Essay

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Overview of *Arabidopsis* Resource Project in Japan

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Subject areas: Resources

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Reviewer: Hiroshi Ezura, University of Tsukuba, Japan; Ryo Akashi, University of Miyazaki, Japan

Editor: Ki Hyun Ryu, Seoul Women's University, Republic of Korea

Received January 01, 2011;
Accepted January 15, 2011;
Published January 20, 2011

Citation: Kobayashi, M. Overview of *Arabidopsis* Resource Project in Japan. IBC 2011, 3:2, 1-4.
doi: 10.4051/ibc.2011.3.1.0002

Funding: The *Arabidopsis* resource project described in this manuscript is supported by the Ministry of Education, Culture, Sports, Science and Technology (MEXT), Japan.

Competing interest: All authors declare no financial or personal conflict that could inappropriately bias their experiments or writing.

SYNOPSIS

Arabidopsis is well-known to the world's plant research community as a model plant. Many significant resources and innovative research tools, as well as large bodies of genomic information, have been created and shared by the research community, partly explaining why so many researchers use this small plant for their research. The genome sequence of *Arabidopsis* was fully characterized by the end of the 20th century. Soon afterwards, the *Arabidopsis* research community began a 10-year international project on the functional genomics of the species. In 2001, at the beginning of the project, the RIKEN BioResource Center (BRC) started its *Arabidopsis* resource project. The following year, the National BioResource Project was launched, funded by the Japanese government, and the RIKEN BRC was chosen as a core facility for *Arabidopsis* resource. Seeds of RIKEN *Arabidopsis* transposon-tagged mutant lines, activation-tagged lines, full-length cDNA over-expresser lines, and natural accessions, as well as RIKEN *Arabidopsis* full-length cDNA clones and T87 cells, are preserved at RIKEN BRC and distributed around the world. The major resources provided to the research community have been full-length cDNA clones and insertion mutants that are suitable for use in reverse-genetics studies. This paper provides an overview of the *Arabidopsis* resources made available by RIKEN BRC and examples of research that has been done by users and developers of these resources.

Keywords: activation-tagged line, cultured cells, full-length cDNA, insertion mutant, natural accession, transposon-tagged line

Introduction

Model organisms are important tools in modern biology, allowing the relationships between phenotypes and genotypes to be extensively examined and discussed. *Arabidopsis* (*Arabidopsis thaliana* (L.) Heynh.) became the first higher plant whose genome sequence was fully characterized (by December 2000). Although *Arabidopsis* was already known as an excellent experimental model plant because of its short life cycle and ability to grow well under laboratory conditions, this remarkable achievement has significantly boosted its value.

In 2001, the international "Multinational Coordinated *Arabidopsis thaliana* Functional Genomics Project" was launched. Under this project, researchers have been elucidating the biological functions of all 27,000 genes in the *Arabidopsis* genome. The Multinational *Arabidopsis* Steering Committee (MASC) supports this project by coordinating the development, preservation, and distribution of genomic resources, such as insertion mutants and full-length cDNA clones. According to the 2010 MASC Annual Report (http://www.arabidopsis.org/portals/masc/2010_MASC_Report.pdf), at least one insertion mutant is available for 96% of the known *Arabidopsis* genes, and fully sequenced full-length cDNA is available for 64% of the genes. The *Arabidopsis* genome information is provided by The *Arabidopsis* Information Resource (TAIR). Most *Arabidopsis* researchers rely on the annotated genome information provided by TAIR, and TAIR collects the results of new research to further improve its annotation resource.

In Japan, RIKEN has developed various *Arabidopsis* resources that are now being distributed by the RIKEN BioResource Center (BRC) through the National BioResource Project (NBRP). This paper gives an overview of the *Arabidopsis* resources provided by RIKEN BRC through the NBRP. It also describes some key achievements of the developers and users of these resources.

