# Review

# The National BioResource Project (NBRP) Lotus and Glycine in Japan

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The objective of the National BioResource Project (NBRP) in Japan is to collect, conserve and distribute biological materials for life sciences research. The project consists of twenty-eight bioresources, including animal, plant, microorganism and DNA resources. NBRP *Lotus* and *Glycine* aims to support the development of legume research through the collection, conservation, and distribution of these bioresources. *Lotus japonicus* is a perennial legume that grows naturally throughout Japan and is widely used as a model plant for legumes because of such advantages as its small genome size and short life cycle. Soybean (*Glycine max*) has been cultivated as an important crop since ancient times, and numerous research programs have generated a large amount of basic research information and valuable bioresources for this crop. We have also developed a "LegumeBase" a specialized database for the genera *Lotus* and *Glycine*, and are maintaining this database as a part of the NBRP. In this paper we will provide an overview of the resources available from the NBRP *Lotus* and *Glycine* database site, called "LegumeBase".

Key Words: NBRP, Lotus japonicus, Glycine max, Glycine soja, LegumeBase, bioresource.

# Introduction

Leguminosae is an enormous plant family consisting of 20,000 species divided into 700 genera with high diversity in morphology (Doyle and Luckow 2003). This family includes important plant species used for grain, feed and oil due to their rich seed composition, plants of medicinal value, and those that can be used as fertilizers. Some examples include soybean (Glycine max), alfalfa (Medicago sativa), peanut (Arachis hypogaea), kudzu (Pueraria lobata) and sesbania (Sesbania aculeata). Lotus japonicus has been promoted as a model legume in the past two decades due to its short life cycle (2–3 months), self-fertility, diploidy (n = 6), small genome size (472.1 Mb), small plant size, ease of hand pollination, and amenability to Agrobacterium-mediated transformation (Handberg and Stougaard 1992). Moreover, the first whole genome sequencing of a legume was reported using L. japonicus Miyakojima MG-20 (Sato et al. 2008). Soybean (Glycine max) is the most important grain legume crop worldwide for its useful seed components such as protein, oil and secondary metabolites and consequently, has been utilized for a large number of basic and applied re-

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search investigations. Recently, a soybean whole-genome shotgun sequence of *G. max* var. Williams 82 was published (Schmutz *et al.* 2010). Another genome sequence project for Japanese soybean (*G. max* var. Enrei) has been conducted in Japan since 2007. Due to the significant numbers of investigations on these two species, we expect research on *Lotus* and *Glycine* to continue being at the forefront of plant science research in the future.

Currently, large numbers of important bioresources such as experimental strains, mutants, DNA libraries, etc., have been developed through numerous independent research programs and scientific research projects. These bioresources will continue to serve as valuable materials for basic and applied studies. The National BioResource Project (NBRP) was launched by the Japanese government in 2002 with the objective of collecting, conserving and distributing such valuable, independent resources and making them easily available to the larger research community. At present, the NBRP is a consortium of twenty-eight core facilities of animal, plant, microorganisms and DNA resources, and an information center (Yamazaki et al. 2010). The NBRP plant consists of nine resources: Arabidopsis (Arabidopsis thaliana), rice (Oryza sativa), Lotus/Glycine, wheat (Triticum aestivum), barley (Hordeum vulgare), tomato (Solanum lycopersicum), Chrysanthemum, morning glory (Ipomoea nil) and algae (Kurata et al. 2010). As part of this

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project, the *L. japonicus* and *G. max* program has been developed since the end of 2003. In this paper, we provide an overview of the extensive resources available for *L. japonicus* and *G. max* from our resource center.

