

# Cereal Resources in National BioResource Project of Japan

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## SYNOPSIS

The National BioResource Project of Japan is a governmental project to promote domestic/international research activities using biological resources. The project has 27 biological resources including three cereal resources. The core center and sub-center which historically collected the cereal resources were selected for each cereal program. These resources are categorized into several different types in the project; germplasm, genetic stocks, genome resources and database information. Contents of rice resources are wild species, local varieties in East and Southwest Asia & wild relatives, MNU-induced chemical mutant lines, marker tester lines, chromosome substitution lines and other experimental lines. Contents of wheat resources are wild strains, cultivated strains, experimental lines, rye wild and cultivated strains; EST clones and full-length cDNA clones. Contents of barley resources are cultivar and experimental lines, core collection, EST/cDNA clones, BAC clones, their filters and superpool DNA. Each resource is accessible from the online database to see the contents and information about the resources. Links to the genome information and genomic tools are also important function of each database. The major contents and some examples are presented here.



Grain samples of rice (left), wheat (middle) and barley (right)

**Keywords:** barley, bioresource, cDNA, genetic resource, genome, germplasm, rice, wheat

## Introduction

The National BioResource Project (NBRP) was established in 2002. In the first phase (2002-2006), representative experimental biological resources were collected and managed at universities and institutions in Japan. The project is now in the second phase (2007-2011). The project has been supported by a national grant from the Ministry of Education, Culture, Sports and Technology (MEXT). The current project office and steering committee are located at the National Institute of Genetics (NIG). At the same time, each project has its organizing committee consisting of the users of the resources and advisors. The committee discusses the demands in the research community and the project plan of each crop.

The major users in the cereal projects of NBRP are interested in biology, genomics, evolution, genetics and breeding. These projects have been collecting and supplying a large number of seed samples and genomic resources and accumulating information for these research purposes.

A series of outstanding studies have been conducted using the cereal resources of NBRP<sup>1-3</sup>. The high impact publications have made the resources and the projects more attractive. In this review we will introduce the current NBRP cereal resource project activities and discuss about the future roles of each program.

## Current Status of NBRP Cereal Resources

### Rice

Objective: Establishment of a structure to collect, manage and release the rice bioresources and information which have been preserved or developed at universities and institutions under the MEXT.

Contents: Accessions of 21 wild species in 9 genomes (1,701); Accessions of local varieties in East and Southwest Asia & wild relatives (4,700); MNU-induced chemical mutant lines (8,800), marker tester lines (782), chromosome substitution lines (356), other experimental lines (370).

Core center and PI: Genetic Strains Research Center, National Institute of Genetics, Prof. Nori Kurata.

### Wheat

Objective: Maintenance and distribution of seed stocks and DNA clones of wheat focusing on wild species and local breeds, and on full-length cDNA clones and EST clones. Collection and characterization (polymorphism detection) of DNA markers to be used in the gene isolation of wheat.

Contents: Wild strains (4,782), cultivated strains (5,418), experimental lines and others (1,316) of wheat; Rye wild and cultivated strains (48); ESTs (556,545 clones); full-length cDNAs (11,902 clones).

Core center and PI: Graduate School of Agriculture, Kyoto University, Prof. Takashi R. Endo.

### Barley

Objective: Maintenance and distribution of a large number of seed stocks with evaluation data, a BAC library, cDNA clones. Development of online applications for the efficient use of these resources.

Contents: Cultivar and experimental lines (5,256 strains); Core collection (380 strains), EST/cDNA (139,933 clones), BAC (300,000 clones) + BAC filter (one library) and BAC superpool DNA.

Core center and PI: Institute of Plant Science and Resources, Okayama University, Prof. Kazuhiro Sato.

## Germplasm Collection

### Rice

A total of ca. 16,700 strains are being managed by NBRP-RICE. The National Institute of Genetics (NIG) acts as the center of the project, Kyushu University consisting of two laboratories acts as a sub-center and two other laboratories in the University of Tokyo and Nagoya University are incorporated as collaborating institutions for strain evaluation and accumulation of biological information.

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 and nine genome types are managed, and of them 281 accessions with detailed information are accessible as a set of core collection. Details about wild *Oryza* resources are described<sup>4</sup>. About 1,400 accessions of Asian wild species (mostly AA genome) closely related to cultivated rice and 3,300 landraces have also been incorporated. These were collected as populations suitable for the study of natural population structure and variations.

