Plant Molecular Biology **54:** 325–334, 2004.

Rice mutant resources for gene discovery

Hirohiko Hirochika[1,*], Emmanuel Guiderdoni[2], Gynheung An[3], Yue-ie Hsing[4], Moo Young Eun[5], Chang-deok Han[6], Narayana Upadhyaya[7], Srinivasan Ramachandran[8], Qifa Zhang[9], Andy Pereira[10], Venkatesan Sundaresan[11] and Hei Leung[12]

[1]*Molecular Genetics Department, National Institute of Agrobiological Sciences, Tsukuba City, Ibaraki 305-8602, Japan (*author for correspondence; e-mail hirohiko@nias.affrc.go.jp);* [2]*CIRAD, Avenue Agropolis, TA 40/03, 34398 Montpellier Cedex 5, France;* [3]*Department of Life Science and National Research Laboratory of Plant Functional Genomics, Pohang University of Science and Technology, Pohang 790-784, South Korea;* [4]*Academia Sinica, Institute of Botany, Yienchuyuan Road, Nankang, Taipei 11529, Taiwan;* [5]*Genomics Division, National Institute of Agricultural Biotechnology, Rural Development Administration, Suweon 441-707, South Korea;* [6]*Plant Molecular Biology and Biotechnology Research Center, Gyeongsang National University, Jinju 660-701, South Korea;* [7]*CSIRO Plant Industry, GPO Box 1600, Canberra ACT 2601, Australia;* [8]*Temasek Life Sciences Laboratory, 1, Research Link, National University of Singapore, Singapore 117604, Singapore;* [9]*National Key Laboratory of Crop Genetic Improvement, Huazhong Agricultural University, Wuhan 430070, China;* [10]*Plant Research International, P.O. Box 16, 6700 AA Wageningen, Netherlands;* [11]*Plant Biology and Agronomy, LSA 1002, University of California, Davis, CA 95616, USA;* [12]*International Rice Research Institute, DAPO Box 7777, Metro Manila, Philippines*

Received 9 January 2004; accepted in revised form 19 January 2004

Key words: deletion mutants, flanking sequence tag, insertional mutants, rice

Abstract

With the completion of genomic sequencing of rice, rice has been firmly established as a model organism for both basic and applied research. The next challenge is to uncover the functions of genes predicted by sequence analysis. Considering the amount of effort and the diversity of disciplines required for functional analyses, extensive international collaboration is needed for this next goal. The aims of this review are to summarize the current status of rice mutant resources, key tools for functional analysis of genes, and our perspectives on how to accelerate rice gene discovery through collaboration.

Abbreviations: FST, flanking sequence tag; International Rice Functional Genomics Consortium (IRFGC)

Introduction

Rice (*Oryza sativa* L.) was proposed as a model for monocot plants because of its small genome size relative to other cereals (430 Mb), the ease of transformation, the large store of molecular and genetic information, and its economic importance. With the completion of genomic sequencing of rice (Delsney, 2003; Sasaki and Sederoff, 2003), rice has been firmly established as a model organism for both basic and applied research. The entire research community appreciates the International Rice Genome Sequencing Project (IRGSP) (Sasaki and Burr, 2000; Feng *et al*., 2002; Sasaki *et al*., 2002) as well as other public and private sequencing efforts (Goff *et al*., 2002; Yu *et al*., 2002) for their contributions in making accurate rice genome sequence data available to the public much ahead of schedule. The next challenge is to uncover the functions of the predicted genes inferred by sequence information. Considering the amount of effort and the diversity of disciplines required for

functional analyses, even more extensive international collaboration is needed for this next goal than was required for the sequencing project.

Knockout of genes by insertional mutagenesis is a straightforward method to identify gene functions. For *Arabidopsis*, whose entire genome sequence is available, the effort to obtain saturation with insertion mutants has involved many groups from different countries. These groups are producing mutant lines using a variety of insertion elements, including T-DNA, *Ac*/*Ds*, and *Spm*/*dSpm* elements. While these mutant lines are important resources for forward genetics studies of gene function, their applications in reverse genetics are even more important with the available *Arabidopsis* genome sequence. Two primary approaches for reverse genetics analysis have been established. One is PCR screening of DNA pools from mutants with primers corresponding to a target sequence. The other is flanking sequence tag (FST) analysis, a strategy in which sequences flanking the insertion elements are determined in each mutant line to develop a database of knockout genes that can be searched electronically. At the early phase of reverse genetics studies, the PCR screening method was widely adopted (Maes *et al*., 1999). However, because of the labor- and time-demanding nature of PCR screening, FST database development is now the preferred and popular strategy. Although the investment in the FST database is high, screening of mutants is easy once the database is established. In several *Arabidopsis* laboratories (http://signal.salk.edu/tabout.html, http://genoplante-info.infobiogen.fr), a large collection of FST data, totaling more than 100 000, has been established and opened to the public (Alonso *et al*., 2003). Mutant lines can be readily identified and seeds requested from the source. Several *Arabidopsis* seed stock centers have played a key role in making mutant lines linked with FST data available to the public (Scholl *et al*., 2000).