### Lotus resource

#### Experimental strains

Gifu B-129 is the first established experimental *L. japonicus* strain, collected by Hirayoshi in Gifu Prefecture Japan, named Gifu B-129 by Grant and self-pollinated 9 times by Stougaard (Handberg and Stougaard 1992, Stougaard and Beuselinck 1996). Secondly, Kawaguchi (2000) established the accession Miyakojima MG-20 by self-pollinating *L. japonicus* strains from Miyakojima Island, Okinawa Prefecture, Japan. This accession is characterized by a short generation time and easily flowers under fluorescent lights. Another strain, *Lotus burttii* B-303, was established as the

third *Lotus* experimental strain, collected in Pakistan and named by Burtt (Sz.-Borsos *et al.* 1972) and self-pollinated 9 times by Kawaguchi *et al.* (2005). There is a great demand for these experimental strains that have played central roles in studying legume-specific characteristics such as nodulation, and large numbers of mutants have been isolated in the past two decades (Charpentier and Oldroyd 2010, Kawaguchi *et al.* 2002, Novák 2010, Popp and Ott 2011, Szczyglowski *et al.* 1998). All of these experimental strains are available from LegumeBase (Table 1).

### Wild accessions

*L. japonicus* ecotype is distributed across East and Central Asia, including Japan, Korea, and China, extending to West Asia into Afghanistan (Pajuelo and Stougaard 2005). Since *Lotus* adapts readily to diverse environmental conditions, such as temperature or soil type, it is thought to possess a broad range of genetic variations. The strains that we

Table 1. L. japonicus and G. max/soja resources preserved in LegumeBase

Name of resource	No. of stocked resources	Depositor	Reference
Lotus resource			
Wild accessions	180	Lotus research community, NBRP	Suginobu et al. 1988
			Kawaguchi et al. 2001
			Hashiguchi et al. 2011
Core collection <sup><i>a</i></sup>	20	NBRP	Kai <i>et al.</i> 2010
Experimental strains (Gifu B-129,	3	M. Kawaguchi, W. Grant	Stougaard and Beuselinck 1996
Miyakojima MG-20, L. burttii B-303)			Kawaguchi 2000
			Kawaguchi et al. 2005
RILs (Gifu B-129 × Miyakojima MG-20)	205	Kazusa DNA research institute	Hayashi et al. 2001
EMS mutants	171	RIKEN	-
Superroot	1	University of Miyazaki	Akashi <i>et al</i> . 1998
$M_2$ bulked seeds	$162^{b}$	NBRP	
Activation tag lines <sup>a</sup>	960	Nihon University	Imaizumi et al. 2005
M. loti STM mutants	6,671	Kazusa DNA research institute	Shimoda et al. 2008
TAC clones	72,192	Kazusa DNA research institute	Sato et al. 2001
BAC clones	14,976	Kazusa DNA research institute	Sato et al. 2007, 2008
cDNA clones	140,544	Kazusa DNA research institute	Asamizu et al. 2004
Binary vectors	6	M. Hayashi	Maekawa et al. 2008
Full-length cDNA clones	104,064	Kazusa DNA research institute	Sakurai et al. unpublished
Glycine resource			
Wild accessions	1,159	Hokkaido University	Hong et al. 2003
		-	Xu et al. 2002
Cultivars	205	Hokkaido University	Abe et al. 2003
RILs (Misuzudaizu × Moshidou Gong 503)	167	Chiba University	Tajuddin et al. 2003
		-	Watanabe et al. 2004
RILs (TK780 × B01176)	96	Hokkaido University	Liu et al. 2007
Edamame (cultivars)	8	Yamagata University	Akazawa and Egashira 2005
Fatty acid mutants	21	Saga University	Takagi et al. 2000
M <sub>2</sub> bulked seeds <sup><i>a</i></sup>	$1^c$	Saga University	Hoshino et al. 2010
Full-length cDNA clones	37,890	<i>Glycine</i> full-length cDNA consortium, RIKEN	Umezawa et al. 2008

<sup>a</sup> This resource is in preparation.

