**Invited Review**

**Collection, preservation and distribution of *Oryza* genetic resources by the National Bioresource Project RICE (NBRP-RICE)**

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Biological resources are the basic infrastructure of bioscience research. Rice (*Oryza sativa* L.) is a good experimental model for research in cereal crops and monocots and includes important genetic materials used in breeding. The availability of genetic materials, including mutants, is important for rice research. In addition, *Oryza* species are attractive to researchers for both finding useful genes for breeding and for understanding the mechanism of genome evolution that enables wild plants to adapt to their own habitats. NBRP-RICE contributes to rice research by promoting the usage of genetic materials, especially wild *Oryza* accessions and mutant lines. Our activity includes collection, preservation and distribution of those materials and the provision of basic information on them, such as morphological and physiological traits and genomic information. In this review paper, we introduce the activities of NBRP-RICE and our database, Oryzabase, which facilitates the access to NBRP-RICE resources and their genomic sequences as well as the current situation of wild *Oryza* genome sequencing efforts by NBRP-RICE and other institutes.

**Key Words:** National Bioresource Project (NBRP), wild *Oryza*, *Oryza sativa*, mutants, methyl nitrosourea (MNU), chromosomal segment substitution lines (CSSLs), nearly isogenic lines (NILs).

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**Introduction**

The National Bioresource Project (NBRP) of Japan is a set of government-supported programs run to collect, preserve and distribute bioresources that are essential to the life sciences (Kurata et al. 2010). One NBRP program is NBRP-RICE. Rice is an indispensable staple food crop that accompanies with human beings under the pressure of selection and breeding. Genetic resources of rice, such as cultivars, landraces, wild *Oryza* species, mutants and other experimental strains, are essential to both breeding and basic science. It is thought that there are over 350,000 cultivated lines of rice in the world (FAO 2010); many of them are stored and maintained in large-scale genetic resource centers such as the International Rice Research Institute (IRRI Philippines), the National Agriculture and Food Research Organization (NARO Japan) and other centers in rice-producing Asian countries. On the other hand, experimental strains and other genetic resources, such as mutants, wild species and other research materials, are maintained in relatively small institutes or universities (Eamens et al. 2004, Hsing et al. 2007, Jeon et al. 2000, Kim et al. 2004, Kolesnik et al. 2004, Kurata and Yamazaki 2006, Li et al. 2017, Miyao et al. 2003, Sallaud et al. 2004, van Enckevort et al. 2005). One such example is NBRP-RICE, operated by the National Institute of Genetics (NIG) and Kyushu University, Japan. NIG has charge of collecting, preserving and providing *Oryza* genetic resources. Kyushu University collects, preserves and provides experimental lines such as mutant strains, chromosomal segment substitution lines (CSSLs) and aneuploids derived from wild *Oryza*. In Japan, the Genebank project in NARO extensively collects landraces and cultivars and, thus, the collections of NBRP-RICE and NARO Genebank complement together to meet the request of resources used for both basic science and breeding.
In order to facilitate and promote the usage of genetic resources, it is important to provide genomic information. The Rice Annotation Project Database (RAP-DB, https://rapdb.dna.affrc.go.jp/) provides a comprehensive set of annotations for the genome sequence of rice, *Oryza sativa*, based on the Os-Nipponbare-reference-IRGSP-1.0 (IRGSP1.0), as well as transcriptome and SNP data of several cultivars of rice (Kawahara et al. 2013, Sakai et al. 2013). RAP-DB enables rice researchers to access genomic data of cultivated species. Oryzabase, a database constructed and operated by NBRP-RICE, focuses mainly on genomic and phenotypic information of wild *Oryza* resources in NBRP-RICE (Kurata and Yamazaki 2006, Yamazaki et al. 2010).

In this review, we introduce the bioresources accompanied with their biological attributes such as genome sequences and trait information, and examples of research conducted using those materials and the activities of NBRP-RICE to promote their usage.

Bioresources and activities in NBRP-RICE

**1) Collection, preservation and distribution of wild *Oryza* species**

NIG maintains more than 1700 wild *Oryza* species, which show a great degree of diversity in morphology (Fig. 1). NBRP-RICE follows the scientific names of wild *Oryza* species proposed by Vaughan and Morishima (2003), with respect to Drs. Oka and Morishima, who launched wild *Oryza* research at NIG. Within this system, NBRP-RICE wild accessions are classified into 21 wild species, among which the African species *O. punctata* is divided into diploid and tetraploid species under the same species name; *O. rufipogon*, the direct wild progenitor of cultivated rice *O. sativa*, includes both annual and perennial types; and annual *O. nivara* is distinguished from perennial *O. rufipogon*. NBRP-RICE holds 20 of the species except *O. schlechteri* and their phylogenetic relationships is shown in Fig. 2 (Nonomura et al. 2010).