With the availability of the rice genome sequence, many rice researchers feel that it is timely to establish a framework for producing and sharing resources essential for functional analysis of rice genes. Rice researchers from different countries sharing this mutual interest have met in a series of meetings. Priorities and models of collaboration were discussed at recent meetings held at the First International Rice Congress in Beijing in September 2002 (organized by H. Leung, IRRI) and at the Global Rice Gene Machine Workshop in Canberra in November 2002 (organized by N. Upadhyaya, CSIRO, see http://www.pi.csiro.au/grgm02/). At the Canberra meeting, it was proposed that IRRI should organize an interim steering committee to formally establish an International Rice Functional Genomics Consortium (IRFGC). The meeting of the interim steering committee was held at the Plant and Animal Genome Conference in San Diego on January 11, 2003 (for more background and details, see http://www.iris.irri.org/IRFGC). As a first step to facilitate coordination of research efforts, the interim steering committee proposed that a summary of the activities of different institutions involved in mutant research to be prepared to inform the research community. Here, we summarize the current status of rice mutant resources and our perspectives on how to accelerate rice gene discovery through collaboration.

Current status of mutant collections

The current status of rice mutant resources and projected activities are summarized in Table 1. The list is not comprehensive as we are aware that additional laboratories are involved in production of mutant libraries. Nonetheless, the list illustrates the research programs active around the world in producing and characterizing rice mutants. Below we highlight the commonly used approaches in producing different types of mutant populations.

Insertional mutants

Ac and *Ds* elements from maize are the first insertional mutagens used in rice. Despite their activity in rice, one problem has been the unpredictable silencing of *Ds* elements observed especially in later generations in the presence of *Ac* transposase (Izawa *et al*., 1997). One solution is to select lines showing active transposition (Nakagawa *et al*., 2000; Greco *et al*., 2003; Kolesnik *et al*., 2004). However, no two mutagenic lines (crosses between immobile *Ac* line and a *Ds* lines) nor two progeny of a given mutagenic line behaved similarly with respect to germinal transposition resulting in varying frequencies of independent stable insertion lines in the progenies (Upadhyaya *et al*., 2002). Methylation has been proposed to be involved in

Table 1. Rice mutant resources developed by the International Rice Research Community.

Institution[a]	Variety	Type	Vector/ mutagen	Population size (current)	Population size (target)	Mutated loci per genome	Mutated sites in total collection	Database and DNA resource	Seed availability	Reference or contact
National Institute of Agrobiological Sciences	Nipponbare	Insertion, endogenous retrotransposon	*Tos17*	50 000	50 000	10	500 000	http://tos.nias.affrc.go.jp Flanking sequence: 16 784 Phenotype: 18 000 Lines DNA pool: 50 000 lines	Open to public (lines linked with FST)	H. Hirochika (E-mail: hirohiko@nias.affrc.go.jp)
Pohang University of Science and Technology	Dongjin and Hwayoung	Gene and enhancer trap, activation tags	T-DNA	50 000 activation lines 50 000 inactivation lines	100 000	4 for T-DNA (4 for Tos17)	150 000 for T-DNA 400 000 for Tos17	http://postech.ac.kr/life/risd Flanking sequence: 7000 Phenotype DNA pool: 40 000 lines	Open to public	G. An (E-mail: genean@postech.ac.kr)
RDA, Gyeongsang National University	Dongjinbyeo	Insertion	*Ac*/*Ds*	30 000	100 000 each of *indica* and *japonica*			Website will be constructed (November 2003) http://www.niab.go.kr		Moo Young Eun (E-mail: myeun@rda.go.kr) Min Hee Nam (E-mail: nammhee@rda.go.kr) Chang-deok Han (E-mail: cdhan@nongae.gsnu.ac.kr)
CSIRO	Nipponbare	Gene and enhancer trap	*Ac*/*Ds*	8 000	5000/ year	2	16 000	http://www.pi.csiro.au/fgrttpub/home.htm Flanking sequence		N.M. Upadhyaya (E-mail: narayana upadhyaya@csiro.au)
CIRAD-INRA-IRD-CNRS, Génoplante	Nipponbare	Enhancer trap	T-DNAs	46 000	46 000	1.4 for T-DNA 3.2 for Tos17	64 400 for T-DNA 147 200 for Tos17	Integrated database: http://genoplante info.infobiogen.fr/oryzatagline/ Flanking sequences: 12 000 Phenotype: 5 000 Lines	Gradually open to public from May 2004	E. Guiderdoni (E-mail: guiderdoni@cirad.fr)
European Union	Nipponbare	Enhancer trap and activation tags	Ac/Ds	10 000	20 000	2–4	25 000	Flanking sequence: 5000	Open to public	Pereira (E-mail: Andy_pereira@wur.nl)
Huazhong Agr. Univ. China	Zhonghua 11	Enhancer trap	T-DNA	42 000	70 000	2	84 000	Flanking sequence: 6 000 http://www.ricefgchina.org	Open to public	Q. Zhang (E-mail: qifazh@mail.hzau.edu. cn)

Table 1. (Continued).

