

# NBRP-Rice

NATIONAL BIORESOURCE PROJECT RICE NEWSLETTER

# 05

March 2022



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Wild *Oryza* species grown in greenhouse. Most wild *Oryza* species are perennial, and it is necessary to maintain the plants in a heated greenhouse during the winter season in Japan.



## Looking back on the activities of the 4<sup>th</sup> phase of NBRP-Rice

SATO, Yutaka

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Thank you very much for your understanding with regard to the activities of the National BioResource Project (NBRP) and for using National BioResource-Rice (NBRP-Rice). One of the roles of bioresources is to serve as a basis for life science research. NBRP is a project of the Ministry of Education, Culture, Sports, Science and Technology that aggregates and preserves bioresources collected and developed through the hard work of researchers, and distributes them to the research community on request. The NBRP is currently home to 31 strategically important bioresources and information centers. One of them is NBRP-Rice. NBRP-Rice handles unique materials that do not overlap with the genetic resource projects of other ministries and agencies. NBRP-Rice collects, preserves, and provides wild *Oryza* genetic resources of the genus *Oryza*, as well as various experimental strains (mutants and experimental strains derived from wild *Oryza* species). For more information on these strains, please visit the NBRP-Rice information website Oryzabase (<https://shigen.nig.ac.jp/rice/oryzabase/> or use the QR code below).

This year is the final year in the 4th phase of five-year NBRP. In the final year of the 3rd phase, I took over the NBRP-Rice work from my predecessor. Within five years, I have experienced the whole cycle from application to evaluation for NBRP project. Now we are in the last year of the 4th phase, and the final evaluation has already been completed, and we were able to receive a better evaluation than in the final evaluation of the 3rd phase. We thank all the users of NBRP-Rice for the better evaluation and appreciate the significant contribution made by these users through utilizing NBRP-Rice resources and publishing a broad range of high-quality basic research papers. I would like to take this opportunity to express my deepest gratitude. Five years ago, our pledge at the time of the application for NBRP 4th phase was to increase the number of NBRP-Rice users. From the beginning of the 4th phase, several projects were started in order to widely inform researchers about NBRP-Rice resources and promote their use. These include the publication of this newsletter and offering tours to open experimental fields growing experimental strains derived from wild *Oryza* species. We have also conducted joint public relations activities among NBRP plant resources, and held workshops and symposiums on plant bioresources at relevant academic meetings. These activities seem to have been effective, and the annual average number of users in the NBRP 4th phase increased by approximately 1.5 times compared with the 3rd phase. During the NBRP 4th

phase, we also worked on developing a genetic transformation system for wild *Oryza* species and on genome sequencing of wild *Oryza* species using next-generation sequencing technology. The outcomes of these activities to upgrade research infrastructure are also distributed via Oryzabase and other sources. We hope this information will be used to further stimulate the research utilizing genetic resources of wild *Oryza* species and others.

When I was appointed to be in charge of NBRP-Rice, it was my first time to deal with wild *Oryza* genetic resources directly. In these five years, I have learned difficulty and felt interest in dealing with these resources. NBRP-Rice has been operated with the support from many part-time technicians, and this support, such as covering wild *Oryza* panicles with paper bags in the summer, harvesting and organizing the seeds, has helped the project to progress smoothly. When you use wild *Oryza* seeds, I hope that you will also think about the dedication of these people. I have made efforts to deepen my own understanding of wild *Oryza* genetic resources as research materials, but I feel that there are still many things I don't yet know. I would like to continue distributing information to rice researchers on the fascinating aspect of wild *Oryza* as much as possible.

At the moment of writing this newsletter, applications for the NBRP 5th phase have closed. It is not yet clear whether the NBRP-Rice project can be continued in the future, but if it can, we will continue our activities to help researchers utilizing the NBRP-Rice resources effectively in their research.



Covering wild *Oryza* panicles with paper bags in summer



Please use this QR code to visit Oryzabase.