Arabidopsis Resource Centers around the World

Most of the *Arabidopsis* resources available to the international research community are distributed by the *Arabidopsis* Biological Research Center (ABRC), the Nottingham *Arabidopsis* Stock Center (NASC), and RIKEN BRC (Table 1). RIKEN BRC provides all three categories of resources (i.e., seed lines, DNA materials, and cultured cells) (Table 2). It also preserves and distributes cDNA clones and cultured cells from non-*Arabidopsis* plants, which will be described in a future paper.

Seed Lines

1) Transposon-tagged lines

Although most of the *Arabidopsis* insertion mutants available from ABRC and NASC are T-DNA tagged mutants, RIKEN *Arabidopsis* transposon-tagged mutant (RATM) lines were developed by the RIKEN Genomic Sciences Center (GSC)[1-3] and are now being distributed by RIKEN BRC. Instead of endogenous transposable elements, the maize activator (Ac)–dissociation (Ds) system described by Smith et al.[4] was used to establish the RATM lines. More than 15,000 lines have been created, and the sequences flanking the Ds element have been characterized to determine the insertion sites. Some of the lines that have a Ds insertion within coding sequences can be used as knock-out mutants to elucidate the function of genes of interest through phenotypic observation. Homozygous seed stocks have been prepared for about 2,400 lines to accelerate their characterization by researchers.

Table 1. Resource centers of *Arabidopsis*

	Country	Seed	cDNA	Cultured cell	URL
ABRC	USA	○	○	○	http://abrc.osu.edu/
NASC	UK	○	-	-	http://arabidopsis.info/
RIKEN BRC	Japan	○	○	○	http://www.brc.riken.jp/lab/epd/

Table 2. *Arabidopsis* resources provided from RIKEN BRC

	Name of resource	Number of available resource
Seeds	RATM line	15,267
	RATM line (homozygous)	2,407
	Activation-tagged line	36,650
	FOX line	6,000
	Natural accession	398
cDNA	RAFL clone	251,382
Cultured cell	T87 cell	1

Unlike the T-DNA tagged mutants from ABRC and NASC, whose genetic background is the Columbia ecotype, the background ecotype of the RATM lines is Nossen, which may lead to ambiguous annotation when determining the insertion site. To overcome this problem, RIKEN BRC confirms the insertion site by means of PCR analysis and informs users when the result is inconsistent with their analysis. More than 2,000 lines had been shipped to the world research community by the end of 2010.

2) Activation-tagged lines

This resource was established by RIKEN GSC and BRC, and is distributed as seed pools for use in phenotypic screening. The T-DNA vector pPCVICEn4HPT[5] was used to create the lines[6]. As in the case of the other T-DNA insertion mutants, the insertion of the T-DNA region from the vector into the *Arabidopsis* genome can disrupt genes and produce recessive phenotypes. In addition, a quartet of 35S-enhancer sequences in the vector increases the expression level of neighboring genes that may produce dominant phenotypes[7-9]. It must be noted, however, that according to the developer's report, the T-DNA insertion sites of dominant mutants were within 10 kb of the first ATG sequence[10]. This indicates that the 35S-enhancer is effective if it is located within or near the promoter of the target genes.

3) FOX lines

Many full-length cDNA overexpresser (FOX) lines were developed by the RIKEN Plant Science Center by transformation of *Arabidopsis* with RIKEN *Arabidopsis* full-length cDNA (RAFL) clones expressed under the influence of the 35S-promoter[11]. After characterization of the inserted cDNA, the lines were deposited in the RIKEN BRC. Currently, 6,000 lines are available for phenotypic screening, and the number will increase to 9,000 by the end of 2011. By that time, users will be able to find over-expressers of genes of interest by searching through BRC's database.

4) Natural accessions

The *Arabidopsis* Information Service (AIS, organized by Prof. A. R. Kranz) first collected and distributed seeds of *Arabidopsis* natural accessions (the AIS collections) in the 1960s. When AIS closed, the accessions were deposited at ABRC, NASC, and the Sendai *Arabidopsis* Seed Stock Center (SASSC, organized by Prof. N. Goto). At the retirement of Prof. Goto, the SASSC accessions were deposited in the RIKEN BRC collection for preservation and distribution.

