Nagoya University*, Furo, Chikusa, Aichi 464-8601, Japan
[4)] *Graduate School of Agriculture and Life Sciences, University of Tokyo*, Tokyo 113-8657, Japan
[5)] *Laboratory of Genetics, Graduate School of Agriculture, Kyoto University*, Kita-Shirakawa Oiwake, Sakyo, Kyoto 606-8502, Japan
[6)] *Barley and Wild Plant Resource Center, Institute of Plant Science and Resources, Okayama University*, 2-20-1 Chuo, Kurashiki, Okayama 710-0046, Japan
[7)] *Frontier Science Research Center, Miyazaki University*, 1-1 Nishi, Gakuen Kibanadai, Miyazaki 889-2192, Japan
[8)] *Gene Research Center, Graduate School of Life and Environmental Sciences, University of Tsukuba*, 1-1-1 Tennodai, Tsukuba, Ibaraki 305-8572, Japan
[9)] *Graduate School of Science, Hiroshima University*, 1-4-3 Kagamiyama, Higashi-Hiroshima, Hiroshima 739-8526, Japan
[10)] *RIKEN BioResource Center*, 3-1-1 Kouyadai, Tsukuba, Ibaraki 305-0074, Japan
[11)] *Graduate School of Science, Kyushu University*, 6-10-1 Hakozaki, Higashi, Fukuoka 812-8581, Japan
[12)] *Environmental Biology Division, National Institute of Environmental Studies*, 16-2 Onogawa, Tsukuba, Ibaraki 305-8506, Japan
[13)] *Center for Genetic Resource Information, National Institute of Genetics*, Yata 1111, Mishima, Shizuoka 411-8540, Japan
[14)] *Plant Breeding Laboratory, Faculty of Agriculture, Kyushu University*, 6-10-1 Hakozaki, Higashi, Fukuoka 812-8581, Japan

The National BioResource Project has been organized and established to promote research activities using valuable bioresources. A total of twenty-eight bioresources for ten animals, nine plants and nine microorganisms/cell lines developed or collected in Japan were selected for the project. Resources are categorized into several different groups in the project; genetic resources, germplasm, genome resources and their information. Choices of how many resources must be preserved and maintained and in which categories are dependent on the status of the research community of each organism. These resources, if utilized systematically and intelligently, are powerful means for leading new scientific discoveries. Some examples can be seen in this paper. This paper reviews plant bioresources with the main focus on rice resource activities within the project.

Key Words: NBRP, plant bioresources, germplasm, line/strain/accession, rice.

Introduction

With the recent accumulation of huge amounts of genome data together with a rapid loss of biodiversity from the earth, a momentum to preserve, analyze and utilize the variety of organisms has been generated. In addition, the availability of genetic engineering technologies and genomic markers made it possible to generate and use valuable genetic lines of model organisms for fundamental and applied research. Although many bioresources have been developed in different universities and research institutions, maintenance and distribution of information and materials mostly depends on the voluntary work of each researcher/laboratory.

The National Bioresource Project (NBRP) was established in 2002 to gather and manage valuable biological resources distributed among many universities. The project has been managing bioresources for eight years; the first five years as phase I while phase II has been running since 2007. The project was first organized under the Ministry of Education, Culture, Sports and Technology (MEXT), then later the organizing office and steering committee were transferred to the National Institute of Genetics (NIG). The project is planned to respond to the demands of research communities for each organism. The major decisions concern what kind of biological resources should be prepared and supplied to the community.

Communicated by H. Yasui
Received August 25, 2010. Accepted November 28, 2010.
*Corresponding author (e-mail: nkurata@lab.nig.ac.jp)

In NBRP, particular types of valuable bioresources have been collected based on the requirements of researchers having interests in basic biology, functional genomics, evolutionary biology and breeding. A large number of genetic strains, accessions and genomic resources have been collected and distributed for various unique studies. Excellent novel findings made using NBRP bioresources of many organisms including rice have been published (Kawakatsu *et al.* 2005, Komatsuda *et al.* 2007, Nomura *et al.* 2005, Suzuki *et al.* 2008, Ueguchi-Tanaka *et al.* 2005) and this in turn has made the resources and the projects more valuable. It is anticipated that new bioresources will be developed, accumulated and incorporated into NBRP leading to new trends in genetics, genomics, breeding and interdisciplinary research. In this review we will introduce NBRP focusing on plant bioresource project activities, mainly in rice, and describe how NBRP is managed.