Kyushu University manages chemical mutant lines and chromosome-modified/genome-substitution lines (See section Genetic stocks). In the first five years in phase I of NBRP, Shizuoka University, the Research Institute for Humanity and Nature (RIHN), Tohoku University and Hirosaki University joined this project and a large number of landraces and close relative wild strains or populations collected in Asian countries were deposited. These resources are now in the current second phase project.

### Wheat

NBRP-WHEAT was originally assigned to the core and sub centers consisting of the Graduate School of Agriculture, Kyoto University (LPGKU, MOZUME), Kihara Institute for Biological Research, Yokohama City University (KIBR), and the Faculty of Agriculture, Tottori University (TACBOW). Wheat researchers in Japan have formed a committee to support the project. In the second phase, the NBRP operation is managed by LPGKU, MOZUME and KIBR.

NBRP-WHEAT conserves and distributes about 12,000 accessions of cultivated and wild wheat (the genus *Triticum*) and its related species (the genus *Aegilops* and the genus *Secale*). The most abundant categories are wild species and wheat landraces consisting of more than 10,000 accessions altogether. The wild species include 27 species of *Aegilops* and four species of *Triticum*. The unique features of the wild species and landraces are that they are original field collections of the explorations organized by Kyoto University during 1955-1996. Accessions are being examined for morphological and genomic characters before seed propagation and conservation. During the first phase, more than 7,000 wheat accessions, including wild species, landraces and genetic stocks, have been collected, propagated and conserved. The laboratory of Plant Genetics, Kyoto University (LPGKU) is in charge of the collection of various seed stocks of wheat that were produced in the past genetic studies on wheat. These stocks have been propagated after cytological examination.

In the second stage of NBRP-WHEAT we are also focusing on collecting polymorphic DNA markers on the core NBRP germplasm that are useful in genetic studies and wheat breeding.

### Barley

Okayama University is the only institution and core center in

NBRP-BARLEY. Cultivated barleys, wild barleys and genetic stocks are maintained and distributed in the project. These materials have been historically collected since 1940s at Okayama University. Cultivated barley was categorized into ten groups by their area or country of origin. The capital letter (initial of area or country) is used to represent the origin in these barleys. East Asian materials were divided into Japan (J), Korean Peninsula (K), China (C), Nepal (N). India is included in the Southwest Asia (I), together with Pakistan, Afghanistan, Iran, Iraq and Syria. Turkey (T) and Ethiopia (E) form respective category. Europe including former USSR (U), North Africa (B), Americas, Australia and others (A) are covering rather large areas. NBRP-BARLEY preserves ca. 5,200 cultivated barley accessions with the categories above. These germplasms have been intensively characterized by the marker traits and some important agronomic traits, such as resistances to powdery mildew. The data set is available online from Barley DB<sup>5</sup>.

These collections are also the basis of the South East Asian sub-collection of the international barley core collection<sup>6</sup>, which represents the whole barley genetic diversity. NBRP-Barley is responsible for the distribution of Southeast Asian subset collection comprised of 380 accessions. Other subset materials can be requested for USDA small grain collection (subset Americas), ICARDA (WANA and ssp. *spontaneum*), IPK, Germany (European) and Nordgen, Sweden (wild barley).

Barley, *Hordeum vulgare* L., is placed in *Hordeum*, which includes ca. 32 species and altogether ca. 45 taxa<sup>7</sup>. NBRP-Barley maintains wild barley samples, but the systematic nomenclature system is different from above<sup>7</sup> and the distribution program is not very active.

The total number preserved in NBRP-Barley is 10,980 for cultivars and landraces, 2,498 for genetic stocks and 628 for wild barleys.

## Genetic Stocks

### Rice

The Plant Breeding Laboratory in Kyushu University is responsible for managing several types of experimental genetic lines, such as recombinant inbred lines, near isogenic lines, backcrossed inbred lines, chromosome substitution lines and

reciprocal translocation lines. Particularly chromosome substitution lines carrying series of chromosome segments from wild AA genome species will be soon incorporated in the rice project. Although several similar chromosome substitution or recombinant inbred lines have been developed in other institutions, cross combinations differ with others, thus contributing to reveal wide natural variation. Several examples of the development and use of genetically recombined series of lines are also introduced<sup>8-12</sup>.

