NBRP-Rice 04

NATIONAL BIORESOURCE PROJECT RICE NEWSLETTER

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Seeds of cultivated rice (center) and wild *Oryza* species. In the wild *Oryza* species, a lot of red rice is included, and there is diversity in the shape and size of the seeds.

Contents

NBRP-Rice 4th phase: Activities in the fourth year

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2020 was a year in which our daily life was greatly affected by the coronavirus calamity. I hope all of you have been well. Because the resources possessed by NBRP Rice have been stably maintained despite the pandemic, there have been no problems in that respect; so, please don't worry. However, the activity of NBRP-Rice itself has not followed the original plan due to countermeasures against the virus. NBRP-Rice is acting to help the rice research community to effectively utilize the wild *Oryza* genetic resources owned by the National Institute of Genetics, the various mutant lines owned by Kyushu Univ., and other resources. One example of NBRP-Rice activities is an open-field tour conducted every year. By actually seeing the genetic resources of NBRP-Rice through this open field tour, we hope that researchers will be inspired to use them in their experiments.

This year, we planned an overseas open-field tour (overseas open-field), in addition to the yearly open-field tours at the National Institute of Genetics and Kyusyu Univ. We prepared for the overseas open-field with the help of, among other researchers, Dr. Yasui of Kyushu Univ. In collaboration with local researchers in Myanmar, it was planned to grow there the wCSSL lines (a series of lines in which chromosome fragments are introduced into a cultivated line background, together covering most of the chromosomes of the wild *Oryza* AA genome - one of the best known resources of NBRP-Rice), in order to observe the wCSSL lines in an environment different from Japan. Our plan also included an opportunity to observe wild *Oryza* growing naturally in Myanmar. We recruited domestic researchers wishing to join the tour; but regretfully, we had to postpone the planned visit. When there is once again no problem in traveling overseas, we will plan this visit again. So, don't miss it!

Because NBRP-Rice carries out projects supported by public research funds, its yearly activity is recorded and reported. In particular, the number of the actual users is important, so the monthly change in the number of the users has been recorded for a long period of time. From these numbers, the effect of the coronavirus pandemic can be clearly seen. Because the material is rice, there is a tendency for a large volume of seed requests in early spring every year. However, in 2020, the number of users significantly decreased compared with a normal spring; this is largely because the first state of emergency was declared in April. On the other hand, the number of requests for the genetic resources recovered somewhat after July. Because the effect of the pandemic is seen even in the number of the users in NBRP-Rice, there may be adverse effects not only in the restaurant business but in all sectors, including research and education. At the moment of writing, the second state of emergency has been declared, so the future from now on is unclear. We just pray that normal daily life can resume as soon as possible!

In the past year, we have been forced to think about how science and scientists face society. There have been several times where science had the chance to appeal to society about fully foreseeable future outcomes. It must be fully examined how this communication functioned and how it was received by society. In other point of view, we faced a situation where the potential of basic science was felt: it appears that development of the vaccine progressed faster than anticipated, doesn't it? It seems that the first RNA vaccination will start in the near future. The RNA vaccine was developed by combining various elementary technologies including stabilization and delivery of RNA. Truly, this is the fruit of basic research. It is reported that mRNA, which is used in the RNA vaccine against the coronavirus, is attached with a cap structure in its 5' terminal. This cap structure was discovered and reported by Dr. Yasuhiro Furuichi in 1975, in his study using a silkworm RNA virus. I have witnessed the actual scene where basic research was combined with other knowledge and technology to generate a breakthrough; with this, I felt the power of basic science and was encouraged to tackle basic research again. I will end this section on that positive note.