<sup>b</sup> Number of batches. We provide seed sets containing of 10–20 batches. Each set consists of 5,000–9,000 M<sub>2</sub> seeds derived from 1,000–2,000 M<sub>1</sub> plants.

<sup>c</sup> Number of batches. One batch containing 250 g; approximately 1,000 grains. The M<sub>2</sub> seed set was derived from approximately 5,000 M<sub>1</sub> plants.

currently maintain and distribute at LegumeBase were collected across several climatic zones from as far north as Rebun Island, Hokkaido (45°17′46″N) to Miyakojima Island, Okinawa (24°43′57″N) to the south. These strains were collected mainly for three purposes: first, to evaluate the potential of *L. japonicus* as a pasture plant by Shimada in 1979 and for the Gene Bank Project of the Ministry of Agriculture, Forestry and Fisheries of Japan in 1981 (Suginobu *et al.* 1988); second, to assess the suitability of this plant species to serve as a model organism for leguminous plants by Kawaguchi and Aoki since 1996 (Kawaguchi *et al.* 2001); and third, to collect *L. japonicus* bioresources for NBRP. At present, 180 accessions are stocked and 108 accessions are available via LegumeBase.

After the launch of NBRP, we studied variations in nine morphological characteristics of *L. japonicus* wild accessions (Hashiguchi *et al.* 2011). Recently, Kai *et al.* (2010) selected 20 accessions to serve as a representative core collection based on SSR (Simple Sequence Repeats) polymorphisms (Table 1). The range of morphological traits in the core collection was representative of that found in the entire collection. This core collection will be useful for genomewide studies and data obtained for this model species should lead to numerous practical applications for crop legumes.

# Recombinant inbred lines (RIL)

A total of 205 "LjMG RI Lines" (Table 1) were derived from an  $F_2$  seed cross between Miyakojima MG-20 and Gifu B-129, and were established at the Kazusa DNA Research Institute by eight times self-pollination. A total of 96 SSR markers were mapped on the chromosomes of *L. japonicus* using the  $F_2$  generation (Hayashi *et al.* 2001), and AFLP and SSR marker-based high-density linkage maps of *L. japonicus* were constructed (Wang *et al.* 2008). Gondo *et al.* (2007) reported the first quantitative trait locus (QTL) analysis of 13 phenotypic traits in two consecutive years in *L. japonicus*, and the data evaluated in this study are available at LegumeBase. In addition, macrosynteny between soybean and *L. japonicus* was analyzed with the objective of applying genomic information of the model legume *L. japonicus* to soybean (Tsubokura *et al.* 2008).

#### EMS mutants and $M_2$ bulked seeds

Ethyl methanesulfonate (EMS)-treated mutants of *L. japonicus* were isolated from Miyakojima MG-20 at the RIKEN Plant Science Research Center. There are two kinds of mutants: above-ground mutants (plantlet, leaf, stem, flower etc.) and root morphological mutants (root elon-gation, root thickness, root hair length and the number of root hairs etc.). At present, 98 homozygous mutants are available, as well as 78 heterozygous mutants. In addition, we have prepared 10 sets of EMS-treated bulked seeds of *L. japonicus* Miyakojima MG-20 (Table 1). Each set consists of 5,000–9,000 M<sub>2</sub> seeds derived from 1,000–2,000 M<sub>1</sub> plants treated with a 0.4% EMS solution for 8 to 10 hours. Users may screen the mutants themselves and use the

screened mutants for their research. Once their study is published, users are required to deposit the isolated mutant lines derived from this resource with our resource center.