Structure and organization of NBRP

What's NBRP? Establishment and principles of NBRP

The National BioResource Project was launched in 2002, to promote the collection, preservation, and provision of bioresources and development of related technologies. Four programs: (1) Core facility upgrading program, (2) genome information upgrading program, (3) fundamental technologies upgrading program, and (4) information center upgrading program were established, with collaboration between them. Simultaneously the principles and the standards of bioresources in NBRP were established. To keep pace with trends in genetic materials, promote biodiversity studies and collect important resources scattered in many universities, well-developed model organisms and traditional Japanese resources were selected as appropriate resources for the project. Organisms and biological resources chosen for the project were those already preserved or developed in Japanese universities or institutions. Most projects for individual organisms are organized by several laboratories in different institutions, each having their own resources, strains, DNA clones/libraries with their information and handling capacities. Several collaborating institutes collaborate with the center facility taking a core activity. Thus, a total of 28 projects were established at 28 core institutions (http://www.nbrp.jp/, Yamazaki *et al.* 2010a): ten genera/species of animals; such as mouse, rat, *Drosophila*, silkworm; nine plant genera; rice (*Oryza*), wheat (*Triticum* and *Aegilops*), barley (*Hordeum*), *Arabidopsis,* tomato (*Solanum*), morning glory (*Ipomea*), *Lotus/Glycine*, *Chrysanthemum* and algae, and nine micro-organisms and cell lines/DNAs (Fig. 1). The kind of bioresources to be collected and managed was investigated and decided within each research community. Research objectives and goals of NBRP are briefly indicated in Fig. 1 (http://www.nbrp.jp/about/about.jsp).

Collection and management of bioresources

Until 2002 when NBRP was implemented, many important experimental materials were stored in various university laboratories. In addition to these materials, a series of hybrid progeny lines, genetically modified strain libraries such as mutant lines, DNA libraries and other molecular resources had been developed and established. The first task of NBRP was the accumulation and arrangement of information about how many and what kind of strains exist, where they are stored and how much data relating to the strains have been accumulated for each organism. Then the steering committee could decide what resources of each organism should be managed in the community.

In the plant bioresource projects, most resources contain a large amount of germplasm collected around the world, mutant libraries, various genetic populations derived from crossed progenies and those substituted for chromosomes. Molecular materials, such as genomic and cDNA libraries, were also deposited in the bioresource bank of NBRP. The objectives and contents of the bioresource project of each plant are summarized in the following section. All information about the resources i.e. accession number, origin of lines, biological and molecular characters of each line/clone and other related data are managed in the "Information Center" for almost all NBRP resources (Yamazaki *et al.* 2010a). Rice resource data is available in the NBRP site; http://www.nbrp.jp/report/reportProject.jsp and is also accessible from Oryzabase (Yamazaki *et al.* 2010b).

Collaboration with other plant resources/germplasm in Japan

There is an active and large-scale resource center for plants not supervised by MEXT. A large amount of agricultural germplasm of wild and cultivated rice, barley, wheat, other grass species, legumes, tuber crops, and forage crops are managed in the Genebank of the National Institute of Agrobiological Sciences (NIAS) under the Ministry of Agriculture, Forestry and Fisheries (MAFF). In addition, many other genomic resources especially for rice; Tos17 mutant populations, full length cDNA libraries and several useful databases are also managed at NIAS. Genomic and biological information for legumes and other crop plants have been also accumulated (http://www.gene.affrc.go.jp/index_j.php). There is good collaboration between the NIAS GeneBank and the NBRP. In the case of rice resources, the NIAS Genebank and the NBRP exchange opinions and information to manage efficient resource banking in both projects for the benefit of researchers in the rice community. There are many other small-scale resources in university laboratories that were not incorporated into the NBRP. When these become recognized as valuable resources within the research communities, NBRP should make efforts to collect and manage them. The organizing and steering committee and facility members for each organism recognize that the organisms and materials currently managed in the NBRP may be replaced with newly developed resources and organisms, responding to the demands of the time. However, judgment on the range of germplasm collection would be complicated and difficult due to the biodiversity problem.