Column

My journey with genetic resources of rice −from chromosome to genome

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Nineteen years have already passed since the National Bioresource Project (NBRP) was started in 2002. I was in charge of the rice project for 14 years from the first period to the third period at the National Institute of Genetics, the core institution of "RICE"; this overlapped with my final period as a researcher. My encounter with rice goes back to the days of my graduate study in 1975. In my doctoral research, being interested in chromosomes in those days, I changed my research material to rice, whose karyotype was hardly known because it was so small. I started the research with observation of the chromosomes. They were very small indeed! Observation by the normal squash method was almost impossible; so, the cell wall of the root tip tissue was digested to make it hypotonic, followed by preparation of a flame dried specimen. In this way, at last I could manage to clarify the karyotype of the somatic cell chromosome! The key to success was that I made use of the chromosome sampling method used for animal cells, which was under development in those days. Determination of the rice karyotype was the first in the world. Most of the 12 (n=12) chromosomes had a centromere around its central region, and the length of those 12 chromosomes changed continuously; so, it was very difficult to distinguish them individually. It was a trisomics series of rice that contributed to solving this problem. The series of trisomics (synonym of trisomy in animals) in which chromosomes are added one by one to a diploid plant was a precious genetic resource that was still in the stage of preparation and storage for many years in the breeding laboratory of Kyushu Univ. In addition, groups of lines having various genes controlling morphological traits had been prepared by many researchers and institutions including Kyusyu Univ. and Hokkaido Univ., where analyses of genetic linkage groups had been carried out. In parallel to this, correlation of the genetic linkage groups with the trisomics had been carried out; so, these genetic resources were available. Thanks to these, I could clarify most of the relationships between the chromosomes and the genetic linkage groups.

I also carried out analysis of the chromosomes in the meiotic pachytene (pachytene stage), which are suitable for observation in more detail than in the somatic cell.

At the International Rice Genetics Symposium held at IRRI in 1986
Dr. Hiko-ichi Oka, the author, Dr. G.S. Kush, and Dr. Nobuo Iwata (from left to right)

By using the chromosomes at the pachytene stage of not only *Oryza sativa japonica* rice (AA genome) but also wild species such as *O. punctata*, *O. officinalis*, and *O. brachyantha* having the BB genome, the CC genome, and the FF genome, I carried out the comparative analysis of these structures to each other. In order to solve the problem of chromosome identification, in 1985 and 1986, I was invited to the International Rice Research Institute (IRRI) by Dr. Kush (Director of the IRRI Breeding Department), and carried out collaborative research as a visiting researcher, staying twice around a month each. The collaborative research aiming to unify the chromosome numbers in IRRI was based on a joint proposal by Dr. Oka, Dr. Kinoshita (Professor of Hokkaido Univ.), Dr. Iwata (Professor of Kyushu Univ.), and Dr. G.S. Kush (Picture). During this period, I had an opportunity to become acquainted with the storage of genetic resources in an international institution as well as with the scale and method of cultivation (Picture). I also recall that by this time Dr. D.S. Brar had already tackled the incorporation of a different genome (BB and CC genome species) into cultivated rice by hybridization. In the 1980s, the Council of Rice Genetics was started internationally with the aim of unifying the naming of genetic traits of rice, genetic linkage groups, and chromosomes, etc. As the bulletin of this organization, the Rice Genetics Newsletter was published by the National Institute of Genetics by the efforts of Dr. Hiko-ichi Oka. Eventually, editorship by the council members was handed over to the NBRP Rice project until it came to an end in 2010.

After my postdoctoral research, I had been engaged in the field of molecular biology using animals and microorganisms for 10 years in the 1980s. From 1990, I joined the rice genome project started by the Ministry of Agriculture, Forestry and Fisheries. I established the gene mapping and physical mapping team in the rice genome project, and was able to produce a highly dense and precise genetic linkage map. After completion of the linkage map, I moved my research lab to the National Institute of Genetics, so I left this project. However, it is said that completion of the total rice nucleotide sequence in 2004 was possible due to this highly precise map; to my great pleasure, which was more than I could have dreamt of. The person who guided me to engage the rice genome project and to research in rice again was Dr. Masahiro Nakagawara, who established the Gene Bank in the Ministry of Agriculture, Forest, and Fisheries. He served as Head of the Gene Bank, and later as Director of the National Institute of Agrobiological Sciences. There is no doubt that his insights into breeding science, genetics, and genetic resource science greatly helped my research and my projects in these fields.

During the decade after the determination of the rice genome nucleotide sequence, development of many methodologies and groups of lines was actively carried out domestically and world-wide. These developments included, among other things, isolation of various genes using the genome sequence, preparation of chromosome segment substitution line groups, preparation of various mutant lines and establishment of methods for exploring them, and theories of breeding line preparation. During the most recent 7 to 8 years, DNA sequence analysis has made remarkable progress, achieving significant reductions in both cost and time. Owing to this progress, the age has come where many genetic resource lines (cultivation lines, as well as lines of existing species, wild species, mutant lines, etc.) can be analyzed in a short time, so that their characteristics can be efficiently determined and utilized, each line can be analyzed by comparing the differences in the genome sequence among many lines.