# Activation tag lines

Activation tagging is a method to produce gain-offunction mutants by random insertion of tandemly repeated CaMV 35S enhancer sequences into the plant genome. This method allows the analysis of functionally redundant gene families and essential genes, whose knockout mutants cannot be obtained. Although this powerful approach has been used in Arabidopsis thaliana (Weigel et al. 2000), its application to leguminous plants was not popular because of the difficulty in genetic transformation of legumes. Imaizumi et al. (2005) improved the transformation technique for L. japonicus and produced more than 3,500 T-DNA insertional lines, demonstrating the possibility of activation tagging with L. japonicus. Activation-tagged populations of this model legume should provide a powerful tool for identifying novel genes involved in morphology, accumulation of seed storage proteins, biosynthesis of legume-specific natural products, symbiotic nitrogen fixation, and mycorrhizal formation. These activation tagged lines will also serve as suitable materials for post-genomic analyses, such as transcriptomics, proteomics and metabolomics, and will be available via LegumeBase in the future (Table 1).

#### Root culture (superroot)

We discovered super-growing roots (superroot: SR) from Lotus corniculatus L. that grow efficiently after removal of the above-ground organs and when cultured in a medium containing no plant hormones (Akashi et al. 1998) (Table 1). SRs are highly competent for plant regeneration. Moreover, protoplasts can be easily obtained from SRs that proliferate well in vitro. These characteristics are still maintained 14 years after the discovery of SR (Akashi et al. 1998, 2003). SRs can be used in physiological research as well as in functional analysis of genes using A. tumefaciens (Tanaka et al. 2008) or A. rhizogenes-mediated transformation (Jian et al. 2009). Himuro et al. (2011) developed 130 Arabidopsis fulllength cDNA overexpressor (FOX)-superroot lines using the FOX hunting system. FOX-superroot lines provide a new tool for genetic analysis and control of root growth in leguminous plants.

# cDNA, TAC and BAC clones

Sato *et al.* (2008) sequenced the entire genome of *L. japonicus* genome using the Miyakojima MG-20 strain. Various material resources such as transformation-competent artificial chromosome (TAC) (Asamizu *et al.* 2003, Kaneko *et al.* 2003, Kato *et al.* 2003, Nakamura *et al.* 2002, Sato *et al.* 2001), bacterial artificial chromosome (BAC) (Sato *et al.* 2007, 2008) and cDNA libraries (Asamizu *et al.* 2000, 2004) were developed during the genome sequencing projects. These important products of *L. japonicus* genome sequence projects are exceedingly

valuable tools for genetic and physiological studies and/or synteny analysis of leguminous plants. These resources have been deposited with our resource center and are available from LegumeBase for researchers (Table 1).

# Full-length cDNA

Full-length cDNAs are useful resources for the functional analysis of genes or proteins and are available for several plants, such as *Arabidopsis* (Seki *et al.* 1998), rice (Kikuchi *et al.* 2003), wheat (Ogihara *et al.* 2004), soybean (Umezawa *et al.* 2008), maize (*Zea mays*; Soderlund *et al.* 2009), tomato (Aoki *et al.* 2010) and barley (Matsumoto *et al.* 2011). *L. japonicus* full-length cDNAs were developed at the Kazusa DNA Research Institute and have been deposited with LegumeBase (Table 1). There are approximately 100,000 *L. japonicus* cDNA clones from a full-length enriched cDNA library, including 3,874 full read sequences that were derived from plants and roots, as well as from *in vitro* cultured cells of *L. japonicus* that were cultured under diverse chemical treatment conditions (Sakurai *et al.* unpublished).

#### Binary vectors

Promoter analysis studies have demonstrated that the polyubiquitin promoter from *L. japonicus* plants (*Ljubq1*) possesses higher activity than the CaMV35S promoter in *L. japonicus* leaves, stems, roots, nodules, and pollen (Maekawa *et al.* 2008). The GATEWAY conversion technology-compatible binary vectors that were constructed in this study for overexpression and RNAi under the control of the *Ljubq1* promoter provide alternative choices for studies in *L. japonicus*. For one of these vectors, Nakagawa *et al.* (2011) investigated expression profiles for the Nod factor (NFs) receptor gene in roots of *L. japonicus* through a complementation test using *Agrobacterium rhizogenes*-mediated transgenic *L. japonicus* with pUB-GW-GFP. In LegumeBase, six kinds of vectors are now available for research (Table 1).