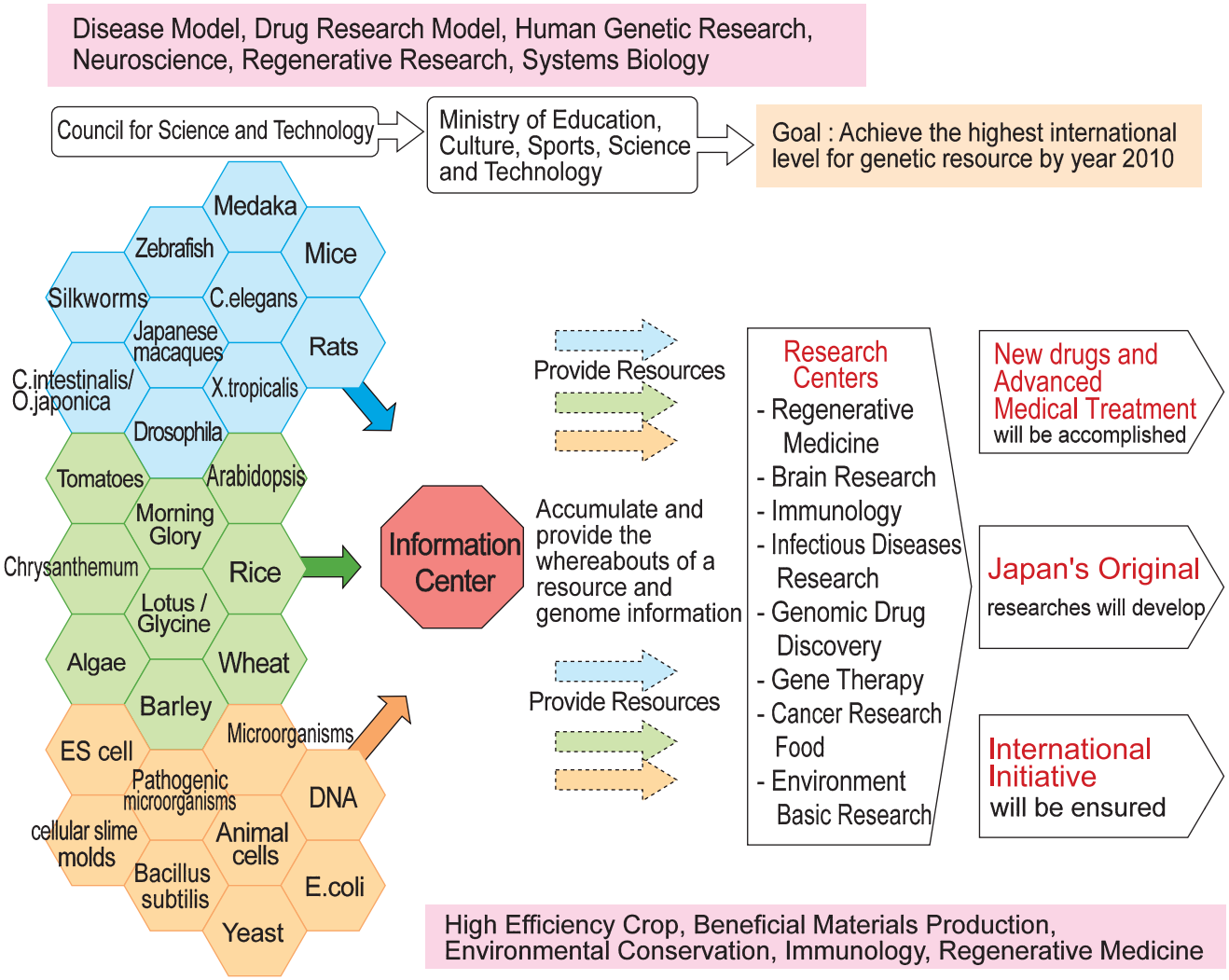

Fig. 1. Structure and organization of NBRP that includes nine plant bioresources (green color).

Plant bioresources in NBRP

At present, the NBRP-PLANT deals with both common and different categories of biological resources in each of nine organisms; rice, wheat, barley, *Lotus/Glycine*, tomato, *Arabidopsis*, *Chrysanthemum*, morning glory and algae. The categories and amount of resources for each organism have been determined depending on the current situation of each community. Brief descriptions for nine plant bioresource projects are shown below focused on (1) objective of the project, (2) contents and number (in parentheses) of bioresources and (3) center facility of each project. The terms strain, line and accession used here refer to different categories of resources. Strain means an established variety with a homogenous genetic background such as a cultivar. Line means a genetically developed independent descendant having the almost similar background such as mutant and tagged lines. Accession means an independently collected and/or propagated seed or plant originating from one or more plants in the natural population. The information is quoted from NBRP information center HP (http://www.nbrp.jp/) and summarized here.

Rice

(1) Objective: Establishment of a structure to collect, manage and release the rice bioresources and information which have been preserved or developed in universities and institutions under the MEXT. (2) Contents: 21 wild species in 9 genomes (1,701 accessions), East, Southeast and Southwest Asian local varieties & wild relatives 4,700 accessions), MNU-induced chemical mutants (8,789 lines), Marker tester lines (1,069), Recombinant inbred lines (599), Chromosome segment substitution lines (445), and other experimental lines (357). Details of the resources are shown in Table 1 and the following section. (3) Center facility: Genetic Strains Research Center, National Institute of Genetics (in charge of the project: Prof. Nori Kurata).

Wheat

(1) Objective: In the second phase, NBRP-WHEAT does

not only continue the maintenance and distribution of seed stocks and DNA clones of wheat that were collected in the first phase of NBRP, but also continue the collection of seed stocks and DNA clones, focusing on wild species and local breeds that have not yet been incorporated into the NBRP project, and on full-length cDNA clones and EST clones. (2) Contents: Wheat wild strains (4,782), Wheat cultivated strains (5,418), Wheat experimental lines and others (1,316), Rye wild and cultivated strains (48), EST (556,545 clones), *Triticeae* full-length cDNA (11,902 clones). (3) Center facility: Graduate School of Agriculture, Kyoto University (in charge: Prof. Takashi Endo).