The wild *Oryza* samples collected for many years by researchers including Dr. Oka and Dr. Morishima in the National Institute of Genetics have been cultivated, multiplied, confirmed, and stored in the first and second periods of the NBRP, and have been organized as genetic resources of NBRP. In the third period, by using these precious gene resources, many fruits could be obtained by wide international collaborations. Examples include: research to explore the origin of cultivated rice (Reference 1); research to analyze mutual evolutionary relationships among the AA, BB, CC, EE, FF, GGHH, HHKK, KKLL, etc. genomes (Reference 2); and research to analyze evolution and difference in genome structures in various wild species having the CC genome (Reference 3). I hope that from now on these genetic resources may continue to be much more widely used.

During the days of my graduate research, I not only had to ask other laboratories to use a high-performance microscope for taking chromosome pictures but also to develop the pictures by myself; so, in those days, I could never even dream that the total nucleotide sequence of rice could be clarified during my life as a researcher. When I look back on those days as a student — now closing my life as a researcher, I feel I am living in a completely different age. I feel excited when I think of how the coming 50 years will progress and how biological research, not limited to plants and rice, will develop. Biological genetic resources will clearly show their value in future research. Rice research in the world could never have advanced without genetic resources. Whatever the age, living things in the natural world will never stop their evolution; so, the value of living and preserved genetic resources will continue to increase. This is shown to us without doubt, as COVID-19 has struck us with world-changing impact. It is always necessary to carry out the genetic resource studies and to reconstruct the project by reviewing various findings and outcomes. The methods of collecting genetic resources, including the scale, must also be considered, and how to prioritize improvement of the research infrastructure and maximum utilization of resources. I hope that the rice genetic resources project will evolve with the aim of utilizing the diversity of genetic resources that is so important for world food production.

Scene of the organization room of rice seed breeding and genetic resources in IRRI as of 1986

References

1. Huang X, Kurata N, et al. A map of rice genome variation reveals the origin of cultivated rice. ***Nature.*** 490: 497-501. 2012.
2. Stein JC, et al. Genomes of 13 domesticated and wild rice relatives highlight genetic conservation, turnover and innovation across the genus Oryza. ***Nat Genet***. 50:285-296. 2018.
3. Shenton M, et al. Evolution and Diversity of the Wild Rice *Oryza officinalis* Complex, across Continents, Genome Types, and Ploidy Levels. ***Genome Biol Evol***. 12:413-428. 2020.

New Findings from Studies utilizing NBRP-Rice Genetic Resources

A reference C genome for the wild rice *Oryza officinalis*

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Introduction

Domestication of crop species results in a reduction in genetic diversity. As the environment changes, there is a need to introduce new or exotic alleles into cultivated crops to maintain or increase yields under changing conditions. Access to crop wild relatives like wild rice species is a source of such diverse genetic material. Unlike the A-genome cultivated rice species (*Oryza sativa* L. and *O. glaberrima* Steud), several species in *Oryza* have a C genome, which makes introgression from these species more difficult. Understanding the C genomes of *Oryza* species, alongside the other genome types in the genus, can provide new understanding of the evolutionary processes that allowed the *Oryza* species to adapt to different environments. This information can help to inform the innovative breeding required to meet the demand for new varieties that can thrive under changing environmental conditions. In this work, we constructed a C genome wild rice reference for *Oryza officinalis*, and investigated diversity in B and C genome species using resequencing data from wild rice accessions in the NIG Genetic Resource Center.

Background

The *Oryza* genus comprises ~21 wild species composed of 10 different genome types, and the species are classified into several complexes based on their characteristics; the largest of these is the *Oryza officinalis* complex (with different combinations of B, C, D and E genomes in diploid and allotetraploid species). The Genetic Resource Center at the National Institute of Genetics maintains stocks of many accessions of these species, collected by former members of NIG on pioneering study tours of different regions of the world starting in the 1950s. The *Oryza* species are distributed in most tropical and subtropical areas of the world, which is reflected in their wide phenotypic diversity. Loci linked to favorable ecological adaptations and disease resistance have already been identified in the *O. officinalis* genome, and among C genome species, *O. officinalis* is the most widely distributed, across South and South-east Asia. Therefore, we targeted the *O. officinalis* genome to further understand evolution and explore genomic and structural divergence in the *O. officinalis* complex[1].