## Column

## Preservation of local varieties that supported traditional rice farming in Indo-China countries

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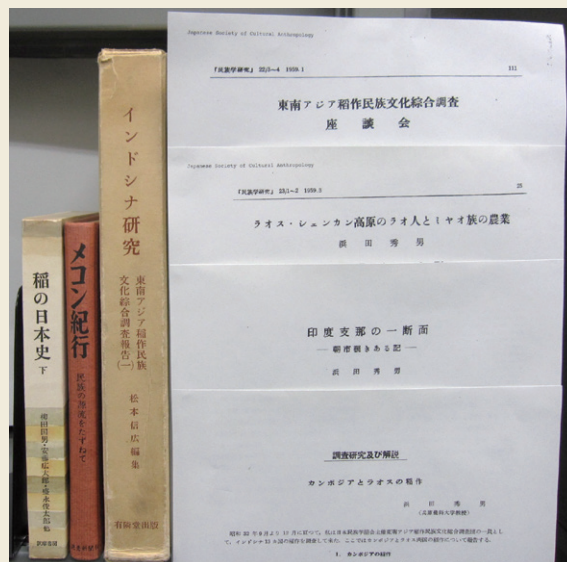
The National Institute of Genetics preserves seeds of local varieties that supported traditional rice farming in Indo-China countries. The seeds comprise 638 accessions numbered between C6006 and C7197. According to the records of the National Institute of Genetics, these strains were obtained from a researcher named H. Hamada on May 9, 1959. About two years ago, I happened to learn that the researcher named H. Hamada was Professor Hideo Hamada of Hyogo University of Agriculture (the predecessor of the Faculty of Agriculture, Kobe University). In this column, I would like to explain how Professor Hamada collected these local varieties and how I came to learn that they were preserved at the National Institute of Genetics.



Samples of seeds and panicles of local varieties collected by Dr. Hamada

Professor Hideo Hamada was born in Kobe in 1899. He studied at the Faculty of Agriculture, Hokkaido Imperial University and in 1938 he received his PhD in Science from Kyoto University. He became an engineer at the Tainan Prefecture Agricultural Research Institute, and after working as a professor at Harbin Agricultural University and then at Hokkaido Imperial University, he received an offer to work as a professor at Hyogo University of Agriculture, which was newly established after the war. He served at Hyogo University of Agriculture from October 1949 to March 1965 and was mainly engaged in research into rice. It is notable that

he participated in the Inasaku-shi Kenkyukai (Rice Farming History Research Meeting, organized by Kunio Yanagita, Hirotaro Ando, Toshitaro Morinaga, and Nobuhiro Matsumoto). The purpose of the meeting was to explore the origins of Japanese rice. The meeting was started in 1955 and continued for eight years, with discussions being held by experts in ethnic science, agriculture, linguistics, and archeology. The records of the discussions are included in the book "Ine no Nihonshi (Japanese History of Rice)" published by Chikumashobo. Another fact of note is that he conducted a survey on agriculture in the countries of Indochina, as described below.



Books and materials related to "Synthetic research team of the culture of rice-cultivating races in Southeast Asian countries"

In the mid-1950s, when reconstruction after World War II had settled, people in Japan eventually became ready to conduct academic research and surveys in foreign countries. In 1955, Kyoto University sent out an overseas academic expedition team to Karakorum-Hindukush with Professor Hitoshi Kihara as the leader. Following this, a full-scale academic expedition was organized. This was the "Southeast Asian Rice Farming Ethnic Culture Research Mission" dispatched by The Japanese Society of Ethnology and sponsored by the Ministry of Education. The first research team comprised 18 experts in linguistics, ethnology, archeology, and other fields. They were dispatched to Vietnam, Cambodia, Laos, and Thailand from 1957 to 1958. One of the researchers in the "Agricultural Team" of this expedition was Professor Hideo Hamada of Hyogo University of Agriculture. From September 1957 to January 1958, Professor Hamada conducted agriculture-related field surveys