Barley

(1) Objective: Supply of all available resources to both local and foreign scientists to accelerate the genome and genetic analysis, and development of new cultivars in barley and related species. (2) Contents: Cultivar and experimental resources (5,256 strains), Core collection (380 strains), EST/cDNA (139,933 clones), BAC (300,000 clones) + BAC filter (one library) and BAC superpool DNA. (3) Center facility: Research Institute of Bioresources, Okayama University (in charge: Prof. Kazuhiro Sato).

Lotus/Glycine

(1) Objective: *Lotus japonicus* (Japanese trefoil) is a wild perennial plant with a small genome and a short life cycle. Soybean (*Glycine max*), on the other hand is one of the major crops for protein and oil production in the world and an ideal leguminous crop model. It originated in East Asia, where a rich genetic diversity of both cultivated and wild forms exists. This project aims to promote legume research through management of useful legume resources. We opened "Legume Base" in Miyazaki University to facilitate utilization of materials by the research community. (2) Contents: *Lotus:* Domestic wild strains (108), RILs & experimental lines (208), EMS mutant lines (171), EMS M_2 bulked seeds (7 lines), BAC library (10,830 clones), TAC library (16,656 clones), cDNA (92,389 clones), transformation vectors (6 clones), *Glycine*: Wild strains (1,159 acc.), RILs & experimental lines (265), cultivar (304 strains), mutant lines (21), full-length cDNA (37,890 clones). (3) Center facility: Frontier Science Research Center, Miyazaki University (in charge: Prof. Ryo Akashi).

Tomato

(1) Objective: Tomato is a model crop for studying *Solanaceae* and fruit development. The tomato genome sequence has been published recently by the International *Solanaceae* Genomics project (SOL). In order to effectively and efficiently utilize the information released by SOL, establishment of functional genomics tools and resources in tomato is urgently needed. In this project, tomato bioresources of Micro-Tom mutants will be established by Tsukuba University and full-length cDNA clones and promoter clones by the Kazusa DNA Research Institute. (2) Contents: Wild and cultivated strains (89), EMS mutants (761 lines), gamma-ray irradiated mutants (60 lines), EMS M_3 bulked seeds (2,300 lines), gamma-ray irradiated M_3 bulked seeds (2,300 lines), full-length cDNA (141,707 clones). (3) Center facility: Graduate School of Life and Environmental Sciences, University of Tsukuba (in charge: Prof. Hiroshi Ezura).

Arabidopsis

(1) Objective: *Arabidopsis* is an experimental plant that is used in many laboratories in the world because of its small genome size and short life cycle. The entire genome sequence of *Arabidopsis* has been completely characterized by the end of 20th century. The Experimental Plant Division of RIKEN preserves and distributes *Arabidopsis* seeds, plant genetic materials and cultured plant cells through the National Bioresource Project. (2) Contents: Transposon-tagged lines (15,267), Activation (T-DNA)-tagged lines (36,650), *Arabidopsis* FOX lines (5,000), natural accessions, mutants and related species (from SASSC) (1,231 lines), individual mutants and transgenic lines (20), *Arabidopsis* full-length cDNA (251,382 clones), *Physcomitrella patens* full-length cDNA (149,363 clones), Tobacco EST/full-length cDNA (22,221 clones). (3) Center facility: RIKEN BioResource Center (in charge: Dr. Masatomo Kobayashi).

Morning glory

(1) Objective: The Japanese morning glory (*Ipomoea nil* or *Pharbitis nil*) was introduced from China, and was established as a floricultural plant in Japan. In the late Edo era (1806~1860), many mutants were isolated and many genetic and physiological studies of the Japanese morning glory were conducted by Japanese biologists. In this project, we collect, develop and distribute mutant strains, EST clones, linkage maps, and transgenic lines of the Japanese morning glory to both local and foreign biologists. These resources will contribute to genome and genetic analysis, and development of new cultivars in the Japanese morning glory and related species. (2) Contents: Related species (108 strains), mutant lines (714), EST (62,235 clones), linkage map information. (3) Center facility: Graduate School of Science, Kyushu University (in charge: Ass. Prof. Eiji Nitasaka).

Chrysanthemum

(1) Objective: *Chrysanthemum sensu lato* belongs to the *Asteraceae*, the largest family among the Angiosperms. They are well-diversified and distributed widely in Eurasia, centering in East Asia. *Chrysanthemum sensu lato* is utilized in a wide range of research fields such as plant genetics and evolutionary biology in relation to polyploidization, plant physiology associated with flower development as well as in medicine, pharmacy, agriculture and horticulture. However, many species of the *Chrysanthemum sensu lato* still have not been introduced to our research fields. In this project, new plant materials characterized through molecular genetic analysis, artificial hybridization and so on are managed. (2)

Contents: *Chrysanthemum* (113 strains), Hybrid (99 lines). (3) Center facility: Graduate School of Science, Hiroshima University (in charge: Prof. Makoto Kusaba).