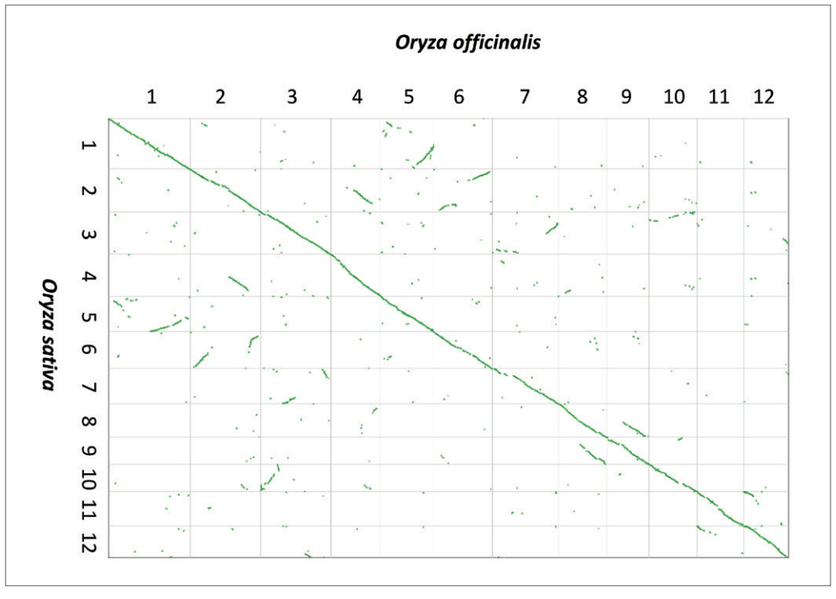

Figure 1. Syntenic relationship between *Oryza officinalis and Oryza sativa*
Dot plot generated by aligning annotated genome sequences of *O. officinalis* and *O. sativa* using the software "SynMap" hosted at CoGe (https://genomevolution.org/coge/)

Reference genome construction

We made our C genome reference by de novo assembly of Pacbio reads and several different libraries of Illumina reads. The de-novo assembled scaffolds were then arranged into chromosome pseudomolecules based on a physical map created by sequencing the ends of BAC library clones, previously prepared by the Arizona Genomics Institute. This allowed us to include the vast majority of *Oryza officinalis* scaffolds into 12 chromosomes, all of which are substantially larger than their *O. sativa* counterparts, but which maintain a high degree of synteny with *O. sativa* (Figure 1), and with species of other genome types.

Diversity and evolution in *Oryza* and the *Oryza officinalis* complex

The valuable materials held at NIG have been organized and classified, and a wild core collection established for the study of these species[2]. Recently, whole genome short read NGS data of ~200 of these accessions from 19 wild *Oryza* species has been prepared by NBRP, and in addition the C genome *Oryza officinalis* reference we created in this study, a series of *Oryza* reference genomes have been created by international groups[3]. Based on these genomes and their annotations, and on resequencing data from 77 NBRP *O. officinalis* complex accessions, we looked at evolution and phylogenetics in *Oryza* (Figure 2) and in the *O. officinalis* complex.

We concluded that the *O. officinalis* genome is 1.6 times larger than the A genome of cultivated *Oryza sativa*, mostly due to proliferation of Gypsy type long-terminal repeat transposable elements, but overall syntenic relationships have been maintained with other *Oryza* genomes (A, B, and F). Using draft genome assemblies of the two other C genome diploid species, *Oryza eichingeri* and *Oryza rhizomatis*, we estimated divergence times in the genus (Figure 2). By analysis of short-read resequencing of a series of other B and C genome species and accessions (Figure 3), we revealed that after the divergence of the C genome progenitor, there was still a substantial degree of variation within the C genome species through proliferation and loss of both DNA and long-terminal repeat transposable elements.

Prospects

For the purpose of exploiting the stress tolerance characteristics of the *O. officinalis* complex, the C genome reference is now available as a tool to aid in molecular breeding of cultivated rice. The genome sequence will aid in the more efficient use of diverse wild rice resources in rice breeding for further improvements. The *Oryza* C genome appears dynamic, based on recent variation caused by TEs and genome innovations associated with polyploidization. In the context of plant breeding, this relative instability provides us with diverse genetic resources that will be invaluable in future rice improvement.