Institution[a]	Variety	Type	Vector/ mutagen	Population size (current)	Population size (target)	Mutated loci per genome	Mutated sites in total collection	Database and DNA resource	Seed availability	Reference or contact
Institute of Botany, Academia Sinica, Taiwan	Tainung 67	Insertion and activation tags	T-DNA	20 000	60 000 (by the end of 2005)	1.5 copy per genome	30 000	http://trim.sinica.edu.tw. Flanking sequence: 1 500		Y.C. Hsing (E-mail: bohsing@gate.sinica.edu.tw)
Temasek Laboratory-Singapore	Nipponbare	Gene trap	*Ac*/*Ds*	18 000	30 000	1	18 000	Flanking sequence: 2 500	Non-commercial use	R. Srinivasan (E-mail: sri@tll.org.sg)
University of California-Davis	Nipponbare	Transposons gene traps	En/Spm *Ac–Ds*	1 200	40 000	1	1 200	http://www-plb.ucdavis.edu/Labs/sundar/index.htm	Public access FST database + seeds	V. Sundaresan (E-mail: sundar@ucdavis.edu)
IRRI	IR64	Deletions and point mutation	Fast neutron γ-ray diepoxybutane EMS	40 000 at M3 or M4	50 000	10	500 000	http://www.iris.irri.org/cgi-bin/MutantHome.pl Phenotype TILLING DNA pool	Open to public	H. Leung (E-mail: H.Leung@cgiar.org)
National Institute of Agrobiological Sciences	Nipponbare	Deletion mutations	γ-ray ion beam	15 000 M2 7000 M2	50 000			DNA pool		M. Nishimura (E-mail: nishimura@affrc.go.jp)

[a]Institutional abbreviation: IRD, Institut de Recherche pour le Développement; CNRS, Centre National de la Recherche Scientifique; INRA, Institut National de la Recherche Agronomique; CIRAD, Centre de Coopération Internationale en Recherche Agronomique pour le Développement.

the silencing; however, the detailed mechanism of inactivation and reactivation is still unknown, making it difficult to select lines to produce a large collection of mutant lines using this method. Repetitive ratooning (Chin *et al*., 1999) and reactivation through tissue culture (Izawa and Shimamoto, 1999; Kim *et al*., 2002) may be alternative methods to overcome the inactivation problem. Recently, an efficient *Ac*/*Ds* tagging system has been reported in rice, where higher germinal transposition rates, and independent *Ds* transpositions among siblings of an F_2 family were observed (Kolesnik *et al*., 2004). In this system several generations of parental and *Ds* insertion lines have been analysed for transposon activity and neither the starter lines nor the transposants showed silencing, the mechanism of which is yet to be determined (Ramachandran *et al*., unpublished). *Ac* itself was shown to be relatively stable in successive generations. By taking advantage of this phenomenon, large collections of mutant lines have been produced (Enoki *et al*., 1999; Greco *et al*., 2001), but the lines are not yet widely available mainly due to limited seed stock.

As in *Arabidopsis*, *Ds* elements can be modified to monitor the expression of tagged genes and screen for enhancers/promoters in rice. By monitoring the GUS reporter gene expression to screen for enhancer traps, about 8% of the lines showed GUS expression in panicles (Chin *et al*., 1999). A large number of insertion mutants has been produced with T-DNA by several groups (Table 1). Some T-DNA vectors have been modified to monitor the expression of tagged genes and screen for enhancers/promoters. For example, GUS reporter gene expression was observed in 1.6–2.1% of organs tested of T-DNA promoter-trap lines (Jeon *et al*., 2000). Recently, Wu *et al*. (2003) used the GAL4/VP16-UAS elements with GUS as the reporter to produce a large population of enhancer trap lines. Another population using the same system but GFP as reporter has also been generated in a collaborative effort between the University of Cambridge and Génoplante (Johnson *et al*., unpublished). Furthermore, the multimerized transcriptional enhancers from the cauliflower mosaic virus 35S promoter were positioned next to the left border of the T-DNA to make activation tagged lines (Jeong *et al*., 2002). Already 50 000 activation-tagged lines have been produced (G. An, unpublished data). Another important mutagen is *Tos17* which is an endogenous retrotransposon (Hirochika, 2001). *Tos17* is activated by tissue culture, but distinct from DNA type transposons such as *Ac*/*Ds* and *Spm*/*dSpm*, the integrated copies of the retrotranspon are never excised, thus inducing only stable mutations. The research team at the National Institute of Agrobiological Sciences (NIAS) has produced 50 000 lines, which together harbor about 500 000 insertions (Miyao *et al*., 2003). Importantly, activation of *Tos17* was also induced in T-DNA insertion lines during transformation and regeneration. For example, 4 and 3.2 copies of newly transposed *Tos17* were found in T-DNA lines produced at Pohang University of Science and Technology (POTECH) and CIRAD, respectively (G. An, E. Guiderdoni, unpublished data). This means that the current populations of *Tos17* and T-DNA lines together may contain more than a million *Tos17* insertions.