Natural accessions are suitable for screening genes that play important roles in the plant's adaptation to its environment. For example, the HMA5 metal-ion transporter was found to be responsible for the tolerance of copper ions through the use of a series of natural accessions provided by RIKEN BRC[12]. To help users select the most appropriate lines for their research purposes, we are now characterizing the responses of the natural accessions to abiotic stresses, such as osmotic stress and acid stress. The preliminary results will be made available to the research community through the RIKEN Web site (http://plant.rtc.riken.jp).

DNA Materials

RIKEN *Arabidopsis* full-length cDNA (RAFL) clones

Since their development and description by RIKEN GSC[13-15], RAFL clones have been intensively utilized by the world *Arabidopsis* community. This resource, which is also referred to as "R-clones" in several databases, has been used to establish derivative resources such as open reading frame (ORF) clones (U-clones), FOX lines, and the *Arabidopsis* DNABook™ (http://rarge.psc.riken.jp/DNA-Book/). More than 20,000 clones have been fully sequenced with support from NBRP and the Multinational Coordinated *Arabidopsis thaliana* Functional Genomics Project. Other clones are accompanied by the end sequences obtained by the clone's developer.

By now, more than 20,000 clones have been distributed around the world by RIKEN BRC. Before shipment, the end sequence of every clone is characterized to confirm the absence of contamination or mishandling. Many of the users of this resource use RAFL clones to establish transgenic lines that over-express specific *Arabidopsis* genes of interest. Others have used full-length cDNA to obtain recombinant proteins, or to develop micro- and macro-arrays for *Arabidopsis* genes[16,17]. It is noteworthy that Ogawa et al.[18] made comprehensive use of RAFL clones, which they over-expressed in T87 cells to investigate metabolic changes.

Cultured Cells

***Arabidopsis* T87 cells**

The T87 cell line was first reported in 1992 as unique cultured cells that showed partial plastid development under light (Figure 1)[19]. This accession was deposited with RIKEN BRC in 1994, and has been maintained for more than 15 years in the same culture medium used by the developer to prevent any changes in the growth and features of the cells. In 2003, Nakamichi et al.[20] reported the occurrence of a circadian rhythm for gene expression in this cell line, which greatly improved the usefulness of the T87 cells. In 2009, RIKEN BRC started international distribution of the T87 cells.

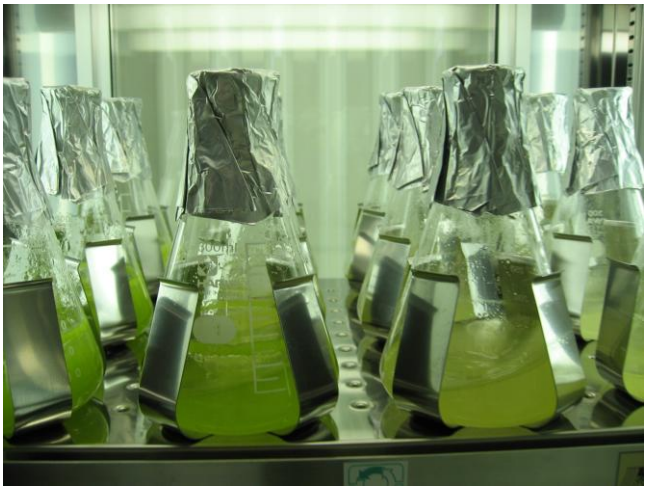

Figure 1. Culture of T87 cells maintained under light condition.

Cultured cells are important for characterizing the cellular localization of proteins, and for the production of useful materials as a result of transformation with foreign genes. As mentioned above, Ogawa et al.[18] used T87 cells in metabolic profiling studies. Further application of cultured cells in basic and applied research is anticipated.

Resources Available from US and European Centers

Insertion mutants are the major seed resource used by the *Arabidopsis* community. Many T-DNA insertion mutants have been established in the USA and in European countries, and are provided by ABRC and NASC. Other transgenic lines, such as RNA interference (RNAi) lines and natural accessions, are also available from these centers.

ABRC also distributes *Arabidopsis* cDNA clones such as ORF clones (U-clones) and T87 cells. It should be noted that ABRC maintains T87 cells using a modified protocol, so that the features of these cells may differ from those maintained by RIKEN BRC.