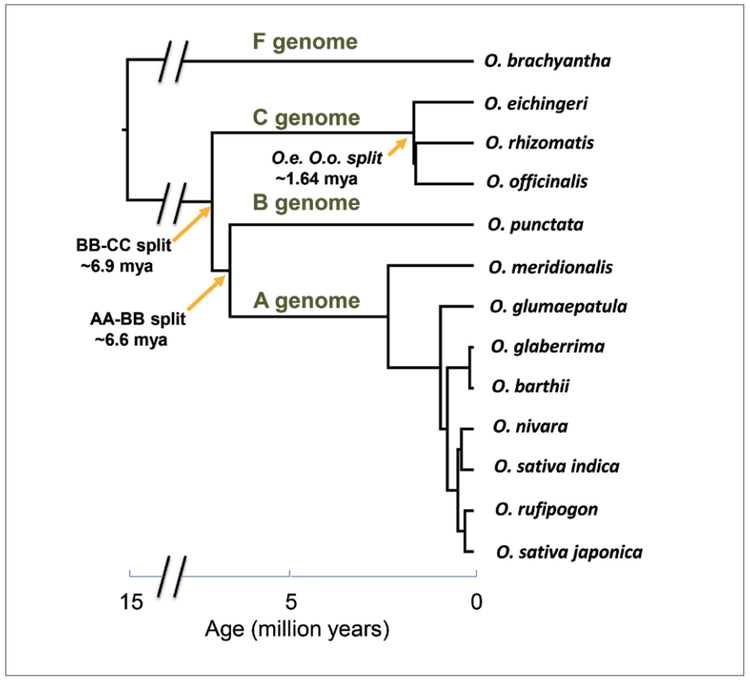

Figure 2. Divergence time of different groups in the *Oryza* genus
Phylogenetic tree based on gene sequences of single-copy orthologs in different *Oryza* species

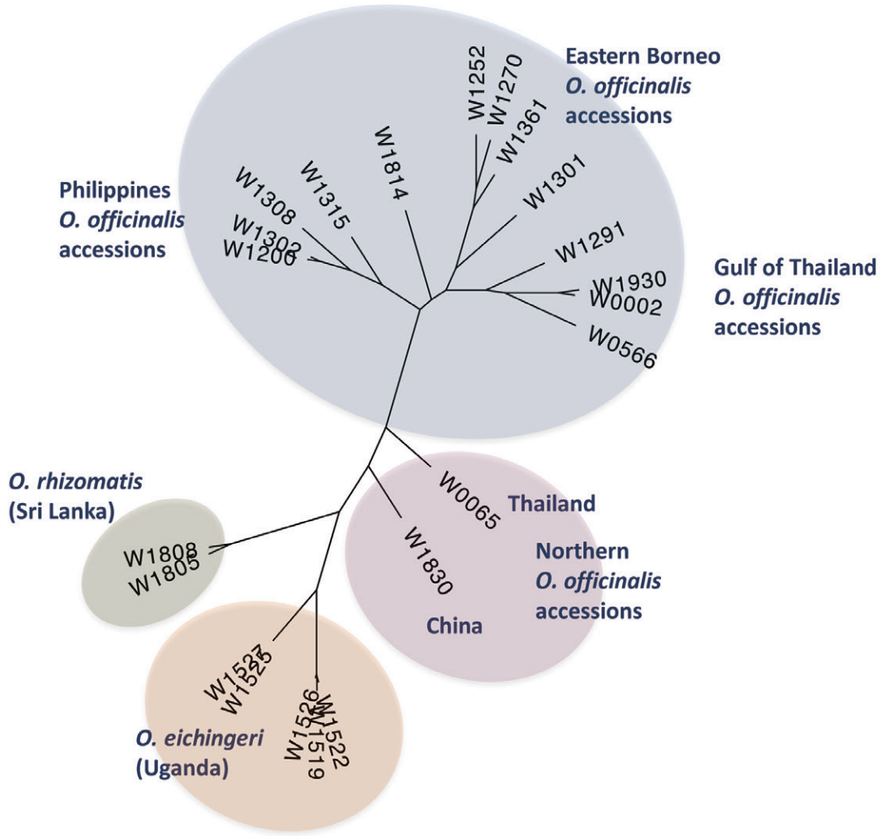

Figure 3. Phylogenetic relationships among diploid C genome *Oryza* species accessions
Unrooted maximum likelihood tree based on representative SNPs among CC diploid accessions, mapped against the *O. officinalis* reference genome.