in the four countries mentioned above, moving mainly over land, and collected 1208 strains of local rice varieties. After returning to Japan, he analyzed a broad range of seed traits among the collected samples and compiled the results in a report titled "Rice in the Mekong Valleys", published as part of "Indo-Chinese studies: Synthetic research of the culture of rice-cultivating races in Southeast Asian countries (I)" in 1965. The results of the field survey are presented in the book "Mekong Kiko (Mekong Travelogue; Yomiuri Shimbun)" and several other research articles.

I (Ishii) am working at the Graduate School of Agricultural Science, Kobe University. In 2006, renovation work was conducted on a university building in the Graduate School of Agricultural Science. At that time, many samples of rice seeds and panicles were discovered in a rarely entered office warehouse. I was informed about it and, from the records related to the samples, I realized that some of the samples included rice collected by Dr. Hamada in his field surveys in Indo-China countries. I received these samples, as well as drafts of reports and handwritten notes that were packed together with them, from the Graduate School of Agricultural Science. Of the 1208 strains collected by Dr. Hamada, seeds of 1129 strains were identified. Unfortunately, however, their germinability had been lost because they had been stored at room temperature. These seeds were collected in 1957-58. After 1958, Indo-China countries were plunged into very chaotic times, including the Vietnam War in the 1960s and the Cambodian Civil War in the 1970s. In addition, after the green revolution in rice in the 1960s, a number of modern varieties were developed and introduced to the region. Considering these facts, it can be said that the seeds collected by Dr. Hamada are a huge collection of local varieties that supported traditional rice farming in Indo-China countries. It is not clear why they were packed away in the warehouse of Kobe University Graduate School of Agricultural Science. According to the materials I have received, it is likely that when Dr. Hamada retired from research in the 1980s, he donated his research materials to the Faculty of Agricultural Science at Kobe University, which had transitioned into a national university from the former Hyogo University of Agriculture. Based on these materials, I could gain a detailed insight into the research history of Dr. Hamada and the Indo-China expedition team.

To change the topic slightly, about two years ago I happened to meet Dr. Yoichiro Sato, a distinguished professor at Kyoto Prefectural University (Professor Emeritus of the Research Institute for Humanity and Nature), at a meeting on domestic rice genetic resources held at the National Institute of Genetics. From him, I heard that he had seen that the seeds Dr. Hamada had collected were still being kept in storage at the National Institute of Genetics. This was good news because Dr. Sato had previously worked at the National Institute of Genetics. Later, upon doing some investigation, I found that 638 strains were registered as genetic resources for cultivated rice. The countries of origin of these

strains were Cambodia (149 strains), Vietnam (316 strains), Laos (84 strains), and Thailand (89 strains). However, many records indicated that seed propagation was last conducted in the 1980s, and only about half of the strains of seeds could be distributed.

As mentioned above, fortunately, it was revealed that the local varieties collected by Dr. Hamada were packed and hidden away in the Institute of Genetics. However, there were many strains that had different passport data regarding their historical trail, such as the name of the variety and the location of collection, compared with the information held by Kobe University. These materials include samples collected in the field, together with those distributed from agricultural research institutes. For this reason, there were cases where Cambodian and Thai varieties had been distributed from agricultural research institutes in Vietnam and then registered as Vietnamese varieties. There were also some typing errors, probably because Dr. Hamada's handwriting is difficult to read. Fortunately, the raw data and collection information were written on the seed bags found at Kobe University. These were written by Dr. Hamada, and are the most trustworthy historical record of the samples. The circumstances surrounding the research are also described in "Japanese History of Rice (Chikumashobo)" and "Mekong Travelogue (Yomiuri Shimbun)," so it is possible to make corrections to passport data in line with this information.