Most *Arabidopsis* resources in the stock centers are searchable from the TAIR home page (http://www.arabidopsis.org/). The SIGnAL T-DNA Express (http://signal.salk.edu/cgi-bin/tdnaexpress). Web site, operated by the SALK Institute, also provides information on insertion mutants and cDNA clones developed in the USA, Europe, and Japan.

Conclusion and Prospects

For 10 years, *Arabidopsis* has been widely used to elucidate the molecular mechanisms of plant growth by the characterization of gene functions in detail. Although the characterization is not yet complete, the current post-genome *Arabidopsis* project is now coming to its end, and researchers are seeking new goals and new subjects with which to establish the next international plant research project.

This past summer (June 2010), more than 1,300 researchers from 32 countries joined in the 21st International Conference on *Arabidopsis* Research (ICAR2010), held in Yokohama, Japan, where they discussed future *Arabidopsis* research projects[21]. They concluded that both the promotion of advanced studies of the unknown functions of the *Arabidopsis* genome and the use of *Arabidopsis* resources and information to support research on other crop and tree species should be considered. *Arabidopsis* resources will therefore remain a powerful tool for advancing plant science in the next 10 years.

Acknowledgements

Both the National BioResource Project and the RIKEN BRC resource project are supported by the Ministry of Education, Culture, Sports, Science and Technology (MEXT), Japan.