Wild genetic resources are useful materials to search for genes or QTLs for resistance to biotic and abiotic stresses, yield and domestication related traits and others for the genetic improvement of cultivated species. Thus, the preservation and distribution of wild crop progenitors such as wild *Oryza* is beneficial for humankind. As environmental destruction and development of wild habitats is decreasing populations of wild *Oryza*, it is noteworthy that NBRP-RICE is dedicated to the ex situ conservation of wild *Oryza*. Seeds, genomic DNA and plantlets of many NBRP-RICE accessions are available via Oryzabase (https://shigen.nig.ac.jp/rice/oryzabase/locale/change?!lang=en). The last section of this review gives detailed instructions on how to order these resources.

**2) Whole genome sequencing of wild *Oryza* species**

Next-Generation Sequencing (NGS) technologies enable the acquisition of the whole genome sequences (WGSs) of any organisms. The availability of WGSs facilitates the evolutionary and functional analysis of genes. For this reason, NBRP-RICE has made efforts to obtain WGSs of
wild *Oryza* species. NBRP-RICE provides WGSs of its resources through Oryzabase (https://shigen.nig.ac.jp/rice/oryzabase/) (Kajiya-Kanegae et al. 2021, Ohyanagi et al. 2016, Yamazaki et al. 2010), as well as its resources to international collaborative efforts on sequencing *Oryza* genomes (Huang et al. 2012, Shenton et al. 2020, Stein et al. 2018, Zhao et al. 2018).

Here, we outline the current landscape of NGS data of wild *Oryza* species available in public databases such as National Center for Biotechnology Information (NCBI) and DNA Data Bank of Japan (DDBJ). We identified 1629 WGS data sets of wild *Oryza* species in NCBI (Supplemental Table 1). The data were generated at 28 research institutes in China, Japan, the USA, France and Korea (Fig. 3A, 3B). The 89.5% (248/277) of NBRP-RICE/NIG data with a sequencing depth of >5× genome coverage enable high-confidence calling of sequence variations (Fig. 3B). Of all 1629 data sets, 96.6% were generated by short-read sequencing platforms and 3.3% by long-read platforms, with sequencing depths ranging from 0.04× to 178.43× genome coverages (Fig. 3C, 3D). WGS data of 794 (48.7%) and 202 (12.4%) wild *Oryza* strains available from NBRP-RICE/NIG and IRRI, respectively, were generated and deposited to public databases such as Oryzabase, DDBJ and NCBI. Thus, high sequencing depth and bioresource availability add further value to the sequence data and bioresources.

Short-read data have been extensively generated to call sequence polymorphisms such as SNPs and indels, and contribute greatly to reveal QTLs controlling agronomic traits (Huang et al. 2018, Wei et al. 2021, Wu et al. 2017), population structures and domestication history (Fawcett et al. 2013, Huang et al. 2012, Xu et al. 2011, Zhang et al. 2014). Most of the NGS data (75%) come from the wild progenitors of cultivated species: *O. rufipogon*, *O. nivara* and *O. barthii* (Fig. 3E). Imputed genome-wide SNPs obtained from low-coverage short reads of 446 *O. rufipogon* species published in Huang et al. (2012) are available at OryzaGenome 2.1 (http://viewer.shigen.info/oryzagenome21detail/index.xhtml) in Oryzabase (see the next section for details) (Kajiya-Kanegae et al. 2021). These sequences helped to reveal that *O. rufipogon* accessions can be divided into three subgroups, Or-I, -II and -III, and that *japonica* cultivars are most closely related to the Or-III population, which is distributed mainly in southern China (Huang et al. 2012).

Reference genomes of wild *Oryza* are important for studies of genome evolution and for the detection of genetic variations in regions that are highly polymorphic or absent in the genomes of cultivated species. To date, reference genomes of 26 accessions of 10 wild *Oryza* species have been deposited (Chen et al. 2013, Reuscher et al. 2018, Shenton et al. 2020, Stein et al. 2018, Wu et al. 2018, Zhao et al. 2018; Supplemental Table 2). Of these, 21 were based on short-read sequencing platforms and 5 on long-read platforms. Through the assembly of 13 *O. rufipogon* genomes,

![Fig. 3. Summary of NGS data of wild *Oryza* deposited in public databases. (A) Countries, (B) major institutes and (C) sequencing platforms generating NGS data of wild *Oryza*. (D) Distribution of read depth of deposited data in each species. (E) Number of accessions generating NGS data in each species.](image-url)
a pan-genome dataset of the *O. sativa*–*O. rufipogon* species complex was released (Zhao et al. 2018). Significant amounts of genome sequence data covering the entire *Oryza* genus have also been accumulated. These were generated in several recent studies focused on non-AA-genome species, and revealed important aspects of genome-size change, phylogenetic relationship, history of polyploidization and outstanding traits such as cold tolerance (Chen et al. 2013, Kitazumi et al. 2018, Reuscher et al. 2018, Shenton et al. 2020, Stein et al. 2018, Wu et al. 2018). Thus, the pan-genome dataset and genus-wide genome information will advance research toward understanding genome evolution and utilization of untapped genetic resources in the genus *Oryza*.