### Mesorhizobium loti STM mutants

The mutant library of *M. loti* was developed by the Kazusa DNA Research Institute through transposon mutagenesis. These transposon insertion mutants were generated using the signature-tagged mutagenesis (STM) technique (Shimoda et al. 2008). At present, 6,671 STM M. loti mutants are available from LegumeBase (Table 1). Detailed information about M. loti ORFs, such as the operon structure, predicted protein domains and orthologous protein groups, is available at RhizoBase (http://bacteria.kazusa.or.jp/ rhizobase/Mesorhizobium/index.html), a database constructed by the Kazusa DNA Research Institute. The M. loti mutant STM5 that contains an inserted transposon at 738 bp of the 1,602-bp PHGDH (3-phosphoglycerate dehydrogenase) gene plays an important role in the development of an effective symbiosis between M. loti and L. japonicus (Thapanapongworakul et al. 2010).

#### **Glycine** Resource

#### Wild accessions

Wild soybean (Glycine soja) is the ancestor of the cultivated soybean (G. max) and is distributed widely in East Asia, growing in riverbanks, open areas and the peripheries of agricultural fields, and is a prostrate or a twining tall herb. Molecular assays have revealed that the wild soybean possesses rich genetic variability compared to cultivated soybean (see Xu et al. 2002, Hyten et al. 2006). In fact, the wild soybean germplasm often has provided unique variants not observed in the cultivated germplasm in seed chemical compositions, such as a storage protein variant lacking all subunits of the 7S-globulin and a variant lacking soyasapogenol A (Hajika et al. 1998, Tsukamoto et al. 1993). The wild soybean collection in LegumeBase consists of samples collected by Hokkaido University and their collaborators from various regions of Japan (Table 1) and has been used in genetic studies of seed compositions (Fukuda et al. 2005, Kanamaru et al. 2008, Shibata et al. 2008), stress tolerance studies (Hamwich and Xu 2008) and evolutionary studies (Abe et al. 1999, Hong et al. 2003, Tozuka et al. 1998, Xu et al. 2002).

#### Cultivars

Cultivated soybean, *G. max*, is the most important leguminous crop in the world due to the high quality of protein, lipid and functional components in its seeds. Nuclear SSR marker analyses have revealed that the Asian cultivated soybean population mainly consists of two sub-populations, the Chinese and Japanese populations, suggesting that genetic resources from different sub-populations could widen the genetic variability of cultivated soybeans (Abe *et al.* 2003). Around 200 accessions of *G. max* introduced from China and Korea are available from LegumeBase (Table 1), and have been evaluated for seed coat color, and fatty acid and seed isoflavone compositions.

#### Recombinant inbred lines

Two sets of soybean recombinant inbred lines (RILs) are available from LegumeBase (Table 1). The first set was developed by the single seed descent method (SSD) from an F<sub>2</sub> population of a cross between Misuzudaizu and Moshidou Gong 503 at Chiba University (Tajuddin et al. 2003, Yamanaka et al. 2000). Misuzudaizu (Norin 51) was released in 1968 from the Nagano Prefectural Agricultural Experimental Station and has a determinate habit, tawny pubescence, white flowers and large and yellow seeds. Moshidou Gong 503 was developed as a forage crop at the Jilin Agricultural Experimental Station, China, and has a semi-determinate habit, tawny pubescence, purple flowers, and small brownish compressed seeds. A total of 1,131 markers were mapped in the RIL population (Hisano et al. 2007, Xia et al. 2007) that have been used for QTL analyses for agronomical traits (Watanabe et al. 2004, Yamanaka et al. 2001, 2005) and for gene isolation (Watanabe et al. 2009,

2011). Presently, 165 lines are available via LegumeBase.