Acknowledgements:

This study was conducted using wild *Oryza* materials from the National Institute of Genetics (NBRP Rice). I would especially like to thank Emeritus Professor Nori Kurata of NIG, and Professor Yutaka Sato for developing the project and for guiding it to its conclusion, respectively. I would also like to thank Hajime Ohyanagi and Professor Kentaro Yano of Meiji University, for their invaluable help, support and collaboration. Many thanks are also due to all of the coauthors, in Japan and internationally. We owe an extra debt of gratitude to collaborators from Arizona, most especially Dario Copetti and Rod Wing.

References

1. Shenton M, Kobayashi M, Terashima S, Ohyanagi H, Copetti D, Hernández-Hernández T, et al. Evolution and Diversity of the Wild Rice *Oryza officinalis* Complex, across Continents, Genome Types, and Ploidy Levels. ***Genome Biol Evol***. 2020 Apr 1;12(4):413–28.
2. Ohyanagi H, Ebata T, Huang X, Gong H, Fujita M, Mochizuki T, et al. OryzaGenome: Genome Diversity Database of Wild *Oryza* Species. ***Plant and Cell Physiology***. 2016 Jan 1;57(1):e1–e1.
3. Stein JC, Yu Y, Copetti D, Zwickl DJ, Zhang L, Zhang C, et al. Genomes of 13 domesticated and wild rice relatives highlight genetic conservation, turnover and innovation across the genus *Oryza*. ***Nature Genetics***. 2018 Feb;50(2):285–96.

Oryzabase Now

The landscape of WGS data in wild *Oryza* species

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Surveying the mountain of WGS data

The genome sequence is one of the most important pieces of information in biological research. Owing to the development of the next generation sequencer, various types of whole genome sequencing (WGS) data are generated today. Many research groups in worldwide, including us, have been releasing WGS data of wild *Oryza* species; but a total image of them has not been clear yet.

A query in the NCBI Sequence Read Archive revealed 1629 records of wild *Oryza* WGS data as of April 2020. It was found that 75% of the data are those of *Oryza rufipogon* and *O. nivara*, the ancestoral species of Asian cultivated rice, and those of *O. barthii*, the ancestor of African cultivated rice (TABLE 1). This is possibly because much research attention has been paid on the domestication process and isolation of genes controlling important agronomical traits. On the other hand, it can be said that the research of other wild *Oryza* species is still a relatively unexplored field. Most of the data are registered from 28 research institutions in 5 countries, i.e., China, Japan, U.S, France, and Korea, suggesting a high level of activity in rice research in these countries (TABLE 2).

Although it is collectively called WGS data, the sequence read length (several tens of bp to 100 kb or more), depth, base calling quality and hence the suitable application vary depending on sequencing methods. In wild *Oryza,* Illumina short reads are commonly used to detect single nucleotide polymorphisms (SNPs) and InDels to carry out phylogenetic analyses as well as QTL/GWAS studies; so, most of the data are short reads (96.6%). The read depth, which is important for reliable polymorphism detection, varies from 0.04 to 178.4. About 90% of the data from the National Institute of Genetics (NIG) have sequence depth of x5 or more (TABLE 2). In addition, about 60% of the total data are from NIG (48.7%) and International Rice Research Institute (IRRI) (12.4%). Accordingly, experimental materials such as seeds and DNA are available upon request from these institutes. It is also worth noting that the metadata such as collection sites and trait information are available in their databases.

Genome	Species	Number of WGS data
AA	*O. barthii*	244 (15 %)
	O. glumaepatula	50 (3.1 %)
	O. longistaminata	76 (4.7 %)
	O. meridionalis	48 (2.9 %)
	O. nivara	321 (19.7 %)
	O. rufipogon	656 (40.3 %)
BB	*O. punctata*	45 (2.8 %)
BBCC	*O. minuta*	5 (0.3 %)
	O. punctata 'tetraploid'	8 (0.5%)
CC	*O. eichingeri*	9 (0.6 %)
	O. rhizomatis	34 (2.1 %)
	O. officinalis	31 (1.9 %)
CCDD	*O. alta*	3 (0.2 %)
	O. grandiglumis	7 (0.4 %)
	O. latifolia	7 (0.4 %)
EE	*O. australiensis*	8 (0.5%)
FF	*O. brachyantha*	33 (2 %)
GG	*O. meyeriana*	5 (0.3 %)
	O. meyeriana var. granulata	31 (1.9 %)
HHJJ	*O. longiglumis*	5 (0.3 %)
	O. ridleyi	3 (0.2 %)