(3) *OryzaGenome 2.1 as an archive of *Oryza* genome information

OryzaGenome 2.1 (http://viewer.shigen.info/oryzaopened/21detail/index.xhtml) (Kajiya-Kanegae et al. 2021), within Oryzabase (https://shigen.nig.ac.jp/rice/oryzabase/), archives genome information of wild *Oryza*, supporting researchers using wild *Oryza* resources, mainly in NBRP-RICE. OryzaGenome 2.1 stores NGS genome information on 217 accessions in 19 wild *Oryza* species. In the Downloads section, information on accessions used for WGS can be retrieved by searching for various conditions such as species, genome type (AA, BB, CC, BBCC, CCDD, EE, FF, GG, etc.), growth habit, rank in the core collection and country of origin. Biological information of the accessions used for WGS can be obtained by clicking on accession numbers, which are linked to Oryzabase, and it is possible to request materials, depending on availability.

The Downloads section also provides polymorphic information on 33 *O. rufipogon* accessions against the reference genome sequence of cultivated rice, Nipponbare, in VCF file format. OryzaGenome 2.1 also provides a SNP viewer of 446 *O. rufipogon* accessions with low-coverage sequence reads (Huang et al. 2012) (http://viewer.shigen.info/oryza Genome21/mapview/MapView.do?action=ini&chromosome No=1) and a SNP effect table for 33 deep-sequenced *O. rufipogon* accessions (http://viewer.shigen.info/oryza genome21detail/snptable/search.xhtml) (Kajiya-Kanegae et al. 2021).

(4) Development of genetic transformation of wild *Oryza* which accelerates molecular genetic analysis and genome editing

Genetic transformation is an important technology for revealing, modulating and utilizing gene function. It is often used for molecular breeding, especially in genome editing. In addition, because of the difficulties posed by genetic crosses between distantly related species, transformation of wild *Oryza* accessions would be particularly valuable. Recently, we reported that a wide range of wild *Oryza* accessions, including those distantly related to cultivated species, can be genetically transformed by the immature-embryo method using *Agrobacterium* (Shimizu-Sato et al. 2020). By using 192 representative wild *Oryza* accessions covering 20 species in NBRP-RICE, we found that 90 accessions in 16 species form calluses from immature embryos and regenerate plantlets in tissue culture under several culture conditions (Hiei and Komari 2008), showing the feasibility of genetic transformation of wild *Oryza*. These accessions include AA, BB, CC, BBCC, CCDD, FF and HHJJ genome species. We also optimized the conditions of *Agrobacterium* infection for 51 accessions in 11 species. Many *Oryza* species could be transformed by the modified immature-embryo method. This method opens the door to genome editing, accelerating the study of wild *Oryza* genetic resources for molecular genetic analysis and future use in molecular breeding.

(5) Collection of mutant strains generated by *N*-methyl-*N*-nitrosourea treatment of fertilized egg cells

The treatment of fertilized egg cells at the single cell stage by *N*-methyl-*N*-nitrosourea (MNU) is one of the most efficient methods to induce nucleotide substitutions with a low rate of chimeric mutants in rice (Satoh et al. 2010). It is empirically shown that the average number of mutations detected in 1 kb genomic region is 7.4 singe nucleotide polymorphisms (SNPs) using 1,000 MNU mutant population made by this method (Suzuki et al. 2008). Thus, it is likely that researchers can obtain mutations in any gene of interest by both reverse and forward genetic screening of mutant pools made by this method and distributed from as NBRP-RICE described below. Mutation pools of rice cultivars ‘Kinmaze’ (3000 lines), ‘Taichung 65’ (3000 lines), ‘Kitaake’ (1100 lines), ‘Yukihikari’ (1300 lines) and ‘IR64’ (1200 lines) are maintained by NBRP-RICE. ‘Kinmaze’ and ‘Taichung 65’ (T65) are standard *japonica* cultivars. The lower photoperiod sensitivity of ‘Kita-ake’ and ‘Yukihikari’, *japonica* cultivars adapted to northern Japan, makes it easier to grow them in plant incubators. ‘IR64’ is a widely cultivated *indica* cultivar. MNU-induced rice lines show a wide spectrum of morphological and physiological phenotypes, such as abnormalities in embryo and endosperm development, panicle architecture, plant height, leaf coloration, seed fertility and tillering. Seeds of most of these lines are available from NBRP-RICE. In addition, NBRP-RICE offers a system for TILLING (Targeting-Induced Local Lesions In Genomes) analysis (McCallum et al. 2000), called TILLING Open Laboratory, where mutations in genes of interest can be screened by using facilities at Kyushu University.