**Mutant lines:** 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<sup>13</sup>.

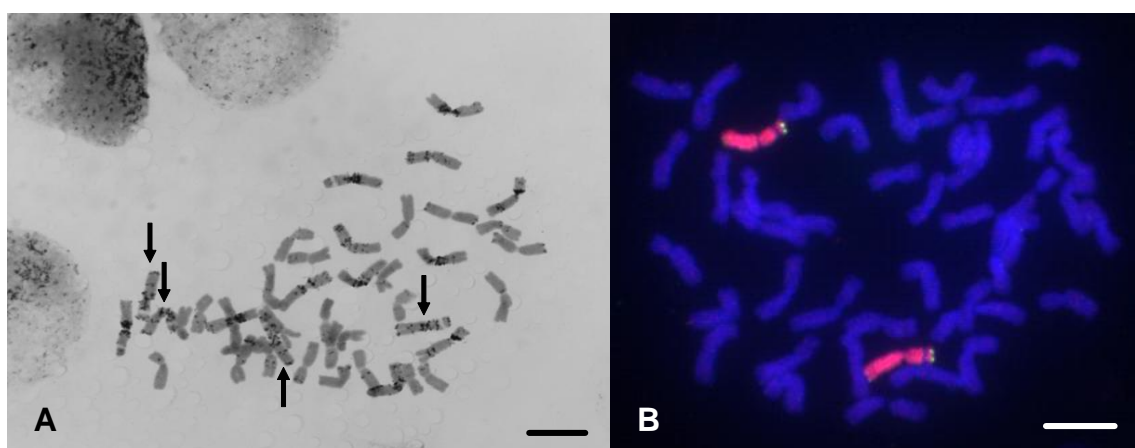
### Wheat

Wheat (*Triticum*) and its related species (*Aegilops*) belong to the Triticeae tribe that is in the Poaceae family. The characteristics of *Triticum* and *Aegilops* are that both genera consist of a series of polyploidy species, namely diploids ( $2n=2x=14$ ), tetraploids ( $2n=4x=28$ ) and hexaploids ( $2n=6x=42$ ). Common wheat or bread wheat is hexaploid, and durum wheat, or macaroni wheat, is tetraploid. NBRP-WHEAT also features a collection of experimental lines produced by many researchers for their studies (Figure 1). The representative experimental lines are "a series of aneuploid stocks of Chinese Spring wheat"<sup>14,15</sup>, "deletion lines of Chinese Spring wheat"<sup>16</sup>, "barley chromosome addition lines of Chinese Spring wheat"<sup>17,18</sup>, "D-genome substitution lines of durum wheat"<sup>19</sup>, and "cytoplasmic substitution lines of Chinese Spring wheat"<sup>20</sup>. NBRP-WHEAT handles a small number of genetic stocks of other genera in Triticeae, such as rye.

### Barley

NBRP-Barley maintains mutants, tetraploids, linkage testers and near isogenic lines for barley. These lines were developed mainly through the research activities in Okayama University and used for the development of linkage maps and genetic analysis of mutant traits<sup>21</sup>. Tetraploids were used to develop a set of trisomic series which is also used to identify chromosome localization of the trait locus. Currently, the distribution of these materials is rather limited, due to the change of materials from original mutants to backcrossed lines, or methods of linkage analysis from mutant markers to DNA markers.

Currently three mapping populations<sup>22-24</sup> are deposited from the



**Figure 1.** Mitotic metaphase cells of wheat aneuploid lines. (A) A C-banded metaphase cell of one of the nullisomic-tetrasomics. This line lacks a pair of 1A chromosomes and instead has two pairs of 1B chromosomes. Chromosomes 1A and 1B are called homoeologous chromosomes, and an extra dose of one chromosome can compensate the loss of the other homoeologous chromosome. The nullisomic-tetrasomics are useful in identifying genes located on individual wheat chromosomes. (B) An in-situ-hybridization metaphase cell of one of the barley chromosome addition lines. This line has a pair of barley 5H chromosomes and is useful in identifying genes located on chromosome 5H. Bars=10  $\mu$ m.

North American barley genome mapping project. These populations were used to develop molecular genetic maps of barley and are used as high though put mapping system by SNP arrays or fast DNA sequencing system.