I have been conducting research mainly on wild rice, and have received many wild strains distributed as research materials from the National Institute of Genetics. These were collected in the field by Dr. Oka and Dr. Morishima and maintained at the Institute of Genetics. I am very grateful for these resources. As mentioned above, Dr. Hideo Hamada, a professor at Hyogo University of Agriculture, the predecessor of the Faculty of Agriculture, Kobe University, also collected local varieties in Indo-China countries more than 60 years ago. When I learned that nearly half of them were maintained and propagated at the National Institute of Genetics, I was very surprised. Even if it happened by chance, I feel like I have been given a mission to connect them. Therefore, I hope I can contribute in a meaningful way to maintaining these genetic resources of local varieties that supported traditional Indo-China rice farming by correcting their historical records and adding basic agricultural trait data.



Seed bag labelled by Dr. Hamada (Variety name, place of collection, and date of collection are included)



# Initiation mechanism of internode elongation elucidated by research on deepwater rice that overcomes flooding

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## Introduction

Among the world's three major grains – rice, wheat, and corn – rice is the only one that can be cultivated in a submerged (paddy field) environment. Deepwater rice, grown mainly in Southeast Asia, can survive in environments enduring long-term flooding during the rainy season, where the water depth can reach several meters. Deepwater rice can elongate its stem (internodes) according to the level of water, raise its leaves away from the water surface to maintain respiration and photosynthesis, and thus survive in this harsh flooded environment (Figure 1A). Until now, we have elucidated part of the mechanism whereby deepwater rice promotes internode elongation, identifying the internode elongation promoting transcription factors *SNORKEL1* and *SNORKEL2*<sup>1)</sup>, and finding the haplotype of the gibberellin biosynthesis enzyme gene (*GA20ox2*) specifically retained by deepwater rice<sup>2)</sup>. However, these are both mechanisms that control the promotion of internode elongation that has already been initiated. At first, it was not known how rice was able to initiate internode elongation. Normal paddy rice initiates internode elongation during the transition from the vegetative growth phase to the reproductive phase. However, deepwater rice can elongate its internodes from the vegetative phase. As such, we thought that there may be a control mechanism to initiate internode elongation which is unique to deepwater rice and attempted to elucidate its molecular mechanisms. In addition, we applied the results obtained in this research to other gramineous crops such as barley. It was shown that the mechanism of internode elongation initiation discovered in deepwater rice (which is a specialized type of rice) may be conserved ubiquitously in gramineous crops.

## 1. Detection of QTLs controlling early internode elongation

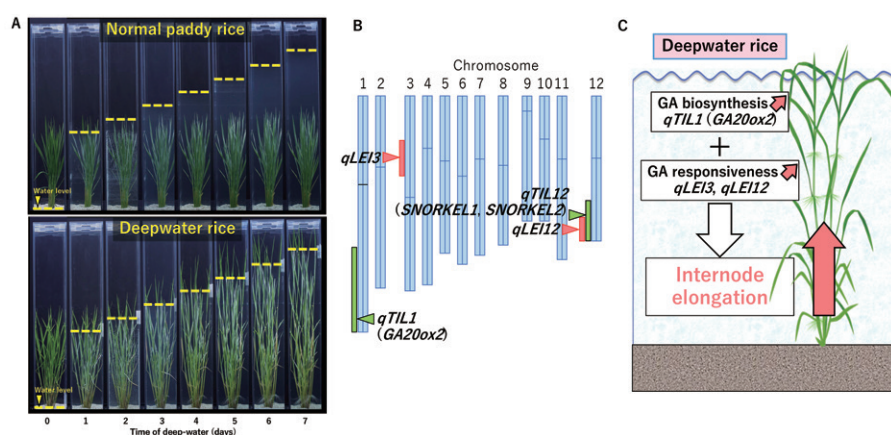
We conducted QTL analysis of internode elongation using an  $F_2$  population of deepwater rice C9285 (Dowai38/9) originating from Bangladesh, and normal paddy rice Taichung 65 (T65) originating from Taiwan. We detected *qTIL1* and *qTIL12* on chromosomes