(6) Recombinant inbred lines, chromosome segment substitution lines (CSSL) and monosomic alien chromosome addition lines distributed by NBRP-RICE

To facilitate genetic analysis and characterization of quantitative trait loci (QTLs), NBRP-RICE developed and made available recombinant inbred lines (RIL) and chromosomal segment substitution lines (CSSLs) that cover the
whole genomic region of donor accessions in the genetic background of recurrent parents (Table 1). Four RILs are derived from an intraspecific cross between subspecies japonica and indica. The CSSLs comprise 14 kinds of introgression lines and originate from six AA-genome Oryza species accessions with the genetic background of O. sativa T65: O. glaberrima, O. glumaepatula, O. meridionalis, O. rufipogon, O. nivara and O. longistaminata. The CSSLs include three pairs derived from reciprocal crossing of T65 and wild relatives (O. glaberrima, O. glumaepatula and O. meridionalis); each pair carries different cytoplasms with a similar nuclear genome composition, useful for studies of genetic interactions between nuclear and organellar genomes. NBRP-RICE also offers monosomic alien substitution lines, called MAALs, which have a single chromosome complement of donor accessions in the genetic region determined the opened or closed shape of panicles. Wu et al. (2018) identified a multi-gene locus in African rice cultivars that changes the plant habit from prostrate to erect in an F$_2$ population of O. glaberrima × NBRP-RICE accession O. barthii W1411, prompting an idea that convergent evolution in plant architecture occurred independently in Asian and African rice cultivars. Using deepwater rice accessions, Hattori et al. (2009) identified SNORKEL1 and SNORKEL2, which encode ethylene response factors that trigger the deepwater response. The SEMIDWARF1 (SD1) allele of a landrace with deepwater response, which differs from the major SD1 allele present in modern cultivars, was selected from O. rufipogon for deepwater rice cultivation in Bangladesh (Kuroha et al. 2018). Similarly, using deepwater accessions, Nagai et al. (2020) identified a mechanism regulating internode elongation, in which the antagonistic function of ACCELERATER of ELONGATION 1 (ACE1) and DECELERATER of ELONGATION 1 (DECI) operates after and before deepwater-induced internode elongation, respectively, and discussed their contributions in domestication by comparing their alleles from O. rufipogon.

Approximately, 30 loci that cause abnormal starch content or endosperm development have been characterized (Satoh et al. 2003). The waxy mutant doesn’t have amylose, which is synthesized by granule-bound starch synthase. dull mutants have a low amylose content, and five loci are known: du1, du2, du3, du4 and du5 (Yano et al. 1988). The sugary1 (sug1) and sugary2 (sug2) mutants accumulate phytoglycogen-type water-soluble polysaccharides instead of starch. Wrinkled-endosperm mutants are typified by

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**Table 1.** Recombinant inbred lines and chromosomal segment substitution lines distributed from NBRP-RICE