Table 1. Number of WGS data in wild *Oryza* species classified by species

Situation of reference genome assemblies

In the case of the AA genome species that are closely related to cultivated rice, such as *O. rufipogon*, short reads are mapped to the Nipponbare reference genome for analysis. However, the read mapping rate declines as the genetic distance from *O. sativa* increases. Reads derived from highly polymorphic regions or from genomic regions not present in Nipponbare are lost during the analysis. In order to avoid this problem and thereby to precisely analyze the diversity of the wild rice genomes, it is important to prepare reference genomes for each species. Recently, an increasing number of the genome assemblies is being constructed using long-read sequencers such as PacBio and Nanopore. To date, 26 wild rice genomes in 10 species have been assembled (TABLE 3). Using these, researches on the domestication history of Asian rice, the pan-genome of the *O. sativa-rufipogon* complex, and genome evolution are progressing. On the other hand, these assemblies appear to be a mixture of varying degrees of completeness, because their contig numbers ranges from of 1,632 to 145,408 (302 in Nipponbare). This situation will be improved by advances on sequencing technologies and assembly methods.
Finally, the Oryza Genome 2.1 database, which is being prepared by NBRP Rice, also provides various information including SNPs. We plan to further update and expand the utilities from now on; so, we welcome your active use of them!

Institute (Country)	Number of WGS data	
	Depth < x5 (*)	Depth > x5 (*)
BGI (China)	18 (15)	19 (16)
CAS (China)	34 (31)	68 (13)
GSC (France)	1 (0)	85 (0)
NCGR (China)	446 (446)	29 (28)
NEW YORK UNIV. (USA)	0 (0)	16 (16)
NIG (Japan)	29 (29)	248 (248)
SAGC (China)	0 (0)	45 (0)
SEOUL NATIONAL UNIV. (Korea)	14 (8)	0 (0)
UNIV. ARIZONA (USA)	103 (40)	62 (40)
UNIV. WUHAN (China)	19 (19)	11 (11)
YUNNAN AGRI. UNIV. (China)	21 (0)	2 (0)
ZHEJIANG UNIV. (China)	1 (0)	277 (0)
Others	0 (0)	81 (35)
Subtotal	686 (588)	943 (407)
Total		1629 (995)

*Number of WGS data whose material strains are maintained in NIG or IRRI.

Table 2. Number of WGS data in wild *Oryza* species registered from research institute in the world

Genome	Species	Acc. No.
AA	*O. barthii*	IRGC105608
	O. glumaepatula	GEN1233
	O. longistaminata	W11, IRGC110404, NA
	O. meridionalis	W2112, Taxon B
	O. nivara	IRGC100897
	O. rufipogon	W1943, W0123-1, W0141, W0170, W1687, W1698, W1739, W1754, W1777, W1979, W2012, W3078-2, W3095-2, Taxon A
BB	*O. punctata*	IRGC105690
CC	*O. officinalis*	W0002
FF	*O. brachyantha*	IRGC101232
GG	*O. granulata*	IRGC102117

Table 3. Wild *Oryza* accessions whose reference genomes are registered in the public database

Technical Tips

Genetic transformation of wild *Oryza* species using immature embryos

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Because wild *Oryza* possesses great genetic diversity, its necessity as research material has been increasing in the fields of basic science and breeding in recent years. There are many wild *Oryza* species that cannot be transformed by the gene transfer method using callus derived from matured seeds. The authors of this paper succeeded in genetic transformation of several wild *Oryza* species by using immature embryos (Shimizu-Sato et al., 2020). Below, important points concerning the process of genetic transformation of wild *Oryza* will be described.

1. Outline

The most important point was to “isolate immature embryos in a suitable state”. For this, growing healthy wild *Oryza* plants was necessary. In a paddy field having a short-day treatment device, the authors grew one individual per accession, while in a plant growth chamber, three individuals were grown. Around 20 good immature embryos could be obtained from about 30 ovaries. Although infection was performed twice independently for certainty, several lines of transformed wild *Oryza* could be obtained in the first round of infection.