Algae

(1) Objective: Algae are defined as an assemblage of polyphyletic organisms that carry out oxygen-evolving photosynthesis excepting land plants. As shown in this definition, algae include various genetic elements that represent bacteria, plants and protozoa, thus living in different habitats including extreme environments. From these characteristics, versatile biological functions may be expected in algae. To facilitate the utilization of such algae, systematic collection of algal strains and strain information is necessary. In this project we will focus on the distribution of genome DNA, addition of strain information, and quality control as well as collecting new and important taxa. (2) Contents: microalgae (2,096 strains), macroalgae (380 strains). (3) Center facility: Environmental Biology Division, National Institute of Environmental Studies (in charge: Dr. Fumie Kasai).

Rice genetic resources in NBRP

A total of about 16,700 strains are being managed in the NBRP-RICE. NIG deals with wild species of rice and Kyushu University with chemical mutant lines and chromosome-modified/genome-substitution lines. NIG acts as the center of the project while Kyushu University with two involved Laboratories serves as a sub-center. In addition, two other Laboratories in the University of Tokyo and Nagoya University are incorporated as collaborating institutions for strain evaluation and accumulation of biological information. During the Phase I of NBRP, Shizuoka University, the Research Institute for Humanity and Nature (RIHN), Tohoku University and Hirosaki University joined us and many land race strains and close relative wild strains or populations collected in Asian countries were deposited. These resources were taken over by the Phase II project.

The rice bioresource project is organized in the "Core facility upgrading program" to promote collection, maintenance, characterization, accumulation of strains' biological and molecular information and distribution of rice bioresources for the scientific community. In the case of rice, several large-scale bioresource projects are being conducted in the GeneBank and NIAS under the MAFF organization. A large amount of cultivars and wild relative strains were collected in the GeneBank, and the full genome sequencing project, full-length cDNA project and Tos17 insertion mutagenesis project have produced a huge amount of resources and information. Therefore, the NBRP-RICE steering committee decided to deal with several unique and valuable bioresources useful for rice scientific community that could complement other large rice resources like those in GeneBank and NIAS. Bioresources selected and managed in NBRP-RICE are shown below and summarized in Table 1. All these biological resources and information are accessible through the NBRP resource database (http://www.nbrp.jp/) and also at the rice comprehensive genome database "Oryzabase" (http://www.shigen.nig.ac.jp/rice/oryzabase/top/top.jsp).

Wild species collection

NIG has a long history of collecting wild species of rice 'genus *Oryza*' from all over the world; Asia, Oceania, Africa and Central and South America. A total of 1,701 accessions consisting of 21 wild species of nine genome types are managed. Of these, 281 accessions have detailed information and accessible as core collection strains. Details about the wild *Oryza* resources are described in this issue (Nonomura *et al.* 2010). About 1,400 accessions of Asian wild species closely related to cultivated rice, most of which have the AA genome, and 3,300 landraces are also incorporated in NBRP-RICE. These are collected as populations suitable for the study of natural population structure and variations at the population level.

RILs, CSSLs, and ILs

The Plant Breeding Laboratory in Kyushu University is responsible for managing several types of experimental genetic stocks, such as recombinant inbred lines (RILs), backcrossed inbred lines, chromosome segment substitution lines (CSSLs) and introgression lines (ILs) (Table 1). ILs carrying series of chromosome segments from wild AA genome species have been incorporated in the rice NBRP project. Since several CSSLs and ILs have been developed in other institutions, these were from different cross combinations thus contributing also in widening natural variation. Several examples were reviewed by Fukuoka *et al.* (2010) in this issue and also introduced by Hirabayashi *et al.* (2010), Shim *et al.* (2010), Yasui *et al.* (2010), and Yoshimura *et al.* (2010).

Mutant collections

The institute of genetic resources in Kyushu University has the role of managing chemical mutant lines in NBRP. Details of MNU-induced mutant lines deposited in NBRP are also described in this issue (Satoh *et al.* 2010). There are two important points about this mutant collection. The first is the common categorization of the mutant phenotypes with that of *Tos17* mutants managed in NIAS. This idea came from the activities of the rice resource management group. This set-up makes for seamless phenotype screening of two different mutant categories. The second point is the utilization of the MNU-induced M_2 mutant lines for SNP screening by TILLING. The MNU-induced M_2 population had a very high mutation rate suitable for screening of nucleotide substitution mutations (Suzuki *et al.* 2008). The high mutation rate of MNU mutagenesis is reflected in that a number of unique mutants found in MNU-induced mutant lines have not been identified in other resources. We have already confirmed the usefulness of the combination of MNU-induced mutant populations and TILLING screening for isolation of mutants of any gene. Therefore, the MNU-induced mutant population and TILLING system should be made available to rice researcher.