| Type of population | Population identifier | Recurrent parent | Species classification (recurrrent parent) | Donor | Species classification (donor) | Cytoplasm | Reference |
|--------------------|-----------------------|------------------|---------------------------------------------|-------|------------------------------|----------|-----------|
| Recombinant inbred lines | RIA | IR24 | O. sativa indica type | Asominori | O. sativa japonica type | [Aso] | Tsumematsu et al. 1996 |
| | RIB | Kinmaze | O. sativa japonica type | DV85 | O. sativa indica type | [Kin] | Ikeda et al. 1998 |
| | RID | Taichung 65 | O. sativa japonica type | DV85 | O. sativa indica type | [T65] | Yasui et al. 2010 |
| | | | | ARC10313 | O. sativa indica type | [T65] | Yasui et al. 2010 |
| Chromosomal segment substitution lines | IAS | IR24 | O. sativa indica type | Asominori | O. sativa japonica type | [IR24] | Kubo et al. 2002 |
| | AIS | Asominori | O. sativa japonica type | IR24 | O. sativa indica type | [Aso] | Kubo et al. 2002 |
| | TACSSL | Taichung 65 | O. sativa japonica type | ARC10313 | O. sativa indica type | [T65] | Yasui et al. 2010 |
| | TDCCSL | Taichung 65 | O. sativa japonica type | DV85 | O. sativa indica type | [T65] | Yasui et al. 2010 |
| | GIL | Taichung 65 | O. glaberrima | IRGC104038 | O. glaberrima | [T65] | Doi et al. 1997 |
| | IRGC103777[GILs] | Taichung 65 | O. sativa japonica type | IRGC103777 | O. glaberrima | [T65] | Yamagata et al. 2019 |
| | IRGC103777[T65] | Taichung 65 | O. sativa japonica type | IRGC103777 | O. glaberrima | [T65] | Yamagata et al. 2019 |
| | GLU-ILs[T65] | Taichung 65 | O. sativa japonica type | IRGC105668 | O. glumaepatula | [T65] | Yoshimura et al. 2010 |
| | GLU-ILs[GLU] | Taichung 65 | O. sativa japonica type | IRGC105668 | O. glumaepatula | [glu] | Yoshimura et al. 2010 |
| | MER-ILs[T65] | Taichung 65 | O. sativa japonica type | W1625 | O. meridionalis | [T65] | Yoshimura et al. 2010 |
| | MER-ILs[MER] | Taichung 65 | O. sativa japonica type | W1625 | O. meridionalis | [mer] | Yoshimura et al. 2010 |
| | WK1962ILs | Taichung 65 | O. sativa japonica type | W1962 | O. rufipogon | [T65] | Yamagata et al. 2019 |
| | IRGC105715ILs | Taichung 65 | O. sativa japonica type | IRGC105715 | O. rufipogon | [T65] | Yamagata et al. 2019 |
| | W1508ILs | Taichung 65 | O. sativa japonica type | W1508 | O. longistaminata | [T65] | Ogami et al. 2019 |
| | W1413ILs | Nipponbare | O. sativa japonica type | W1413 | O. longistaminata | [Nip] | Thein et al. 2019 |
CSSLs distributed by NBRP-RICE find extensive use in exploiting and isolating QTLs for various traits unique to wild accessions. A QTL for resistance to green rice leafhopper was delimited within a 31.2-kbp region by using CSSLs derived from T65 × O. rufipogon, WK1962ILs (Phi et al. 2019). To reveal the genetic basis for promoting out-crossing, Ogami et al. (2019) analyzed anther length in O. longistaminata, which has the longest anther length among AA genome species, by using CSSLs derived from T65 × O. longistaminata, W1508ILs, and identified several QTLs that explain the longer length.

These are only a few examples of research supported by NBRP-RICE resources. Over 300 reports based on NBRP-RICE resources have been published since 2002 (https://rc.nbrp.jp/projects/12?lang=en).

How to request Oryza genetic resources in NBRP-RICE

Oryzabase (https://shigen.nig.ac.jp/rice/oryzabase/) (Yamazaki et al. 2010) is an integrated database for resources distributed by NBRP-RICE, and a portal for ordering the bioresources from NBRP-RICE. Researchers can search by accession number or species name, and can gain access to a series of core collections covering the wide genetic diversity in Oryza. By clicking on accession numbers, basic information on accessions of interest can be obtained. Users can then order resources through the “Add” button.

Author Contribution Statement

Y.S., K.T., Y.Ya., H.M., Y.Yo., K.N.T., T.S., M.N.-T., T.K., K.-I.N., H.Y. and T.K. contributed to the collection, preservation and distribution of resources. H.K.-K. and S.K. contributed to the construction of the database. Y.S., T.K., A.A. and S.S.-S. analyzed genome and phenotype data, and contributed to design and production of Figures and Tables. Y.S., K.T., Y.Y., A.A., S.S.-S., T.K., K.-I.N., Y.H. and T.K. wrote the manuscript.

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Literature Cited

Chen, J., Q. Huang, D. Gao, J. Wang, Y. Lang, T. Liu, B. Li, Z. Bai, J.L. Goicoechea, C. Liang et al. (2013) Whole-genome sequencing of Oryza brachyantha reveals mechanisms underlying Oryza genome evolution. Nat. Commun. 4: 1595.

Doi, K., N. Iwata and A. Yoshimura (1997) The construction of chromosome substitution lines of African rice (Oryza glaberrima Steud.) in the background of Japonica rice (O. sativa L.). Rice Genet. NewsL. 14: 39–41.

Eamens, A.L., C.L. Blanchard, E.S. Dennis and N.M. Upadhyaya (2004) A bidirectional gene trap construct suitable for T-DNA and Ds-mediated insertional mutagenesis in rice (Oryza sativa L.). Plant Biotechnol. J. 2: 367–380.

FAO (2010) The Second Report on the State of the World’s Plant Genetic Resources for Food and Agriculture. Rome, p. 245.

Fawcett, J.A., T. Kado, E. Sasaki, S. Takuno, K. Yoshida, R.P. Sugino, S. Kosugi, S. Natsume, C. Mitsuoka, A. Uemura et al. (2013) QTL map meets population genomics: an application to rice. PLoS ONE 8: e83720.

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

Hiei, Y. and T. Komari (2008) Agrobacterium-mediated transformation of rice using immature embryos or calli induced from mature seed. Nat. Protoc. 3: 824–834.