1 and 12, respectively, that control the total internode length (TIL), and the sum of the lengths of elongated internodes under a flooded environment. We also detected *qLEI3* and *qLEI12* on chromosomes 3 and 12, respectively, that control the position of the lowest elongated internode (LEI), an index of early internode elongation (Figure 1B). In the subsequent analysis, we identified *GA20ox2*, one of the gibberellin biosynthesis enzymes, as the gene responsible for *qTIL1*<sup>2)</sup>. Both *qTIL12* and *pLEI12* were detected near the end of chromosome 12. However, plants over-expressing *SNORKEL1* and *SNORKEL2*, the genes responsible for *qTIL12*, had longer internodes but showed no reduction in LEI (early internode elongation). Therefore, we thought that the functions of *SNORKEL1* and *SNORKEL2* were to promote the lengthening of internodes where elongation had already been initiated and that the gene responsible for *qLEI12* that controls early internode elongation exists at a different locus. We created near-isogenic lines (NILs) for each QTL and treated them with gibberellin. In NIL3 and NIL12 harboring *qLEI3* and *qLEI12*, respectively, early internode elongation was induced in response to gibberellin. These results indicated that, in deepwater rice, biosynthesis of gibberellin by *GA20ox2* is increased in a flooded environment, and initiation of internode elongation is promoted by the genes responsible for *qLEI3* and *qLEI12* in response to gibberellin (Figure 1C)<sup>3)</sup>.

## 2. Identification and functional analysis of the genes responsible for *qLEI3* and *qLEI12*, that control the initiation of internode elongation

We conducted positional cloning to identify the genes responsible for *qLEI3* and *qLEI12*. As a result, we identified *ACCELERATOR OF INTERNODE ELONGATION1* (*ACE1*), which encodes a protein of unknown function, as the gene responsible for *qLEI3*. We also identified *DECELERATOR OF INTERNODE ELONGATION1* (*DEC1*), which encodes a  $C_2H_2$  type zinc-finger transcription factor, as the gene responsible for *qLEI12*<sup>3)</sup>. Analysis of the mechanisms of *ACE1* and *DEC1* revealed that

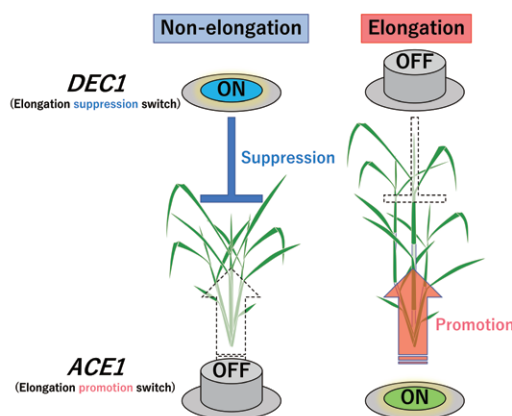
these factors are involved in the activation of the internode intercalary meristem, which is required for the initiation of internode elongation. Expression of *ACE1* is increased by gibberellin and activates cell division in the intercalary meristem, whereas expression of *DEC1* decreases



**Figure 1. Internode elongation of deepwater rice**

(A) Comparison between elongation in normal paddy rice and deepwater rice during flooding  
(B) QTL that controls deepwater rice internode elongation  
(C) Relationship between QTL and internode elongation of deepwater rice

in the presence of gibberellin, also resulting in increased cell division activity in the internode intercalary meristem (Figure 2). These were the first results to reveal the existence of molecular switches with antagonistic functions on cell division control in the internode intercalary meristem. On the other hand, it was shown that, in normal paddy rice, mutations have occurred in the coding sequence of *ACE1*, and functional *ACE1* protein is not synthesized. Furthermore, it was found that internode elongation does not occur during vegetative growth, even in the presence of gibberellin, because of a high level of *DEC1* expression (Figure 2). However, once normal rice enters the reproductive phase, the expression of an *ACE1* homolog, *ACE1-LIKE1*, is increased and the expression of *DEC1* decreases, resulting in the initiation of internode elongation (Figure 2).