The recombinant chromosome substitution lines (backcross introgression lines) are useful sources to identify genes on the specific region of the genome to precisely study and map the locus. NBRP-BARLEY is preparing to release a few sets of lines in the near future<sup>25,26</sup>.

## Genomic Resources and Databases

### Rice

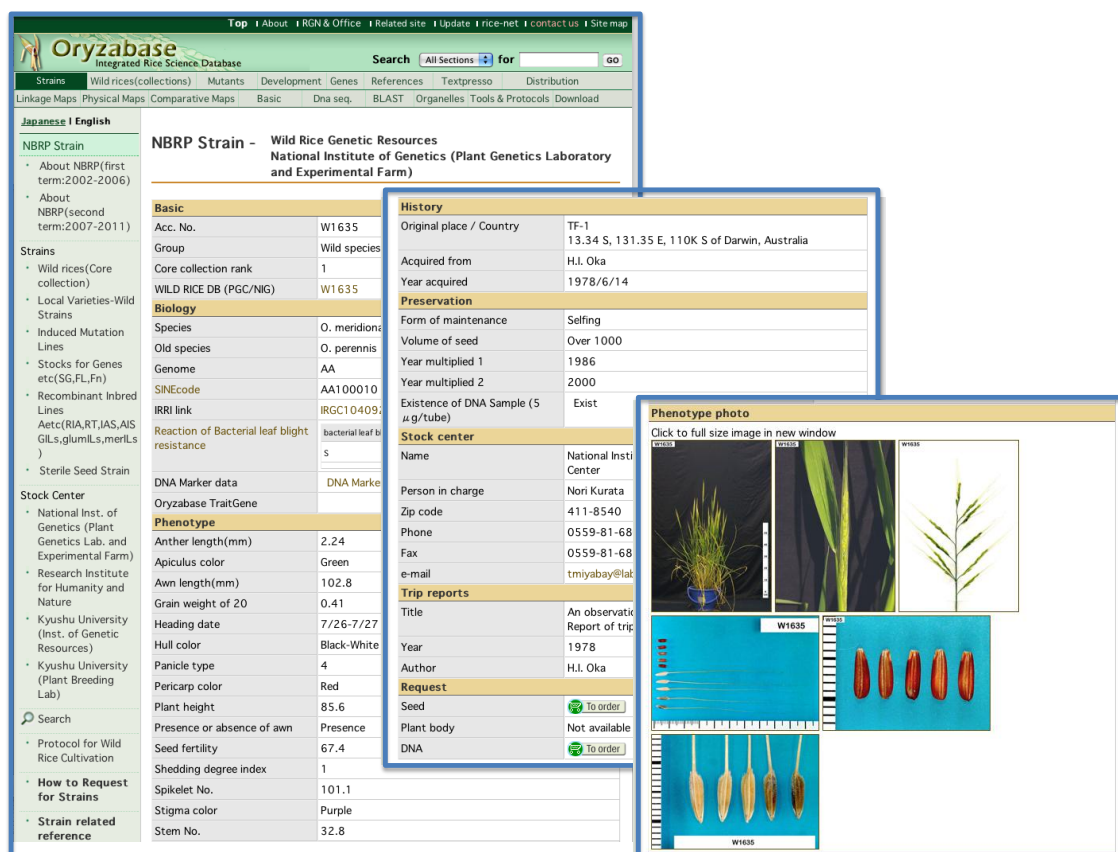
In addition to NBRP-RICE, National Institute of Agrobiological Sciences (NIAS) under the Ministry of Agriculture, Forestry and Fisheries in Japan has large-scale rice bioresource programs. Especially, the full genome sequencing project, full-length cDNA project and Tos17 insertion mutagenesis project have produced a huge amount of resources and information. Therefore, the NBRP-RICE steering committee decided to deal with several unique and valuable resources useful for rice scientific community that could complement other large rice resources like those in NIAS. All these biological resources and information are accessible through the NBRP resource database<sup>27</sup>.

The rice comprehensive database "Oryzabase"<sup>28</sup> is a successful database of trait loci and their genetic stocks used to identify the loci, which was launched in 1995. Genomic information has been added to the database on germplasm and loci so that it has become comprehensive and functional as the NBRP database<sup>29</sup>.