Hsing, Y.I., C.G. Chern, M.J. Fan, P.C. Lu, K.T. Chen, S.F. Lo, P.K. Sun, S.L. Ho, K.W. Lee, Y.C. Wang et al. (2007) A rice gene activation/knockout mutant resource for high throughput functional genomics. Plant Mol. Biol. 63: 351–364.

Huang, J., J. Li, J. Zhou, L. Wang, S. Yang, L.D. Hurst, W.-H. Li and D. Tian (2018) Identifying a large number of high-yield genes by pedigree analysis, whole-genome sequencing and CRISPR-Cas9 gene knockout. Proc. Natl. Acad. Sci. USA 115: E7559–E7567.

Ikeda, K., J.-K. Lei, H. Tsunematsu, Y. Aida, H. Yasui and A. Yoshimura (1998) Rice QTL analysis for days to heading using different RI (Recombinant Inbred) lines. Breed. Res. 48 (Suppl. 1): 72 (In Japanese).

Ishii, T., K. Numaguchi, K. Miura, K. Yoshida, P.T. Thanh, T.M. Hun, M. Yamasaki, N. Komeda, T. Matsumoto, R. Terauchi et al. (2013) OsLG1 regulates a closed panicle trait in domesticated rice. Nat. Genet. 45: 462–465.

Isshiki, M., M. Nakajima, H. Satoh and K. Shimamoto (2000) dull: rice mutants with tissue-specific effects on the splicing of the waxy pre-mRNA. Plant J. 23: 451–460.

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

Kajiy-a-Kanegae, H., H. Ohyanagi, T. Ebata, Y. Tanizawa, A. Onogi, Y. Sawada, M. Yokota Hirai, Z.-X. Wang, B. Han, A. Toyoda et al. (2021) OryzaGenome2.1: database of diverse genotypes in wild Oryza species. Rice (N Y) 14: 24.

Kawagoe, Y., A. Kubo, H. Satoh, F. Takaiwa and Y. Nakamura (2005) Roles of isoamylase and ADP-glucose pyrophosphorylase in starch granule synthesis in rice endosperm. Plant J. 42: 164–174.

sug1, sug2, shrunklen1 and shrunklen2 (Nakagami et al. 2017, Yano et al. 1984). More than 10 causal genes for these 30 mutant loci have been identified (Isshiki et al. 2000, Kawagoe et al. 2005, Kubo et al. 2005, Nakagami et al. 2017, Ohdan et al. 2005, Tuncel et al. 2014).

Sato, Tsuda, Yamagata, Matsusaka, Kajiy-a-Kanegae, Yoshida, Agata, Ta, Shimizu-Sato, Suzuki et al.
Kawahara, Y., M. de la Bastide, J.P. Hamilton, H. Kanamori, W.R. McCombie, S. Ouyang, D.C. Schwartz, T. Tanaka, J. Wu, S. Zhou et al. (2013) Improvement of the *Oryza sativa* Nipponbare reference genome using next generation sequence and optical map data. *Rice (N Y)* 6: 4.

Kim, C.M., H.L. Piao, S.J. Park, N.S. Chon, B.I. Je, B. Sun, S.H. Park, J.Y. Park, E.J. Lee, M.J. Kim et al. (2004) Rapid, large-scale generation of *Ds* transposant lines and analysis of the *Ds* insertion sites in rice. *Plant J.* 39: 252–263.

Kitazumi, A., I.C.M. Pabuayon, H. Ohyanagi, M. Fujita, B. Osti, M.R. Shenton, Y. Kakei, Y. Nakamura, D.S. Brar, N. Kurata et al. (2018) Potential of *Oryza officinalis* to augment the cold tolerance genetic mechanisms of *Oryza sativa* by network complementation. *Sci. Rep.* 8: 16346.

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

Kubo, A., S. Rahman, Y. Utsumi, Z. Li, Y. Mukai, M. Yamamoto, M. Ugaki, K. Harada, H. Satoh, C. Konik-Rose et al. (2005) Complementation of *sugar-y*1 phenotype in rice endosperm with the wheat *isoamylase1* gene supports a direct role for isoamylase1 in amylopectin biosynthesis. *Plant Physiol.* 137: 43–56.

Kubo, T., Y. Aida, K. Nakamura, H. Tsunematsu, K. Doi and A. Yoshimura (2002) Reciprocal chromosome segment substitution series derived from *Japonica* and *Indica* cross of rice (*Oryza sativa* L.). *Breed. Sci.* 52: 319–325.

Kurata, N. and Y. Yamazaki (2006) Oryzabase. An integrated biological and genomic information database for rice. *Plant Physiol.* 140: 12–17.

Kurata, N., H. Satoh, H. Kitano, Y. Nagato, T. Endo, K. Sato, R. Akashi, H. Ezura, M. Kasaba, M. Kobayashi et al. (2010) NBRP, National Bioresource Project of Japan and plant bioresource management. *Breed. Sci.* 60: 461–468.