2. Growing wild *Oryza*

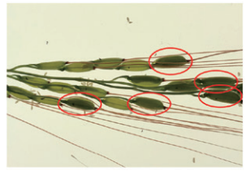
The wild *Oryza* were grown either in a paddy field with a short-day treatment device or in a plant growth chamber. In the case of the plant growth chamber, one seedling was planted in a pot with an approximate size as follows; 5 cm in diameter and 9 cm in height, in order to facilitate spreading of the roots, about 6 holes were made in the bottom of the pot. The growth conditions were: 11 hours for the light period (temperature: 30°C, humidity: 70%) and 13 hours for the dark period (temperature: 25°C, humidity: 70%). Although the heading day differed among accessions, in many cases, the heading started at 50 to 90 days after the imbibition. Basically, the immature embryo was isolated from the ovary about 10 days after pollination. In some accessions, many sterile flowers and insufficient growth of the ovary were observed at 10 days after pollination. On the first flowering day, the authors marked the panicle with a piece of masking tape which the first flowering date were written on. The marked panicle was collected at 10 to 12 days after the first flowering date. The developmental state of the ovary was checked by looking at the unhulled rice on a light box (Picture 1).

3. Isolation of immature embryos

Typical wild *Oryza* ovaries are shown in Picture 2. These are the ovaries after 1, 3, 5, and 8 days from pollination (from left to right). The ovary suitable for sampling was developed fully in the hull, and its endosperm was in a liquid state. The ovary with endosperm which had already become solid was not suitable for sampling. If the time of ovary sterilizing by treatment with hypochlorous acid was too long, the immature embryo was dead, so careful attention was necessary. After sterilization, the immature embryo was isolated from the ovary under observation with a stereoscopic microscope placed in a clean bench. The immature embryo suitable for infection could be easily removed from the endosperm and taken out from the ovary (Picture 3).

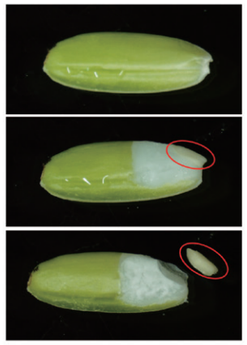
4. Supplement

There are further notable points in infection, callus induction, and regeneration, which could not be explained here; so, those who have an interest are cordially invited to contact us at NBRP (https://shigen.nig.ac.jp/rice/oryzabase/about/contactUs).



Picture 1. Panicle of wild *Oryza*
We put the panicle on a light box to select the ovaries developed fully in the hull (marked with red circles).

Picture 2. Ovary development in wild *Oryza*
Ovaries after 1, 3, 5, and 8 days from pollination (from left to right) are shown. We isolated immature embryos from the ovaries after 8 days from pollination.



Picture 3. Isolation of immature embryo from ovary
We removed pericarp and seed coat with forceps and isolated immature embryo. The immature embryo suitable for infection was easily removed from the endosperm and kept the shape of the embryo after isolation.

References
Shimizu-Sato S. et al. (2020) Agrobacterium-mediated genetic transformation of wild *Oryza* species using immature embryos. ***Rice*** 13:33.

Activity Reports 2020

Public Relations at Academic Societies

84th Annual Meeting of Botanical Society of Japan (Web meeting in September)

43rd Annual Meeting of the Molecular Biology Society of Japan (Web meeting in December)

NBRP-Rice online exhibition at the 43rd Annual Meeting of the MBSJ

NBRP Rice Advisory Committee

The annual committee was held as web meeting on 15th December 2020.

Events 2021

NBRP Rice Public Relations

Several events such as symposium and Open Fields (Study tour of wild *Oryza* genetic resources) introducing NBRP-Rice genetic resources are under consideration to be held in actual and/or web meeting. We will make an announcement in details as soon as the plans are set.

Past issues

Past issues and Japanese versions of this Newsletter are available on Oryzabase (https://shigen.nig.ac.jp/rice/oryzabase/).

Vol.1

- Colum: Professor Morishima and field of diversity
- Oryzabase Now: How to use the new OryzaGenome database
- New findings from studies utilizing NBRP-Rice genetic resources: Detection of quantitative trait loci controlling grain zinc concentration using Australian wild rice *Oryza Meridionalis*
- Technical Tips: Distribution of wild rice species from Oryzabase

Vol.2

- Colum: Genetic resources for insect resistance in rice
- Oryzabase Now: History and current status of Oryzabase
- New findings from studies utilizing NBRP-Rice genetic resources: The presence of a strain-specific gene is involved in sterility of interspecific hybrids
- Technical Tips: Basics of wild rice cultivation: germination

Vol.3

- Colum: The ritual of Overseas Scientific Investigation
- New findings from studies utilizing NBRP-Rice genetic resources: *Leaf Gas Film1* (*LGF1*) gene confers flood tolerance to rice by enhancing gas exchange in submerged leaves
- Technical Tips: Basics of wild rice cultivation: heading and day length

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