For efficient gene knockout, target site specificity of each insertion element must be considered. Available data indicate that insertions of *Ac*/*Ds*, T-DNA and *Tos17* are biased toward low-copy, gene-rich regions (Enoki *et al*., 1999; Miyao *et al*., 2003; An *et al*., 2003; Kolesnik *et al*., 2004; Salland *et al*., in press). For *Tos17* insertion, some hot spots were observed. We have estimated the number of independent insertions required to tag every gene by assuming that these elements are preferentially inserted into genes. By using the formula $P = 1 - (1 - 1/N)^{nf}$, where P is the probability of tagging a gene ($P = 0.95$), N is the number of predicted genes ($N = 60\,000$; Sasaki *et al*., 2002), and f is the average number of insertions per genome ($f = 1$), the number of insertions (n) can be calculated. A total of 180 698 insertions are required to find insertions into every gene with a 95% probability. However, as it is likely that many insertions will lie in intergenic regions, it is useful to calculate the number required assuming that there is no preference for genes. In that case, with a genome size of ~400 Mb, the number of insertions required to disrupt a gene of 2.6 kb with 95% probability will be ~460 000, calculated as $1\text{-}(1\text{-}2.6/400\,000)^{460000} = 0.95$. These two estimates will likely represent the lower and upper limits for the required number of insertions.)

In addition to these insertion lines, several laboratories have developed launching pads by controlling the activation of transposons to con-

duct saturation mutagenesis in a specific chromosomal region (*Ac*/*Ds* system by C.D. Han and N.M. Upadhyaya, *Spm*/*dSpm* by V. Sundaresan, and chemically induced activation system of *Ac*/*Ds* by G.L. Wang and P. Ronald). A newly designed dual orientation gene trap constructs suitable for both initial genome-wide T-DNA insertion and subsequent localized *Ds* trapping have yielded a 11-fold increase in the trapping efficiency (Eamens *et al*., in press). Use of transiently expressed transposase as an inducer of *Ds* transposition in *Ds* containing callus lines has also been explored and initial results are promising (N.M. Upadhyaya *et al*., unpublished). These specialized stocks can create abundant mutations in a localized manner and will contribute toward saturation mutagenesis of the genome. Thus, with existing mutant stocks and on-going projects, the number of insertion mutant lines produced appears to be sufficient to cover the whole genome.

Chemical and irradiation-induced mutations

To maximize the efficiency of characterization of rice genes, we need functional polymorphism in the mutant collection. Phenotypic expression of gene function often depends on the genetic background such that we can only detect function if mutations are created in a specific genotype. Chemical and irradiation mutagenesis offers the advantage of producing a large number of functional variants in any genotype. With advances in high-throughput genotyping, new approaches are available for both forward and reverse genetics using deletion and point mutation stocks (Liu *et al*., 1999; Borevitz *et al*., 2003). The recent development of the TILLING method makes it possible to screen point mutations in desired genes using EMS-induced mutants (Colbert *et al*., 2001; Till *et al*., 2003). This method is applicable to any organisms and is being applied to rice (L. Comai, H. Leung and S. Henikoff). A main advantage of TILLING is that it provides an efficient way to generated an allelic series at a genetic locus.

Methods have also been developed for using deletion mutants in reverse genetics. As has been demonstrated in *Arabidopsis* and rice, nested PCR can be used to selectively amplify the DNA of a deletion mutant in DNA pools of a mutant collection (Li *et al*., 2001). Modification of the techniques has led to successful detection of deletions in specific defense genes through screening of DNA pools (Manosalva *et al*., 2003).

Deletions may be increasingly important for functional analysis of tandemly repeated genes. The genomic sequence data indicate that 22% of genes are tandemly duplicated (Goff *et al*., 2002). In general, tandemly duplicated genes are not diverged compared to dispersed duplicated genes, suggesting functional redundancy. This implies that two or more duplicated genes have to be disrupted to discover their gene function. Large deletions induced by fast neutron or ion beam could be particularly useful for analyzing such duplicated genes. Currently, only IRRI has a large collection of deletion mutants which has been distributed widely (Leung *et al*., 2001). NIAS is making deletions lines induced by ion beam and is also planning to make this resource publicly available.