Kuroha, T., K. Nagai, R. Gamuyao, D.R. Wang, T. Furuta, M. Nakamori, T. Kitaoa, K. Adachi, A. Minami, Y. Mori et al. (2018) Ethylene-gibberellin signaling underlies adaptation of rice to periodic flooding. *Science* 361: 181–186.

Li, G., R. Jain, M. Chern, N.T. Pham, J.A. Martin, T. Wei, W.S. Schackwitz, A.M. Lipzen, P.O. Duong, K.C. Jones et al. (2017) The sequences of 1504 mutants in the model rice variety *Kitaake* facilitate rapid functional genomic studies. *Plant Cell* 29: 1218–1231.

McCallum, C.M., L. Comai, E.A. Greene and S. Henikoff (2000) Targeted screening for induced mutations. *Nat. Biotechnol.* 18: 455–457.

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

Nagai, K., Y. Mori, S. Ishikawa, T. Furuta, R. Gamuyao, Y. Niimi, T. Hobo, M. Fukuda, M. Kojima, Y. Takebayashi et al. (2020) Antagonistic regulation of the gibberellic acid response during stem growth in rice. *Nature* 584: 109–114.

Nakagami, T., H. Yoshihara, T. Nakamura, Y. Utsumi, T. Sawada, N. Fujita, H. Satoh and Y. Nakamura (2017) Biochemical analysis of new-type mutants of japonica rice that accumulate water-soluble α-glucans in the endosperm but retain full starch debranching enzyme activities. *Starch/Stärke* 69: 1600159.

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

Ogami, T., H. Yasui, A. Yoshimura and Y. Yamagata (2019) Identification of anther length QTL and construction of chromosome segment substitution lines of *Oryza longistaminata*. *Plants (Basel)* 8: 388.

Ohdan, T., P.B. Francisco, Jr., T. Sawada, T. Hirose, T. Terao, H. Satoh and Y. Nakamura (2005) Expression profiling of genes involved in starch synthesis and sink and source organs of rice. *J. Exp. Bot.* 56: 3229–3244.

Ohyanagi, H., T. Ebata, X. Huang, H. Gong, M. Fujita, T. Mochizuki, A. Toyoda, A. Fujiyama, E. Kaminuma, Y. Nakamura et al. (2016) OryzaGenome: genome diversity database of wild *Oryza* species. *Plant Cell Physiol.* 57: e1.

Phi, C.N., D. Fujita, Y. Yamagata, A. Yoshimura and H. Yasui (2019) High-resolution mapping of *GRH6*, a gene from *Oryza nivara* (Sharma et Shastro) conferring resistance to green rice leaffopper (*Nephotettix cincticeps* Uhler). *Breed. Sci.* 69: 439–446.

Reuscher, S., T. Furuta, K. Bessho-Uehara, M. Cosi, K.K. Jena, A. Toyoda, A. Fujiyama, N. Kurata and M. Ashikari (2018) Assembling the genome of the African wild rice *Oryza longistaminata* by exploiting synteny in closely related *Oryza* species. *Commun. Biol.* 1: 162.

Sakai, H., S.S. Lee, T. Tanaka, H. Numa, J. Kim, Y. Kawahara, H. Wakimoto, C.C. Yang, M. Iswamoto, T. Abe et al. (2013) Rice annotation project database (RAP-DB): An integrative and interactive database for rice genomics. *Plant Cell Physiol.* 54: e6.

Sallaud, C., C. Gay, P. Larmande, M. Bès, P. Piffanelli, B. Piegu, G. Droc, F. Regad, E. Bourgeois, D. Meynard et al. (2004) High throughput T-DNA insertion mutagenesis in rice: a first step towards in silico reverse genetics. *Plant J.* 39: 450–464.

Satoh, H., A. Nishi, N. Fujita, A. Kubo, Y. Nakamura, T. Kawasaki and T.W. Okita (2003) Isolation and characterization of starch mutants in rice. *J. Appl. Glycsci.* 50: 225–230.

Satoh, H., H. Matsusaka and T. Kumamaru (2010) Use of *N*-methyl-2-nitrosoacetamide treatment of fertilized egg cells for saturation mutagenesis of rice. *Breed. Sci.* 60: 475–485.

Shenton, M., M. Kobayashi, S. Terashima, H. Ohyanagi, D. Copetti, T. Hernández-Hernández, J. Zhang, N. Ohmido, M. Fujita, A. Toyoda et al. (2020) Evolution and diversity of the wild rice *Oryza officinalis* complex, across continents, genome types, and ploidy levels. *Genome Biol. Evol.* 12: 413–428.