Another set of RILs, RIL MxS, was developed by SSD from an  $F_2$  population of a cross between TK780 (*G. max* parent) and B01167 (Hidaka 4) (*G. soja* parent) at Hokkaido University. The *G. max* parent has a determinate habit, tawny pubescence, and large and yellow seeds. The *G. soja* parent is an inbred pureline selection from a wild population near the Saru River in Hokkaido and has an indeterminate and twinning habit, tawny pubescence, and small and black compressed seeds. A total of 282 markers were mapped for 98 RILs, and QTL analyses for agronomic traits have been carried out (Liu *et al.* 2007). This RIL population has also been used for mapping genes isolated through the use of the high level of genetic polymorphism between the *max* and *soja* parents (Kong *et al.* 2010, Liu *et al.* 2008, 2010, Matsuura *et al.* 2009).

### Fatty acid mutants

A total of 21 mutants for fatty acid compositions of seed oil are available from LegumeBase (Table 1). These mutants were developed by X-ray mutagenesis from a cultivar "Bay" at Saga University. The mutants include low- $\alpha$ -linolenic acid mutants (Anai et al. 2005, Takagi et al. 2000) and higholeic acid mutants (Anai et al. 2008). All of the lines were confirmed to be homozygous for their respective genes by progeny tests. A mutant line for high oleic acid content, M23, possesses a dysfunctional allele of GmFAD2-1A that results in a higher level of oleic acid and a reduced level of linolenic acid (Anai et al. 2008). M23 has been used to develop a high-oleic acid line (with a seed oleic acid content greater than 80%) in combination with a dysfunctional allele GmFAD2-1B in another copy of the FAD2 gene, through the use of genetic resources (Pham et al. 2010, 2011) and a reverse genetic approach using mutagenesis (Hoshino et al. 2010). These mutant lines that have variable fatty acid compositions are valuable in soybean breeding for improving seed oil quality.

# $M_2$ bulked seeds

EMS-treated bulked seeds of *G. max* were developed from a cultivar "Fukuyutaka" at Saga University. The  $M_2$ seed set is derived from approximately 5,000 M<sub>1</sub> plants. Part of this M<sub>2</sub> population was used in a reverse genetic screening technique called TILLING (Hoshino *et al.* 2010). Users could screen the mutants themselves for use in their research. After their studies are published, users are required to deposit the isolated mutant lines derived from this resource in our resource center. These soybean M<sub>2</sub> bulked seeds will be available from LegumeBase in the near future.

# Edamame (vegetable soybean)

LegumeBase includes a list of characteristics affecting the quality of edamame, vegetable soybean, for 39 accessions collected in Yamagata Prefecture in northern Japan (Akazawa and Egashira 2005). A local variety of edamame named 'Dadachamame,' which was established in Yamagata Prefecture not less than 150 years ago, has a particular aroma, sweetness, and tastiness. The sweetness and tastiness are due to high contents of sucrose, alanine and glutamine. Different Dadachamame varieties vary in the amount of these constituents and in the dates of their harvest, thereby providing edamame varieties from summer to early fall and supporting a traditional local culture in areas where these varieties are cultivated. At present, eight edamame strains are available from LegumeBase (Table 1).

#### Full-length cDNA

The Soybean Full-Length cDNA Research Consortium has assembled a large-scale collection of full-length cDNA clones derived from the Japanese soybean cultivar, Nourin No. 2. This Consortium developed approximately 40,000 soybean cDNA clones from a full-length enriched cDNA library, including 4,711 full-read sequences, which were obtained from soybean plants grown under various developmental and environmental conditions (e.g., flower buds, roots, nodules, developing seed, drought stress, salt stress, chilling stress, low temperature, etc.) (Umezawa *et al.* 2008). All of clones are available from LegumeBase (Table 1).

The list of stock and their depositors in LegumeBase for *Lotus* and *Glycine* are summarized in Table 1, including resources that are in preparation.