DNA resources and databases for reverse genetics

For reverse genetics, DNA pools for PCR screening or an FST database are needed to identify mutations in genes of interest. The feasibility of PCR screening has been reported for *Ac*-tagged lines (Enoki *et al*., 1999), *Tos17*-tagged lines (Sato *et al*., 1999) and T-DNA (G. An, unpublished). DNA pools from 50 000 *Tos17*-insertion lines have been constructed and, so far, results from screening these DNA pools suggest a success rate of 50% for a given target sequence (H. Hirochika, unpublished). A similar efficiency is observed using the pooled DNA from T-DNA tagged lines (G. An, unpublished data). Due to the finite nature of the DNA pool, it is not practical to distribute DNA pools publicly. In addition, PCR screening is labor- and time-intensive. Thus, generating a FST database as a public resource is a more desirable and practical strategy. Research groups constructing insertional mutant lines have started or planned to construct FST databases. FST data are expected to be anchored to the public IRGSP genome sequence (BACs and chromosome pseudomolecules) and positions of both predicted genes and FST data will be shown graphically on the physical map. As shown in Table 1, a considerable amount of data has already been obtained in several laboratories. As of now, the *Tos17* FST database at NIAS (together

with mutant lines identified by FST database search) and the T-DNA FST database at Pohang University are open to the public. A list of FSTs homologous to publicly available sequences can be found at CSIRO Plant Industry's Rice Functional Genomics project website (http://www.csiro.au/fgrttpub/). It is expected that additional databases (e.g., Génoplante, CSIRO) will be opened. The members of the IRFGC agreed to put their FST data on their respective websites provided that they have sufficient stock to supply the corresponding mutant lines for research use by the scientific community.

In addition to FST databases, it is equally important to make databases on phenotypes. To maximize the utility of phenotype databases, a common vocabulary for describing mutants should be adopted among the mutant producers in different projects with feedback from users of the databases. The database should be searchable by specific biological traits (e.g., plant architecture, seed embryo, response to stresses, etc). The Plant Ontology Consortium vocabulary provides such a common framework and integrates different fields of expertise specific to plants. This vocabulary is already adopted by popular databases such as Gramene, TAIR, and Oryzabase. If the phenotype database is linked to the FST database, functional assignment of genes will be greatly accelerated. A database housing information on reporter gene expression in gene/enhancer trap lines will further stimulate functional characterization of rice genes. Construction of this type of integrated database should be considered an important priority for rice functional genomics.

Availability of resources and future needs

Figure 1 summarizes the overall distribution of different types of mutants in several rice varieties. Considering the insertion target bias demonstrated in different mutant populations, using different insertional mutagens would be complementary. So far, at least three insertional mutagens (*Tos17*, T-DNA, *Ac–Ds*) are utilized, which will give a greater chance of achieving saturation mutant libraries. Although a large number of mutants has been produced, some obvious gaps exist. First, the number of activation lines remains small relative to the number of knockout lines. Since activation lines have the power of unlocking potential variability that cannot be done by any other form of mutagenesis, it is worthwhile to expand the collection of activation tags in rice genes. Second, despite the economic importance of *indica* rice, it has not attracted the attention of researchers because of its low transformation efficiency. Other than the IR64 mutant collection, there are no other sources of *indica* rice mutants. Though technically more challenging, an effort to produce activation lines in *indica* rice will represent a unique and important contribution to the overall mutant resource.

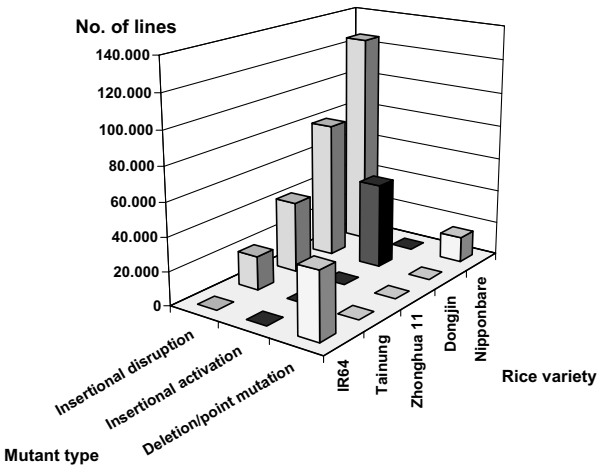

Figure 1. Categories of rice mutants produced as of August 2003.

With the existing wealth of resources, an important task is to design a mechanism to share these mutants and associated tools in a sustainable manner. As discussed above, one feasible way is to make database of FSTs covering all known genes and supply corresponding seed materials to the scientific community. For technical and quality control reasons, seed stocks should be maintained by the mutant producers. While some research groups are committed to this task, additional resources will be needed to support the development of FST databases and to sustain the distribution effort. Selective phenotyping of mutant lines with known insertion sites can help reduce the work load of propagating and distributing large mutant collections by a few laboratories. Following this model, a set of T-DNA lines with known disrupted defense genes from G. An's collection has been provided to IRRI to evaluate responses to insect and disease stresses that cannot be conducted in Korea. This arrangement eases the burden of mutant producers and at the same time capitalizes on the expertise in phenotyping in different geographic locations. The IRFGC can facilitate such complementary activities between laboratories, particularly with research and breeding institutions in developing countries where the need for useful agronomic traits is greatest.