**Figure 2. Antagonistic control of internode elongation by *ACE1* and *DEC1***  
The action of *ACE1* can be thought of as a promoting switch, and that of *DEC* as a suppressing switch for internode elongation.

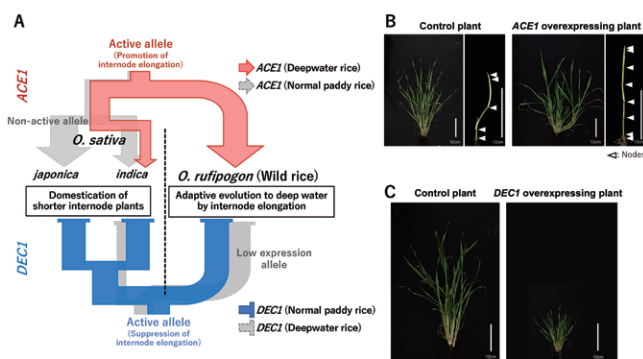
### 3. Selection of *ACE1* and *DEC1* during domestication and environmental adaptation of rice

We have verified the selection process of *ACE1* and *DEC1* during evolution and domestication by comparing the genetic information of 1203 varieties of domesticated rice, *O. sativa*, and 28 varieties of wild rice, *O. rufipogon*. The results showed that deepwater rice type *ACE1* (with an internode elongation promoting effect), normal paddy rice type *ace1* (without the internode elongation promoting effect due to mutations); as well as deepwater rice type *DEC1* (with enhanced internode elongation due to its reduced expression) and normal paddy rice *DEC1* (with reduced internode elongation due to its high expression) were all present in wild rice. It was also shown that normal paddy rice types *ace1* and *DEC1* were selected during the domestication process to make the plants shorter. In environments where flooding occurs during rainy seasons, however, deepwater rice types *ACE1* and *DEC1* have been selected (Figure 3A)<sup>3</sup>. These results indicated that *ACE1* and *DEC1* have played an important role in the adaptation strategy of rice to environments that experience flooding and that flood-resistant rice can be bred by introducing these genes into common rice by crossbreeding.

### 4. Functions of *ACE1* and *DEC1* in gramineous crops

The stems of gramineous plants are built by stacking nodes and internodes vertically. The stem elongates by cell division and subsequent cell elongation in the intercalary meristem located at the basal side of each internode. We verified the function of *ACE1* and *DEC1* in the internode elongation of gramineous plants with stem structures similar to rice<sup>3</sup>. When rice *ACE1* was overexpressed in purple false brome (*Brachypodium distachyon*), a species related to wheat, barley, and sugarcane, internode elongation was promoted as in rice (Figure 3B). In addition, when the expression of the purple false brome *ACE1* homolog was decreased in the plant by RNAi, internode elongation was suppressed. Internode elongation was also suppressed in barley by the overexpression of rice *DEC1* (Figure 3C). Based on these results, the mechanism that controls the initiation of internode elongation involving *ACE1* and *DEC1* discovered in rice is thought to be common among gramineous plants.

In this study, a special rice, deepwater rice, was the key to elucidating the control mechanism of stem elongation of common rice and other gramineous plants. By applying the knowledge discovered in this study, it is expected that high yield deepwater rice cultivars can be developed and the technology will be established to adjust the plant height of rice and other gramineous crops in response to various environmental changes.