References

1. Ito, T., Motohashi, R., Kuromori, T., Mizukado, S., Sakurai, T., Kanahara, H., Seki, M., and Shinozaki, K. (2002). A new resource of locally transposed Dissociation elements for screening gene-knockout lines in silico on the *Arabidopsis* genome. *Plant Physiol* 129, 1695-1699.
2. Ito, T., Motohashi, R., Kuromori, T., Noutoshi, Y., Seki, M., Kamiya, A., Mizukado, S., Sakurai, T., and Shinozaki, K. (2005). A resource of 5,814 dissociation transposon-tagged and sequence-indexed lines of *Arabidopsis* transposed from start loci on chromosome 5. *Plant Cell Physiol* 46, 1149-1153.
3. Kuromori, T., Hirayama, T., Kiyosue, Y., Takabe, H., Mizukado, S., Sakurai, T., Akiyama, K., Kamiya, A., Ito, T., and Shinozaki, K. (2004). A collection of 11 800 single-copy Ds transposon insertion lines in *Arabidopsis*. *Plant J* 37, 897-905.
4. Smith, D., Yanai, Y., Liu, Y.G., Ishiguro, S., Okada, K., Shibata, D., Whittier, R.F., and Fedoroff, N.V. (1996). Characterization and mapping of Ds-GUS-T-DNA lines for targeted insertional mutagenesis. *Plant J* 10, 721-732.
5. Hayashi, H., Czaja, I., Lubenow, H., Schell, J., and Walden, R. (1992). Activation of a plant gene by T-DNA tagging: auxin-independent growth *in vitro*. *Science* 258, 1350-1353.
6. Nakazawa, M., Ichikawa, T., Ishikawa, A., Kobayashi, H., Tsuhara, Y., Kawashima, M., Suzuki, K., Muto, S., and Matsui, M. (2003). Activation tagging, a novel tool to dissect the functions of a gene family. *Plant J* 34, 741-750.
7. Takase, T., Nakazawa, M., Ishikawa, A., Kawashima, M., Ichikawa, T., Takahashi, N., Shimada, H., Manabe, K., and Matsui, M. (2004). ydk1-D, an auxin-responsive GH3 mutant that is involved in hypocotyl and root elongation. *Plant J* 37, 471-483.
8. Takase, T., Nakazawa, M., Ishikawa, A., Manabe, K., and Matsui, M. (2003). DFL2, a new member of the *Arabidopsis* GH3 gene family, is involved in red light-specific hypocotyl elongation. *Plant Cell Physiol* 44, 1071-1080.
9. Zhao, L., Nakazawa, M., Takase, T., Manabe, K., Kobayashi, M., Seki, M., Shinozaki, K., and Matsui, M. (2004). Overexpression of LSH1, a member of an uncharacterised gene family, causes enhanced light regulation of seedling development. *Plant J* 37, 694-706.
10. Ichikawa, T., Nakazawa, M., Kawashima, M., Muto, S., Gohda, K., Suzuki, K., Ishikawa, A., Kobayashi, H., Yoshizumi, T., Tsumoto, Y., et al. (2003). Sequence database of 1172 T-DNA insertion sites in *Arabidopsis* activation-tagging lines that showed phenotypes in T1 generation. *Plant J* 36, 421-429.
11. Ichikawa, T., Nakazawa, M., Kawashima, M., Iizumi, H., Kuroda, H., Kondou, Y., Tsuhara, Y., Suzuki, K., Ishikawa, A., Seki, M., et al. (2006). The FOX hunting system: an alternative gain-of-function gene hunting technique. *Plant J* 48, 974-985.
12. Kobayashi, Y., Kuroda, K., Kimura, K., Southron-Francis, J.L., Furuzawa, A., Iuchi, S., Kobayashi, M., Taylor, G.J., and Koyama, H. (2008). Amino acid polymorphisms in strictly conserved domains of a P-type ATPase HMA5 are involved in the mechanism of copper tolerance variation in *Arabidopsis*. *Plant Physiol* 148, 969-980.
13. Sakurai, T., Satou, M., Akiyama, K., Iida, K., Seki, M., Kuromori, T., Ito, T., Konagaya, A., Toyoda, T., and Shinozaki, K. (2005). RARGE: a large-scale database of RIKEN *Arabidopsis* resources ranging from transcriptome to phenome. *Nucleic Acids Res* 33, D647-650.
14. Seki, M., Carninci, P., Nishiyama, Y., Hayashizaki, Y., and Shinozaki, K. (1998). High-efficiency cloning of *Arabidopsis* full-length cDNA by biotinylated CAP trapper. *Plant J* 15, 707-720.
15. Seki, M., Narusaka, M., Kamiya, A., Ishida, J., Satou, M., Sakurai, T., Nakajima, M., Enju, A., Akiyama, K., Oono, Y., et al. (2002). Functional annotation of a full-length *Arabidopsis* cDNA collection. *Science* 296, 141-145.
16. Seki, M., Narusaka, M., Ishida, J., Nanjo, T., Fujita, M., Oono, Y., Kamiya, A., Nakajima, M., Enju, A., Sakurai, T., et al. (2002). Monitoring the expression profiles of 7000 *Arabidopsis* genes under drought, cold and high-salinity stresses using a full-length cDNA microarray. *Plant J* 31, 279-292.
17. Seki, M., Ishida, J., Narusaka, M., Fujita, M., Nanjo, T., Umezawa, T., Kamiya, A., Nakajima, M., Enju, A., Sakurai, T., et al. (2002). Monitoring the expression pattern of around 7,000 *Arabidopsis* genes under ABA treatments using a full-length cDNA microarray. *Funct Integr Genomics* 2, 282-291.
18. Ogawa, Y., Suzuki, H., Sakurai, N., Aoki, K., Saito, K., and Shibata, D. (2008). Cryopreservation and metabolic profiling analysis of *Arabidopsis* T87 suspension-cultured cells. *Cryo Letters* 29, 427-436.
19. Axelos, M., Curie, C., Mazzolini, L., C., B., and Lescure, B. (1992). A protocol for transient gene expression in *Arabidopsis thaliana* protoplasts isolated from cell suspension cultures. *Plant Physiol Biochem* 30, 123-128.
20. Nakamichi, N., Matsushika, A., Yamashino, T., and Mizuno, T. (2003). Cell autonomous circadian waves of the APRR1/TOC1 quintet in an established cell line of *Arabidopsis thaliana*. *Plant Cell Physiol* 44, 360-365.
21. Shinozaki, K. (2010). The ICAR2010 for development of *Arabidopsis* research beyond 2010. *J Plant Res* 123, 265-266.