Oryzabase has detailed phenotype data and images on wild rice collections and mutant lines with known trait loci (Figure 2). Oryzabase also has links to two external databases: Gramene<sup>30</sup> (genetic information) and IRRI database (wild rice information at International Rice Information System<sup>31</sup>) to share and confirm the rice resource information. Updates of trait genes with data extracted from published papers including accession numbers of DNA sequences, ORF numbers of genome projects, and displays of chromosome by means of physical maps. Oryzabase also allows BLAST searches on a whole rice genome or by chromosome basis. Basic information on rice, including a definition of the tissue-specific developmental stages and information on tissue and/or developmental stage specific gene expression is also available. Also a text-mining system for scientific papers for rice is available providing a total of ca. 20,000 rice-related papers (abstracts and titles) in PubMed.

### Wheat

NBRP-WHEAT collects cDNA clones, expressed sequence tags (ESTs), full-length cDNA sequences and DNA markers as genomic resources. The cDNA libraries are obtained from different tissues of plants grown under various abiotic stresses. NBRP-WHEAT is also in charge of the maintenance of the previously collected cDNA libraries and the genomic library of Chinese Spring wheat with the transformation-competent artificial chromosome (TAC vector). Every year clones and sequences of new ESTs and full-length cDNA are increasing in number<sup>32-34</sup>. To make the genetic stocks more valuable for molecular genetic studies, NBRP-WHEAT has started to collect simple sequence repeat (SSR) markers (ca. 400 markers per year) to add genotype information to the genetic stocks. The SSR markers have been surveyed with 48 accessions of wheat and its relatives to establish a set of SSR markers that are suitable



**Figure 2.** Oryzabase provides detailed information on wild rice accessions gathered as a core collection. This shows an example of the data site of W1635 containing the above ten phenotypic measurements, color photos for several organs, collected site, year, researcher, availability of the seed stock and request form.



for surveying DNA polymorphisms among wheat strains<sup>35</sup>. The obtained SSR data are released every year on the web site KOMUGI<sup>36-38</sup>.

All genetic resources, i.e., seed stocks, cDNA clones and sequence information, collected by NBRP-WHEAT, are in the wheat genetic resources database and web site KOMUGI. In the database, all the strains of seed stocks are categorized into "wild species", "landraces" and "experimental lines" and also can be selected by the four institutions (LPGKU, MOZUME, TACBOW and KIBR) that were in charge of the collection of specific seed stocks. The database also includes DNA clones, sequence information (ESTs and markers) and related information, such as a catalogue of gene symbols for wheat, chromosome maps, BLAST and microarrays. Researchers can order the seed stocks and cDNA clones electronically from the database web site only by the handling and shipping fees.

### Barley

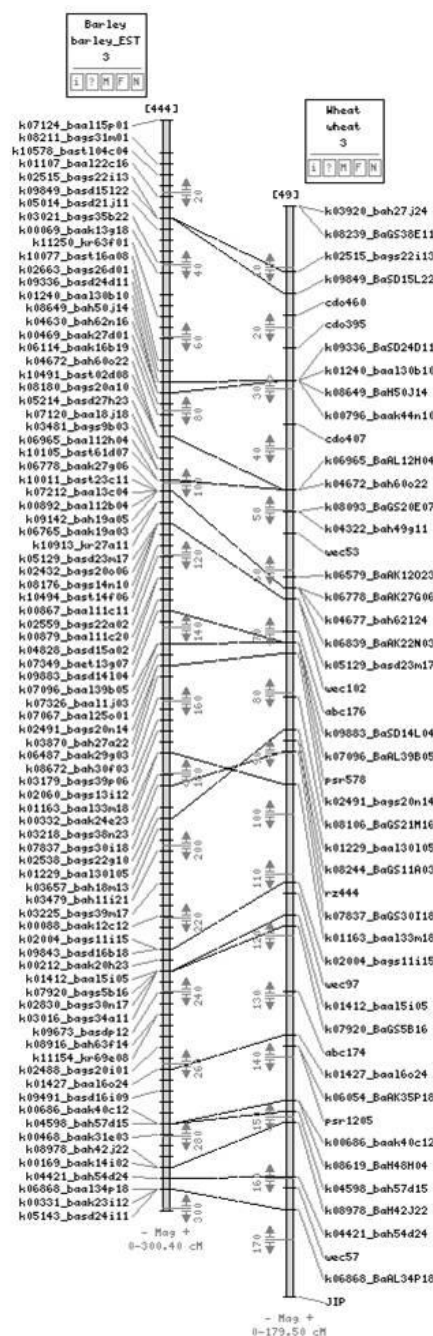
Ca. 135,000 ESTs have been sequenced and published in databases. Nine cDNA libraries from the specific organs in three different haplotypes (Haruna Nijo, Akashinriki, and H602) were used for sequencing from both ends of each clone. The EST information was also published in the HarVEST<sup>39</sup> as an effort of the international EST consortium and was used to develop Affymetrix Barley1 GeneChip<sup>40,41</sup> and SNP array system to map EST-derived unigenes on the genetic map<sup>42,43</sup> also mapped 2,890 ESTs on the single mapping population mainly by SNPs. The mapping effort of these ESTs on the diploid wheat<sup>44</sup> made an important link from barley genome to wheat genome (Figure 3). A collection of 5,006 full length (FL) cDNA sequences was developed from 15 mRNA samples from various organs and treatments were pooled to develop a cDNA library using the CAP trapper method<sup>45</sup>. The high homology between FLcDNAs and mapped barley ESTs enabled assigning linkage map positions to 151 - 233 FLcDNAs on each of the seven barley chromosomes. From these cDNA resources, 80,000 clones were distributed in NBRP-Barley.