Although linking the FST database to the phenotype database will undoubtedly accelerate research, background mutations induced during production of mutant lines and presence of inserts at multiple loci make it unreliable to directly correlate phenotype with insertion sites. Background mutations may be introduced by tissue culture, or may result from footprints induced by excision of *Ac*/*Ds* elements or abortive integration of T-DNA. Consequently, only five successful examples of forward genetics studies of gene function have been reported (Agrawal *et al*., 2001; Nonomura *et al*., 2003; Tanaka *et al*., 2003; Zhu *et al*., 2003; Margis-Pinheiro *et al*., in press). New strategies, such as chemically induced activation of transposition, will help circumvent these problems. Another important challenge is to develop an *in planta* transformation method to produce T-DNA insertion mutant lines with low background mutations and to bypass the generally low efficiency of tissue culture and transformation in *indica* cultivars.

Saturation mutagenesis of the whole rice genome is an achievable goal yet too large a task to be accomplished by a single laboratory or individual institutions. Since mutant resources are the basic ingredients underpinning identification of gene function, maximizing the use of the various mutant collections will benefit the community at large. Currently, different institutions have various forms of material transfer agreements to define the scope of usage of the mutant stocks. The IRFGC will continue to strive for broad sharing of resources to accelerate the complete functional characterization of all rice genes. As more and more stocks are becoming publicly available, it is possible to develop simple generic transfer agreements in the community to make rice mutants broadly accessible as a key component of the public research platform for rice gene discovery.

Acknowledgements

We thank Guoliang Wang for providing information on current work and Jan Leach for helpful comments on the manuscript.

References

Agrawal, G.K., Yamazaki, M., Kobayashi, M., Hirochika, R., Miyao, A. and Hirochika, H. 2001. Screening of the rice viviparous mutants generated by endogenous retrotransposon *Tos17* insertion. Tagging of a zeaxanthin epoxidase gene and a novel *OsTATC* gene. Plant Physiol. 125: 1248–1257.

Alonso, J.M., Stepanova, A.N., Leisse, T.J., Kim, C.J., Chen, H., Shinn, P., Stevenson, D.K., Zimmerman, J., Barajas, P., Cheuk, R. *et al*. 2003. Genome-wide insertional mutagenesis of *Arabidopsis thaliana*. Science 301: 653–657.

An, S., Park, S., Jeong, D.-H., Lee, D.-Y., Kang, H.-G., Yu, J.-H., Hur, J., Kim, S.-R., Kim, Y.-H., Lee, M., Han, S., Kim, S.-J., Yang, J., Kim, E., Wi, S.J., Chung, H.S., Hong, J.-P., Choe, V., Lee, H.-K., Choi, J.-H., Nam, J., Kim, S.-R., Park, P.-B., Park, K.Y., Kim, W.T., Choe, S., Lee, C.-B. and An, G. 2003. Generation and analysis of end-sequence database for T-DNA tagging lines in rice. Plant Physiol. 133: 2040–2047.

Borevitz, J.O., Liang, D., Plouffe, D., Chang, H.S., Zhu, T., Weigel, D., Berry, C.C., Winzeler, E. and Chory, J. 2003. Large-scale identification of single-feature polymorphisms in complex genomes. Genome Res. 13: 513–523.

Chin, H.G., Choe, M.S., Lee, S.H., Park, S.H., Park, S.H., Koo, J.C., Kim, N.Y., Lee, J.J., Oh, B.G., Yi, G.H. *et al*. 1999. Molecular analysis of rice plants harboring an *Ac*/*Ds* transposable element-mediated gene trapping system. Plant J. 19: 615–623.

Colbert, T., Till, B.J., Tompa, R., Reynolds, S., Steine, M.N., Yeung, A.T., McCallum, C.M., Comai, L. and Henikoff, S. 2001. High-throughput screening for induced point mutations. Plant Physiol. 126: 480–484.

Delseney, M. 2003. Towards an accurate sequence of the rice genome. Curr. Opin. Plant Biol. 6: 101–105.

Eamens, A.L., Blachard, C.L., Ramm, K., Shivakkumar, R., Dennis, E.S. and Upadhyaya, N.M. in press. Dual orientation T-DNA/*Ds* gene traps for rice functional genomics. Mol. Genet. Genomics.

Enoki, H., Izawa, T., Kawahara, M., Komatsu, M., Koh, S., Kyozuka, J. and Shimamoto, K. 1999. *Ac* as a tool for the functional genomics of rice. Plant J. 19: 605–613.