Table 1. Rice genetic stocks maintained under National Bioresourse Project—NBRP-RICE—

Genetic stock	Origin/Donor	Category ID	No. of strain	Seed avairability[a]
Wild relatives of genus *Oryza*	Worldwide	W	1,701	NBRP, NIG
Wild relatives, -Core collection-	Worldwide	W	(281)	NBRP, NIG
Wild relatives with AA genome	East and Southeast Asia		1,400	NBRP, NIG
Landrace	East and Southeast Asia		3,300	NBRP, NIG
Induced mutant line	Taichung65, Kinmaze	CM, TCM, KCM	8,789	NBRP, KU
Gene marker line		SG, FL, Fn (T65)	1,069	NBRP, KU
Chromosomal aberrations	Reciprocal translocation, Primary trisomics, Monosomic alien addition lines	RT, Triplo, MAAL	357	NBRP, KU
Recombinant inbred lines	Intersubspecific cross	RIA, RIB, RIC, RID	399	NBRP, KU
Backcrossed inbred lines	Interspecific cross	GBIL	200	NBRP, KU
Chromosome segment substitution lines	Intersubspecific cross	IAS, AIS,TD-CSSL, TA-CSSL	257	NBRP, KU
Introgression lines	Interspecific cross	GILs, GLU-ILs, MER-ILs	188	NBRP, KU

[a] NIG: National institute of Genetics, KU: Institute of Genetic Resources and Plant Breeding Laboratory, Faculty of Agriculture, Kyushu University

NBRP-RICE resources of other categories and those to be established in step with the demands of the times

Several other categories of experimental strains utilized for more than twenty years in classical genetics have been collected in the NBRP-RICE. They are gene marker lines (strain ID is SG, FL and Fn (T65) in Table 1) equipped with reciprocal translocation lines, primary trisomics and other chromosome aberrant lines such as monosomic alien addition lines.

To promote future studies in basic and applied sciences, we will need to incorporate additional resources into NBRP-RICE. Accessibility and utilization of the plant resources will be facilitated by genome-based strain information like full genome sequence data. High-throughput sequencing will soon make it possible to analyze the genome structure of a considerable number of wild strains and mutant populations. This information will considerably aid future planning in functional genomics, evolutionary studies, breeding sciences and resource management.

Practical use, improved value of resources and project activities

Our NBRP-RICE resources have been distributed to worldwide users and used to obtain novel and excellent findings ranging from basic genomics, developmental genetics, evolutionary genetics to unique gene identification and characterization. BAC libraries were constructed in several wild species for genomic studies (Ammiraju *et al.* 2006). Many wild rice accessions have been utilized in evolutionary studies to reveal divergent pathways and genetic characteristics of diverged accessions (Cheng *et al.* 2003). An excellent example of very useful novel gene isolation and its distribution in wild species is reported recently (Hattori *et al.* 2009). Very basic, unique and important gene isolation and characterization have been also achieved using MNU-induced mutants: for hormone biosynthesis or signaling pathways (Ueguchi-Tanaka *et al.* 2005); for developmental genetics in embryo formation (Nagasaki *et al.* 2007); heterochrony (Kawakatsu *et al.* 2006); panicle formation (Kurakawa *et al.* 2007); and gene nomenclature (Kurata *et al.* 2005). These exciting findings most certainly promote utilization of NBRP-RICE bioresources, and in turn encourage the advancement of bioresource projects.

Future directions

Managing rice bioresources

In recent years, bioresources is generally divided into two categories; (1) genetic resources which are genetically rearranged strains, such as insertion mutant lines, chemical mutant lines, chromosome segment substitution lines, recombinant inbred lines etc. and (2) germplasm collections representing natural genetic variation with no genetic modification. The bioresources in NBRP are composed of two such major categories; one is germplasm collected from all over the world and maintained for a long period and the other is genetic resources produced at genome-wide scales in recent years. The germplasm collection is of particular value because it captures the natural diversity of the organisms at the time of collection. The natural diversity is not static, and some germplasms may disappear or lost unless artificially preserved. Undoubtedly, wild strains of rice possess useful genes. However, their fundamental genome structures have not yet been well characterized and useful genes may be lost or changed in cultivated strains. Therefore long term and careful maintenance of wild relatives and rare landraces is extremely important as part of the resource project. On the other hand, genetic resources produced by modern technology can easily be surpassed by next generation resources newly developed with more efficient and improved methods/tools. In addition, a more simple ways to select minimum number of mutant lines and genetically recombined or substituted lines would be adopted in the near future. These new methodologies have the potential to allow new strategies

minimizing time and labor costs in selecting and maintaining genetic resources. The NBRP-RICE organizing committee is keen to incorporate such valuable new genetic resources and facilities under the support of NBRP.