A barley bacterial artificial chromosome (BAC) library, using the Japanese malting barley variety 'Haruna Nijo' consisted of 294,912 clones arrayed in 768 384-well micro titer plates<sup>46</sup>. The average size of each cloned insert was estimated to be 115.2kb. The genomic coverage of the library was estimated to be about 6.6 genome-equivalents. In order to rapidly identify specific BAC clones, we developed a screening strategy that combined PCR analysis of pooled BAC DNAs with colony hybridization. Membrane screening system (16 large filters) and DNA plate pools for PCR screening are also distributed.

The database of NBRP-Barley is named Barley DB<sup>5</sup> and is mainly comprised of the germplasm database (seed samples) and cDNA/ESTs. Advanced genomic information related to cDNA/ESTs is also provided in the mapping of full-length cDNAs (FLcDNAs) on rice genome (Figure 4) and electronic genetic maps<sup>47</sup> of ESTs developed at Okayama University (Figure 3). Simple protocols (only in Japanese) for users unfamiliar to barley study are also available from the web site.

The germplasm database has functions of browsing accessions by the categories of areas. A list of accessions and passport data are available by browsing. The database also provides images of seeds and spikes which are useful for identification of materials by the recipients. Search engine of the database can select accessions by ca. 30 characters or query keywords. The same system is available for the South-East Asian subset of core collection.

The database of full-length cDNA and EST/cDNA also has a browsing function to show basic clone information for each cDNA



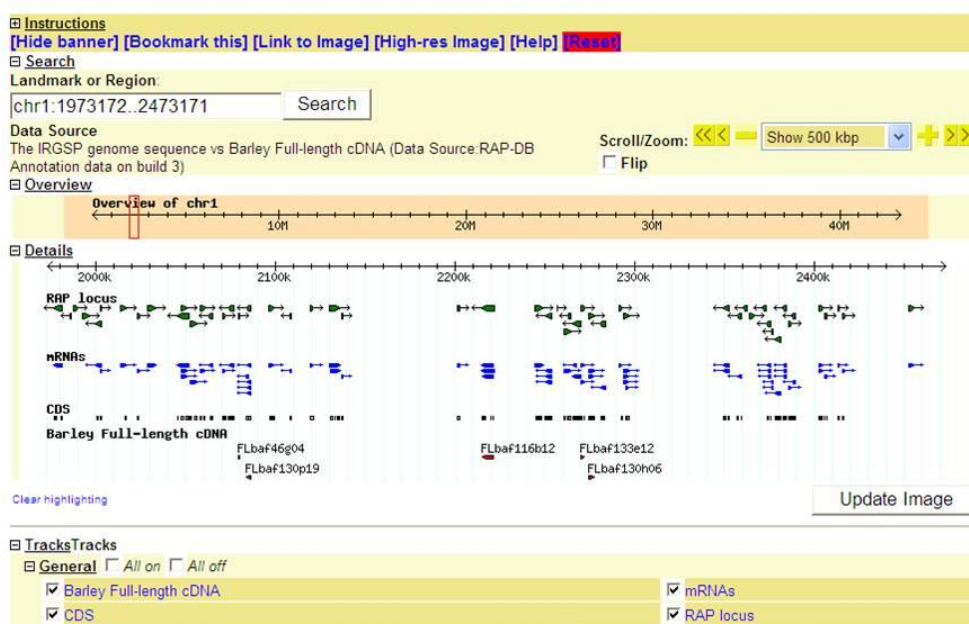
**Figure 3.** Electronic map (cMAP, Fang et al.<sup>47</sup>) of homeoeological chromosomes of barley 3H (left) and diploid wheat 3A. Lines connecting chromosomes are barley EST markers genetically mapped on barley<sup>43</sup> and diploid wheat<sup>44</sup>.