Feng, Q., Zhang, Y., Hao, P., Wang, S., Fu, G., Huang, Y., Li, Y., Zhu, J., Liu, Y., Hu, X. *et al.* 2002. Sequence and analysis of rice chromosome 4. Nature 420: 316–320.

Goff, S.A., Ricke, D., Lan, T.H., Presting, G., Wang, R., Dunn, M., Glazebrook, J., Sessions, A., Oeller, P., Varma, H. *et al.* 2002. A draft sequence of the rice genome (*Oryza sativa* L. ssp. *japonica*). Science 296: 92–100.

Greco, R., Ouwerkerk, P.B.F., Taal, A.J.C., Favalli, C., Beguiristain, T., Puigdomènech, P., Colombo, L., Hoge, J.H.C. and Pereira, A. 2001. Early and multiple *Ac* transpositions in rice generated by an adjacent strong enhancer. Plant Mol. Biol. 46: 215–227.

Greco, R., Ouwerkerk, P.B.F., de Kam, R.J., Sallaud, C., Favalli, C.L., Guiderdoni, E., Meijer, A.H., Hoge, J.H.C. and Pereira, A. 2003. Transpositional behaviour of an *Ac*/*Ds* system for reverse genetics in rice. Theor. Appl. Genet. 108: 10–24.

Hirochika, H. 2001. Contribution of the *Tos17* retrotransposon to rice functional genomics. Curr. Opin. Plant Biol. 4: 118–122.

Izawa, T. and Shimamoto, K. 1999. *Ac*/*Ds* as tools for rice molecular biology. In: K. Shimamoto (Ed.), Molecular Biology of Rice, Springer-Verlag, Heidelberg/Berlin/New York, pp. 59–76.

Izawa, T., Ohnishi, T., Nakano, T., Ishida, N., Enoki, H., Hashimoto, H., Itoh, K., Terada, R., Wu, C., Miyazaki, C. *et al.* 1997. Transposon tagging in rice. Plant Mol. Biol. 35: 219–229.

Jeon, J.S., Lee, S., Jung, K.H., Jun, S.H., Jeong, D.H., Lee, J., Kim, C., Jang, S., Yang, K., Nam, J. *et al.* 2000. T-DNA insertional mutagenesis for functional genomics in rice. Plant J. 22: 561–570.

Jeong, D.H., An, S., Kang, H.G., Moon, S., Han, J., Park, S., Lee, H.S., An, K. and An, G. 2002. T-DNA insertional mutagenesis for activation tagging in rice. Plant Physiol. 130: 1636–1644.

Kim, C.M., Je, BIl., Piao, H.L., Park, S.J., Kim, M.J., Park, S.H., Park, J.Y., Park, S.H., Lee, E.K., Chon, N.S., Won, Y.J., Lee, G.H., Nam, M.H., Yun, D.W., Lee, M.C., Cha, Y.S., Lee, K.H., Eun, M.Y. and Han, C.D. 2002. Reprogramming of the activity of the *activator*/*dissociation* transposon family during plant regeneration in rice. Mol. Cells 14: 231–237.

Kolesnik, T., Szeverenyi, I., Bachmann, D., Kumar, C.S., Jiang, S., Ramamoorthy, R., Cai, M., Ma, Z.G., Sundaresan, V. and Ramachandran, S. 2004. Establishing an efficient *Ac*/*Ds* tagging system in rice: large-scale analysis of *Ds* flanking sequences. Plant J. 37: 301–314.

Leung, H., Wu, C., Baraoidan, M., Bordeos, A., Ramos, M., Madamba, S., Cabauatan, P., Vera Cruz, C.M., Portugal, A., Reyes, G. *et al.* 2001. Deletion mutants for functional genomics: progress in phenotyping, sequence assignment, and database development. In: G.S. Khush, D.S. Brar and B. Hardy (Eds.), Rice Genetics IV, Science Publishers, New Delhi/International Rice Research Institute, Manila, pp. 239–251.

Li, X., Song, Y., Century, K., Straight, S., Ronald, P., Dong, S., Lassner, M. and Zhang, Y. 2001. A fast neutron deletion mutagenesis-based reverse genetics system for plants. Plant J. 27: 235–242.

Liu, L.X., Spoerke, J.M., Mulligan, E.L., Chen, J., Reardon, B., Westlund, B., Sun, L., Abel, K., Armstrong, B., Hardiman, G. *et al.* 1999. High-throughput isolation of *Caenorhabditis elegans* deletion mutants. Genome Res. 9: 859–867.

Maes, T., De Keukeleire, P. and Gerats, T. 1999. Plant tagnology. Trends Plant Sci. 3: 90–96.

Manosalva, P., Ryba-White, M., Wu, C., Lei, C., Baraoidan, M., Leung, H. and Leach, J. 2003. A PCR-based screening strategy for detecting deletions in defense response genes in rice. Phytopathology 93: S57.