**Figure 3. Selection process and ubiquitous functions of *ACE1* and *DEC1***

(A) The process of selecting *ACE1* and *DEC1* in domestication based on short plant stems and in adaptation to environments that experience flooding by internode elongation

(B) Phenotype of rice *ACE1* overexpressed in barley

(C) Phenotype of rice *DEC1* overexpressed in barley

The figure was modified from Nagai et al. (2020)<sup>3</sup>

### Acknowledgements

In this study, deepwater rice and wild rice were distributed from the National Institute of Genetics (NBRP-RICE). The research on barley was supported by the Institute of Plant Science and Resources, Okayama University (NBRP-BARLEY). In addition, we received many valuable pieces of advice from the late Dr. Keiko Morishima (National Institute of Genetics) on deepwater rice and wild rice. We would like to express our deepest gratitude.

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## Mutant screening on the Internet ~Utilization of the NBRP Mutant Library~

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### From PCR selection to *in silico* selection

In recent years, the development and dissemination of genome editing technology has raised expectations about the elucidation of gene functions. However, especially for crops that are mainly grown outdoors, there are several issues that remain to be addressed, such as the biosafety of the product of gene editing and the requirements of much efforts and times for gene manipulation. Under these circumstances, mutant resources that can be easily cultivated will still be one of the options for functional analysis. Our Institution of Kyushu University has been collecting mutant resources derived from the chemical mutagen MNU, and providing the mutant seeds. Since these mutants are derived from fertilized eggs, they have a higher mutation rate and a lower frequency of chimeras compared with mutants derived from seed treatment. In the case of reverse genetics, PCR screening (e.g. TILLING method) from several thousands of individuals is required to find the mutation at a gene of interest. This screening step takes one to two weeks and costs for reagents and consumables. Since the development of NGS, the analyzable amount of data has increased and its cost has been dropping rapidly. Accordingly, it has become practical to construct a mutant library consisting of multiple samples, in which all mutation sites are determined by whole genome sequencing.

### MiRiQ database: To make mutant screening simple and easy

Kyushu University has developed *in silico* TILLING system (hereafter referred to as the MiRiQ database) that enables web-based screening of mutants based on whole genome sequence information. The reference genome cultivar "Nipponbare" was used as the strain material, and M1 individuals that was obtained by MNU treatment of fertilized eggs has been sequenced. The sequence information, including SNP mutations, is compiled into the MiRiQ database where it is possible to find mutants in a gene of interest using the search functions and genome browser. JBrowse is used as the genome browser, and it is possible to visually capture a variety of information, including SNP location within the gene structure, its effects on gene/protein functions, and short-read alignment by using the gene ID (both RAP ID and MSU7 IDs are acceptable) as a search keyword. It is also possible to find the SNP mutations of the entire genome of a mutant individual by using the search function based on the individual number of the mutant. The MiRiQ database enables users to easily search for desired mutants without special knowledge or skill. Currently, trial run is being conducted on a small scale (N=253). The mutant pool and database will be expanded in

the future, including updates scheduled for the near future. The current goal is to construct a mutant library consisting of more than 1000 individuals. Please note that MiRiQ database will be available for registered users. The mutant seeds in M2 and M3 generations will be provided. If you would like to use the MiRiQ database, please use the QR code below for registration.



### You can access the database from the Internet in three steps

1. Access the MiRiQ database
2. Click on "Search" or "Genome browser" from the TOP page (Fig. 1)
3. Enter keywords and location information (Fig. 2)