library, including annotation data by blast search on NCBI nt, uniprot and rice ORF by TIGR and RAP. The database has search engines by clone name or blast search on clones in each cDNA library.

Some additional functions are also included in the Barley DB. Of these, Reference Feedback is extremely important for NBRP-Barley project evaluation since the list of publications by using the resources can be uploaded from the website. Users can also see the list journal papers published using resources of NBRP-Barley.

## The IRGSP genome sequence vs Barley Full-length cDNA (Data Source:RAP-DB Annotation data on build 3)

Showing 500 kbp from chr1, positions 1,973,172 to 2,473,171



**Figure 4.** FLCDNAs mapped on the rice pseudomolecule to show homology to rice locus, mRNAs and CDS. The Gbrowse data set is accessible online<sup>5</sup>.

## Cereal Resources in the Future

### Rice

NBRP-RICE has been collecting experimental strains utilized in classical genetics. They are phenotype-based gene marker lines, reciprocal chromosome translocation lines, primary trisomic series and some chromosome aberrant lines. To promote future studies in basic and applied sciences, we will need to incorporate these additional resources into NBRP-RICE. Accessibility and utility of the plant resources will be facilitated by genome-based strain information like individual 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 be a considerable aid in future planning in functional genomics, evolutionary studies, breeding sciences and resource management.

### Wheat

Wheat is one of the most important cereals produced in the world and will remain important during the 21<sup>st</sup> century. More genetic variation of wheat is needed to cope with the extreme climate changes that will be occurring. NBRP-WHEAT has more than 12,000 germplasm accessions including wild species and landraces, which are the valuable sources of genetic diversity. NBRP-WHEAT will maintain all the present genetic stocks, collect new seed stocks on demand and continue to distribute them to the world. NBRP-WHEAT will continue genotyping analysis of the collected genetic stocks to make them more user-friendly. Thanks to the recent rapid progress in the "next-generation" sequencing technologies, in the near future we will obtain some tools for genotyping multiple markers throughout the genome simultaneously. In that event, forward genetics, i.e. from phenotypes of interest to responsible genes, will easily be realized in bread wheat with a huge genome, and then the importance of the genetic stocks of NBRP-WHEAT will appreciate in value.

### Barley

Barley is the fourth largest cereal crop used for brewing, food and animal feed. Barley can be grown in an extremely wide range of areas excluding the tropics. Barley is originated from the Middle East and shows high adaptability to drought and salinity stresses, which are becoming more serious problems due to global warming. Cultivated barley is a true diploid and has been extensively studied genetically. Barley genome has the same set of chromosomes as hexaploid (bread) wheat and its related species<sup>48</sup> and thus serves as a diploid model of cultivated species in Triticeae. The research community of Triticeae (barley and wheat) will be the biggest in plants in the world. The trends of research moving from model species to industrial crops may raise the numbers of barley users in the near future.

According to the schedule of International Barley Sequencing Consortium (IBGS)<sup>49</sup>, the draft genome sequence will be available in 2011 and a nearly complete sequence will be available by 2015. The uses of barley genome sequence will greatly accelerate the isolation and functional analysis of genes in barley and wheat.

The main important role of barley resource in the near future is to provide germplasm with information of genetic variation in target traits, genomic information on the germplasm (mainly DNA markers and polymorphisms) and genetic materials for gene isolation. Some of these materials have already been developed and can be provided in the near future<sup>25,26</sup>.

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