Margis-Pnheiro, M., Zhou, X.-R., Zhu, Q.-H., Dennis, E.S. and Upadhyaya, N.M. in press. Isolation and characterization of a rice GA responsive dwarf mutant defective in an early step of the gibberellin biosynthesis pathway.

Miyao, A., Tanaka, K., Murata, K., Sawaki, H., Takeda, S., Abe, K., Shinozuka, Y., Onosato, K. and Hirochika, H. 2003. Target site specificity of the *Tos17* retrotransposon shows a preference for insertion within genes and against insertion in retrotransposon-rich regions of the genome. Plant Cell 15: 1771–1780.

Nakagawa, Y., Machida, C., Machida, Y. and Toriyama, K. 2000. Frequency and pattern of transposition of the maize transposable element *Ds* in transgenic rice plants. Plant Cell Physiol. 41: 733–742.

Nonomura, K., Miyoshi, K., Eiguchi, M., Suzuki, T., Miyao, A., Hirochika, H. and Kurata, N. 2003. The *MSP1* gene is necessary to restrict the number of cells entering into male and female sporogenesis and to initiate anther wall formation in rice. Plant Cell 15: 1728–1739.

Sallaud, C., Gay, C., Larmande, P., Bès, M., Piégu, B., Regad, F., Vignes, H., Venturoli, F., Cobo, S., Crouzet, M.P., Rio, M.A., Portefaix, M., Mathieu, T., Hémery, C., Chaine, C., Sabau, X., Bourgeois, E., Ghesquière, A., Delseny, M. and Guiderdoni, E. in press. High throughput T-DNA insertion mutagenesis in rice: a first step towards *in silico* reverse genetics. Plant J.

Sasaki, T. and Burr, B. 2000. International Rice Genome Sequencing Project: the effort to completely sequence the rice genome. Curr. Opin. Plant Biol. 3: 138–141.

Sasaki, T. and Sederoff, R.R. 2003. Genome studies and molecular genetics. The rice genome and comprehensive genomics of higher plants. Curr. Opin. Plant Biol. 6: 97–100.

Sasaki, T., Matsumoto, T., Yamamoto, K., Sakata, K., Baba, T., Katayose, Y., Wu, J., Niimura, Y., Cheng, Z., Nagamura, Y. *et al.* 2002. The genome sequence and structure of rice chromosome 1. Nature 420: 312–316.

Sato, Y., Sentoku, N., Miura, Y., Hirochika, H., Kitano, H. and Matsuoka, M. 1999. Loss-of-function mutations in the rice homeobox gene *OSH15* affect the architecture of internodes resulting in dwarf plants. EMBO J. 18: 992–1002.

Scholl, R.L., May, S.T. and Ware, D.H. 2000. Seed and molecular resources for *Arabidopsis*. Plant Physiol. 124: 1477–1480.

Tanaka, K., Murata, K.Y., Onosato, K., Miyao, A. and Hirochika, H. 2003. Three distinct rice cellulose synthase catalytic subunit genes required for cellulose synthesis in the secondary wall. Plant Physiol. 133: 73–83.

Till, B.J., Reynolds, S.H., Greene, E.A., Codomo, C.A., Enns, L.C., Johnson, J.E., Burtner, A.R., Young, K., Taylor, N.E., Henikoff, J.G. *et al*. 2003. Large-scale discovery of induced point-mutations with high-throughput TILLING. Genome Res. 13: 524–530.

Upadhyaya, N.M., Zhou, X.-R., Zhu, Q.-H., Ramm, K., Wu, L., Eamens, A., Sivakumar, R., Kato, T., Yun, D.-W., Santhoshkumar, C., Narayanan, K.K., Peacock, J.W. and Dennis, E.S. 2002. An *iAc*/*Ds* gene and enhancer trapping system for insertional mutagenesis in rice. Funct. Plant Biol. 29: 547–559.

Wu, C., Li, X., Yuan., W, Chen, G., Kilian, A., Li, J., Xu, C., Li, X., Zhou, D-X., Wang, S. and Zhang, Q. 2003. Development of enhancer trap lines for functional analysis of the rice genome. Plant J. 35: 418–427.

Yu, J., Hu, S.-N., Wang, J., Wong, G.K.-S., Li, S.-G., Liu, B., Deng, Y.-J., Deng, Y.-J., Dai, L., Zhou *et al*. 2002. A draft sequence of the rice genome (*Oryza sativa* L. ssp. *indica*). Science 296: 79–91.

Zhu, Q.-H., Hoque, M.S., Dennis, E.S. and Upadhyaya, N.M. 2003. Isolation and characterization of a *Ds* transposon tagged rice (*Oryza sativa* L.) mutant defective in anther dehiscence. BMC Plant Biol. 3: 6.