Problems in the rice bioresource project

The problems associated with almost all the bioresources are the limits of resource number and capacity in the given project. However, these problems should be solved by selecting and focusing resources as efficiently as possible for each research community. The kinds of resources required for collection and the problems to be solved differ with each project depending on the situations of the organism and the research community. In the case of rice, the most important problem is the maintenance of biodiversity of wild species accessions. Though rice is a self-pollinating plant, most wild species have partial out-crossing ability, so the seeds of wild species contain a high level of genetic heterogeneity. Even among progeny plants derived from open-pollinated seeds of one plant, there is phenotypic variability. Therefore, it is extremely difficult to maintain the original biodiversity. Purification of progeny lines to keep the original genetic diversity requires laborious work and a huge number of lines. One strategy to solve this problem is to maintain seeds in two ways. One method is mixed seed propagation, that is, more than 30 plants derived from one original population are grown in high planting density. All seeds are harvested from these plants after open-pollination and mixed together. They are treated as one accession. The other method is to separate all segregated lines discriminated by phenotypic characteristics. Users may request lines according to their research objectives. In actual practice, however, seed propagation of wild species is very difficult; space, equipment and labor limitation does not allow us to fully employ the two strategies.

Rice germplasm and genetic resources in the world

There are many other rice germplasm, genetic resources, genome/molecular information and valuable databases in the world besides those in NBRP. As for germplasm, institutes belonging to international organizations such as the International Rice Research Institute have accumulated a lot of cultivars, landraces and wild species. Recently the functional genomics consortium has also gathered information about mutant resources including EMS induced mutants, T-DNA tagged lines, *Ac/Ds* insertion lines and *Tos17* insertion lines (Kurishnan *et al.* 2009). Since materials and information become more and more available, researchers can choose suitable resources based on their objectives. However, more than 200,000 tagged and/or insertion lines so far obtained have not resulted to mutations in all of the rice genes. On the other hand, only 1,000 MNU-induced mutant lines contain nine nucleotide substitutions for every 1 kb length of the chromosomes, suggesting that we should be able to identify mutants of any rice gene from this population by Tilling (Suzuki *et al.* 2008). Likewise, irradiated mutant lines are also promising for the identification of mutations in almost all genes. Rapid and efficient development of bioresources and screening methods will be achieved successively in the coming decade, and strategies and resources employed in NBRP will be widely recognized and used.

Acknowledgement

We would like to thank Dr. Matthew Shenton for his critical reading and revising the manuscript. This work is fully supported by the National Bioresource Project, Japan.

Literature Cited

Ammiraju, J.S.S., M. Luo, J.L. Goicoechea, W. Wang, D. Kudrna, C. Muller, J. Talag, H. Kim, N.B. Sisneros, B. Blackmon *et al.* (2006) The *Oryza* bacterial artificial chromosome library resource: Construction and analysis of 12 deep-coverage large-insert BAC libraries that represent the 10 genomes types of the genus *Oryza*. Genome Res. 16: 140–147.

Cheng, C., R. Motohashi, S. Tsuchimoto, Y. Fukuta, H. Ohtsubo and E. Ohtsubo (2003) Polyphyletic origin of cultivated rice: based on the interspersion pattern of *SINE*s. Mol. Biol. Evol. 20: 67–75.

Fukuoka, S., Y. Nonoue and M. Yano (2010) Germplasm enhancement by developing advanced plant materials from diverse rice accessions. Breed. Sci. 60: 509–517.

Hattori, Y., K. Nagai, S. Furukawa, X.J. Song, R. Kawano, H. Sakakibara, J. Wu, T. Matsumoto, A. Yoshimura, H. Kitano *et al.* (2009) The ethylene response factors *SNORKEL1* and *SNORKEL2* allow rice to adapt to deep water. Nature 460: 1026–1030.

Hirabayashi, H., H. Sato, Y. Nonoue, Y. Kuno-Takemoto, Y. Takeuchi, H. Kato, H. Nemoto, T. Ogawa, M. Yano, T. Imbe *et al.* (2010) Development of Introgression lines derived from *Oryza rufipogon* and *O. glumaepatula* in the genetic background of *japonica* cultivated rice (*O. sativa* L.) and evaluation of resistance to rice blast. Breed. Sci. 60: 604–612.

Kawakatsu, T., J. Itoh, K. Miyoshi, N. Kurata, N. Alvarez, B. Veit and Y. Nagato (2006) *PLASTOCHRON2* regulates leaf initiation and maturation in rice. Plant Cell 18: 612–625.

Komatsuda, T., M. Pourkheirandish, C. He, P. Azhaguvel, H. Kanamori, D. Perovic, N. Stein, A. Graner, T. Wichker, A. Tagiri *et al.* (2007) Six-rowed barley originated from a mutation in a homeodomain-leucine zipper I-class homeobox gene. Proc. Natl. Acad. Sci. USA 104: 1424–1429.