Shimizu-Sato, S., K. Tsuda, M. Nosaka-Takahashi, T. Suzuki, S. Ono, K.N. Ta, Y. Yoshida, K. Nonomura and Y. Sato (2020) *Agrobacterium*-mediated genetic transformation of wild *Oryza* species using immature embryos. *Rice (N Y)* 13: 33.

Stein, J.C., Y. Yu, D. Copetti, D.J. Zwickl, L. Zhang, C. Zhang, K. Chougule, D. Gao, A. Iwata, J.L. Goicoechea et al. (2018) Genomes of 13 domesticated and wild rice relatives highlight genetic conservation, turnover and innovation across the genus *Oryza*. *Nat. Genet.* 50: 285–296.

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

Thein, H.W., Y. Yamagata, T. Van Mai and H. Yasui (2019) Four resistance alleles derived from *Oryza longistaminata* (A. Chev. & Roehrich) against green rice leaffopper, *Nephotettix cincticeps* (Uhler) identified using novel introgression lines. *Breed. Sci.* 69:
Tsunematsu, H., A. Yoshimura, Y. Harushima, Y. Nagamura, N. Kurata, M. Yano, T. Sasaki and N. Iwata (1996) RFLP framework map using recombinant inbred lines in rice. Breed. Sci. 46: 279–284.

Tuncel, A., J. Kawaguchi, Y. Ihara, H. Matsusaka, A. Nishi, T. Nakamura, S. Kuhara, Y. Nakamura, B. Cakir et al. (2014) The rice endosperm ADP-glucose pyrophosphorylase large subunit is essential for optimal catalysis and allosteric regulation of the heterotetrameric enzyme. Plant Cell Physiol. 55: 1169–1183.

van Enckevort, L.J., G. Droc, P. Piffanelli, R. Greco, C. Gagneur, C. Weber, V.M. Gonzalez, P. Cabot, F. Fornara, S. Berri et al. (2005) EU-OSTID: A collection of transposon insertional mutants for functional genomics in rice. Plant Mol. Biol. 59: 99–110.

Vaughan, D.A. and H. Morishima (2003) Biosystematics of the genus Oryza. In: Smith, C.W. and R.H. Dilday (eds.) Rice: Origin, History, Technology, and Production, John Wiley & Sons, Inc., New York, pp. 27–65.

Wei, X., J. Qiu, K. Yong, J. Fan, Q. Zhang, H. Hua, J. Liu, Q. Wang, K.M. Olsen, B. Han et al. (2021) A quantitative genomics map of rice provides genetic insights and guides breeding. Nat. Genet. 53: 243–253.

Wu, W., X. Liu, M. Wang, R.S. Meyer, X. Luo, M.N. Ndjiondjop, L. Tan, J. Zhang, J. Wu, H. Cai et al. (2017) A single-nucleotide polymorphism causes smaller grain size and loss of seed shattering during African rice domestication. Nat. Plants 3: 17064.

Wu, Y., S. Zhao, X. Li, B. Zhang, L. Jiang, Y. Tang, J. Zhao, X. Ma, H. Cai, C. Sun et al. (2018) Deletions linked to PROGI gene participate in plant architecture domestication in Asian and African rice. Nat. Commun. 9: 4157.

Xu, X., X. Liu, S. Ge, J.D. Jensen, F. Hu, X. Li, Y. Dong, R.N. Gutenkunst, L. Fang, L. Huang et al. (2011) Resequencing 50 accessions of cultivated and wild rice yields markers for identifying agronomically important genes. Nat. Biotechnol. 30: 105–111.

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

Yano, M., Y. Isono, H. Satoh and T. Omura (1984) Gene analysis of sugary and shrunken mutants of rice, Oryza sativa L. Japan. J. Breed. 34: 43–49.

Yano, M., K. Okuno, H. Satoh and T. Omura (1988) Chromosomal location of genes conditioning low amylose content of endosperm starches in rice, Oryza sativa L. Theor. Appl. Genet. 76: 183–189.

Yasui, H. and N. Iwata (1998) Development of monotelosomic and monoacrosomic alien addition lines in rice (Oryza sativa L.) carrying a single chromosome of O. Punctata Kotsch. Japan. J. Breed. 48: 181–186.

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

Zhang, Q.-J., T. Zhu, E.-H. Xia, C. Shi, Y.-L. Liu, Y. Zhang, Y. Liu, W.-K. Jiang, Y.-J. Zhao, S.-Y. Mao et al. (2014) Rapid diversification of five Oryza AA genomes associated with rice adaptation. Proc. Natl. Acad. Sci. USA 111: E4954–E4962.

Zhao, Q., Q. Feng, H. Lu, Y. Li, A. Wang, Q. Tian, Q. Zhan, Y. Lu, L. Zhang, T. Huang et al. (2018) Pan-genome analysis highlights the extent of genomic variation in cultivated and wild rice. Nat. Genet. 50: 278–284.