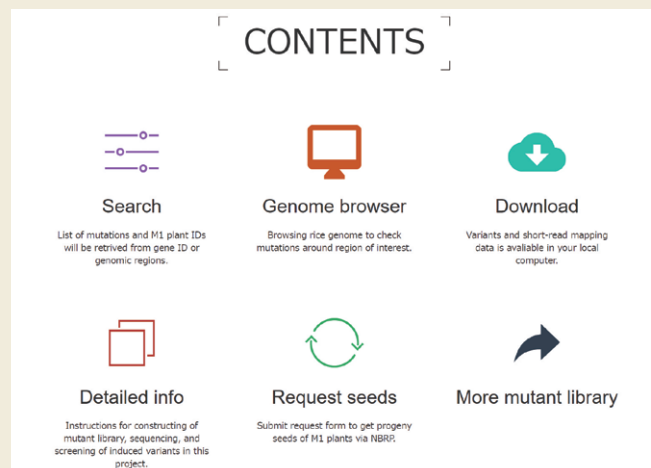


Fig. 1 The content screen of the mutant database MiRiQ

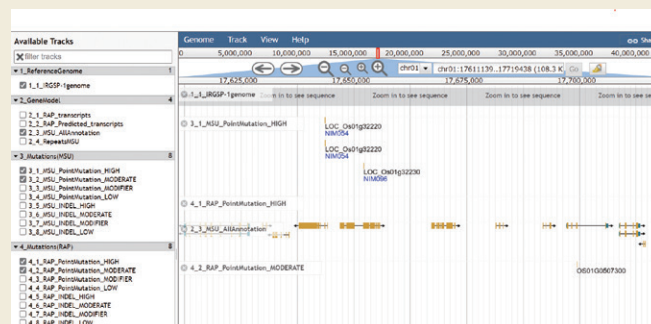


Fig.2 Genome browser screen opened from MiRiQ. The mutant line number with the SNP is displayed.



## Activity Reports 2021

### Special Exhibition, Plants, Mainstays of the Planet

From July 10 to September 20, 2021 “Special Exhibition, Plants, Mainstays of the Planet” was held at the National Museum of Nature and Science, and wild *Oryza* from NIG was exhibited as a specimen. The exhibition featured wild *Oryza* panicles with seed shattering and cultivated rice panicles without seed shattering, and many people were interested to find out what they were.



Special Exhibition, Plants, Mainstays of the Planet



Display comparing panicles of cultivated rice and wild *Oryza*

### A meeting for research on wild *Oryza* species

An NIG meeting entitled “Meeting for research on ecology, genetics, and evolution of closely related wild *Oryza* species in genus *Oryza*” will be held in March as a web meeting (Language: Japanese). You are welcome to join us.

### Activities of NBRP-Rice were introduced in the academic journal “Breeding Science”

We introduced the activities of NBRP-Rice in the academic journal of Japanese Society of Breeding. A beautiful picture of wild *Oryza* panicles was selected as the cover picture of the journal.

### Public Relations at Academic Societies

85th Annual Meeting of the Botanical Society of Japan (as web meeting in September 2021)

45th Annual Meeting of the Molecular Biology Society of Japan (at Makuhari Messe in November 2021)

63rd Annual Meeting of The Japanese Society of Plant Physiologists (as web meeting in March 2022 (scheduled))

### NBRP Rice Advisory Committee

The annual committee was held as web meeting on October 26, 2021.

## Announcements

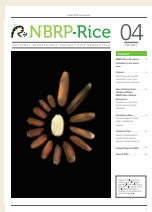
Several events such as symposium and Open Fields (Study tour of wild *Oryza* genetic resources) introducing NBRP-Rice resources are under consideration to be held in actual and/or web meeting. We will make an announcement in details as soon as the plans are set.

#### NBRP Rice Public Relations

Events introducing genetic resources of NBRP-Rice will be held at several academic societies. You are welcome to join us.

#### Past issues

Past issues and Japanese versions of this newsletter are available on Oryzabase (<https://shigen.nig.ac.jp/rice/oryzabase/> or use the QR code).



#### Vol. 4

- Column: My journey with genetic resources of rice—from chromosome to genome
- New Findings from Studies utilizing NBRP-Rice Genetic Resources: A reference C genome for the wild rice *Oryza officinalis*
- Oryzabase Now: The landscape of WGS data in wild *Oryza* species
- Technical Tips: Genetic transformation of wild *Oryza* species using immature embryos

Visit Oryzabase using this QR code.



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March 2022

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