Kurakawa, T., N. Ueda, M. Maekawa, K. Kobayashi, M. Kojima, Y. Nagato, H. Sakakibara and J. Kyozuka (2007) Direct control of shoot meristem activity by a cytokinin-activating enzyme. Nature 445: 652–655.

Kurata, N., K. Miyoshi, K.I. Nonomura, H. Yasui, Y. Yamazaki and Y. Ito (2005) Rice mutants and genes related to organ development, morphogenesis and physiological traits. Plant Cell Physiol. 46: 48–62.

Kurishnan, A., Guiderdori, E. An, G. Hsing, Y-I. Han, H-D. Lee, M.-C. Yu, S-M. Upadhyaya, N.S. Ramachandran, Q. Zhang *et al.* (2009) Mutant resources in rice for functional genomics of the grasses. Plant Physiol. 49: 165–170.

Nagasaki, H., J. Itoh, K. Hayashi, K. Hibara, N. Satoh-Nagasawa, M. Nosaka, M. Mukouhata, M. Ashikari, H. Kitano, M. Matsuoka *et al.* (2007) The small interfering RNA production pathway is required for shoot meristem initiation in rice. Proc. Natl. Acad. Sci. USA

104: 14867–14871.

Nomura, T., A. Ishihara, R.C. Yanagita, T.R. Endo and K. Imamura (2005) Three genomes differentially contribute to the biosynthesis of benzoxazinones in hexaploid wheat. Proc. Natl. Acad. Sci. USA 102: 9842–9847.

Nonomura, K.I., H. Morishima, T. Miyabayashi, S. Yamaki, M. Eiguchi, T. Kubo and N. Kurata (2010) The wild *Oryza* collection in National BioResource Project (NBRP) of Japan: History, biodiversity and utility. Breed. Sci. 60: 502–508.

Sakaguchi, J. and H. Fukuda (2008) Cell differentiation in the longitudinal veins and formation of commissural veins in rice (*Oryza sativa*) and maize (*Zea mays*). J. Plant Res. 121: 593–602.

Satoh, H., H. Matsusaka and T. Kumamaru (2010) Use of *N*-methyl-*N*-nitrosourea treatment of fertilized egg cells for saturation mutagenesis of rice. Breed. Sci. 60: 475–485.

Shim, R.A., E.R. Angeles, M. Ashikari and T. Takashi (2010) Development and evaluation of *Oryza glaberrima* Steud chromosome segment substitution lines (CSSLs) in the background of *O. sativa* L. cv. Koshihikari. Breed. Sci. 60: 613–619.

Suzuki, T., M. Eiguchi, T. Kumamaru, H. Satoh, H. Matsusaka, K. Moriguchi, Y. Nagato and N. Kurata (2008) MNU-induced mutant pools and high performance TILLING enable finding of any gene mutation in rice. Mol. Genet. Genomics 279: 213–223.

Ueguchi-Tanaka, M., M. Ashikari, M. Nakajima, H. Itoh, E. Katoh, M. Kobayashi, T.Y. Chow, Y.I. Hsing, H. Kitano, I. Yamaguchi *et al.* (2005) *GIBBERELLIN INSENSITIVE DWARF1* encodes a soluble receptor for gibberellin. Nature 437: 693–698.

Yamaki, S., T. Miyabayashi, M. Eiguchi, H. Kitano, K.I. Nonomura and N. Kurata (2010) Diversity of panicle branching patterns in wild relatives of rice. Breed. Sci. 60: 586–596.

Yamazaki, Y., R. Akashi, Y. Banno, T. Endo, H. Ezura, K. Fukami-Kobayashi, K. Inaba, T. Isa, K. Kamei, F. Kasai *et al.* (2010a) NBRP databases: databases of biological resources in Japan. Nucleic Acids Res. 38: D26–D32.

Yamazaki, Y., S. Sakaniwa, R. Tsuchiya, K.I. Nonomura and N. Kurata (2010b) Oryzabase: an integrated information resource for rice science. Breed. Sci. 60: 544–548.

Yasui, H., Y. Yamagata and A. Yoshimura (2010) Development of chromosome segment substitution lines derived from *indica* rice donor cultivars DV85 and ARC10313 in the genetic background of *japonica* cultivar Taichung 65. Breed. Sci. 60: 620–628.

Yoshimura, A., H. Nagayama, Sobrizal, T. Kurakazu, P.L. Sanchez, K. Doi, Y. Yamagata and H. Yasui (2010) Introgression lines of rice (*Oryza sativa* L.) carrying a donor genome from the wild species, *O. glumaepatula* Steud. and *O. meridionalis* Ng. Breed. Sci. 60: 597–603.