# Database

We have constructed a web page for NBRP Lotus and Glycine "LegumeBase" (http://www.legumebase.brc.miyazaki-u.ac. jp/) at our resource center, that is composed of two databases, the "Lotus japonicus database" (http://www.legumebase. brc.miyazaki-u.ac.jp/lotus/) and the "Glycine max/soja database" (http://www.legumebase.brc.miyazaki-u.ac.jp/glycine/) (Table 2). In this database, users may pick the strains of interest by passport data, morphological data, meteorological data of the collecting site, and seed components or genotype. Sequence data for DNA resources are also available for each database: L. japonicus Genomic clone: miyakogusa.jp, L. japonicus cDNA: Lotus japonicus EST index and G. max full-length cDNA clone: soybean full-length cDNA database (Table 2). In addition, there are several related websites, such as the social bookmark site "Worldwide Legume Science Information Desk" or sites providing lists of relevant papers in the research area, such as Research Resource Circulation lotus/glycine that was established by the NBRP Information Center at the National Institute of Genetics (Yamazaki et al. 2010) (Table 2). The latter site provides useful information about legume research using the resources of NBRP Lotus and Glycine.

# Conclusions

We have developed extensive resources for two important leguminous plants, *Lotus japonicus* and *Glycine max*, and

Name of database	Contents	URL
LegumeBase	Main page of NBRP Lotus and Glycine	http://www.legumebase.brc.miyazaki-u.ac.jp/
Lotus japonicus database	Database for L. japonicus in NBRP	http://www.legumebase.brc.miyazaki-u.ac.jp/lotus/
Glycine max/soja database	Database for G. max and soja in NBRP	http://www.legumebase.brc.miyazaki-u.ac.jp/glycine/
NBRP Information Site	Website of NBRP	http://www.nbrp.jp/
Worldwide Legume Science Information Desk	Social bookmark site of legume-related webpage	http://www.shigen.nig.ac.jp/infodesk/topSpeciesAction. do?speciesId=4
Research Resource Circulation lotus/ glycine	Database of papers related to the NBRP resources	http://www.shigen.nig.ac.jp/rrc/gatewayAction.do? speciesId=17
RIKEN Bioresource Center	Distribution of <i>L. japonicus</i> culture cell lines	http://www.brc.riken.jp/lab/epd/Eng/species/lotus
Miyakogusa. jp	Genetic map and clone list of L. japonicus	http://www.kazusa.or.jp/lotus/
Lotus japonicus EST index	EST information for L. japonicus	http://est.kazusa.or.jp/en/plant/lotus/EST/
Rhizobase	Database for Rhizobium genome	http://genome.kazusa.or.jp/rhizobase/
Marker BD-Glycine max/soybean-	Linkage map and marker information of <i>G. max</i>	http://www.kazusa.or.jp/soymarker/
Soybean Full-Length cDNA Database	Information for full-length cDNA clones of <i>G. max</i>	http://rsoy.psc.riken.jp/

Table 2. NBRP Lotus and Glycine-related websites and databases

have constructed a database called LegumeBase at our resource center for researchers. NBRP Lotus and Glycine aims to facilitate rapid progress in legume research by collecting research materials and resources readily available to the legume research community. We make all efforts to collect valuable resources for legume research, maintain them in good condition and provide superior quality resources. When using our resources, the user is required to sign a material transfer agreement (MTA) and to explicitly acknowledge our resource center as the source in any publication that ensues from the study. We started collecting handling fees for providing resources in April, 2010. The fees can be paid online using credit cards or by transferring funds to a bank account. Care will be taken to adhere to the protective conditions that were recommended by the depositor when distributing the bioresources. Previously, researchers wasted a lot of time with labor costs for procuring and maintaining their resources. NBRP Lotus and Glycine LegumeBase will alleviate these problems by accepting valuable research materials, maintaining the resources and distributing them as and when needed.

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