



NBRP-Rice

NATIONAL BIORESOURCE PROJECT RICE NEWSLETTER

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Panicles of *Oryza meridionalis* with long awns. *O. meridionalis* is an annual species distributed in northern Australia. Among the *Oryza* species with AA genome, including cultivated rice, *O. meridionalis* is considered to be genetically distant from all other species.

Starting the 5th phase of National BioResource Project Rice

SATO, Yutaka

Project Leader of National BioResource Project-Rice
Plant Genetics Laboratory, National Institute of Genetics (NIG)

The National BioResource Project (NBRP) is a project supported by the Ministry of Education, Culture, Sports, Science and Technology (MEXT) to collect, preserve, and distribute experimental animals and plants that are important to maintain strategically. The NBRP Rice, one of the NBRP projects, operates the collection and preservation of wild *Oryza* and various experimental lines, and distribution of these genetic resources to rice researchers in Japan and overseas. The NBRP Rice started from 2002, and it has been evaluated in a five-year period. After the evaluation, we applied to the next phase, and if it is adopted, the next phase started. Repeating this process, NBRP Rice has been conducted since 2002 to present. The 4th phase of NBRP Rice was completed last year, and the 5th phase has just started this year after applying and judgment. Comments on the 4th phase from the evaluators were generally positive. In particular, because many findings from the studies using the resources distributed by the NBRP Rice were published in high impact scientific journals, it was recognized that our project were distributing important genetic resources that supported basic science research in Japan. This is due to the tremendous efforts of the researchers involved in the collection and development of the resources and the users. We would like to take this opportunity to thank them. As a project leader of the NBRP Rice, I am also pleased to find that users are making effective use of the resources.

On the other hand, a small number of users of the NBRP Rice resources continues to be a major issue, as the mission of the NBRP is not to maintain the resources, but to collect, preserve, and distribute resources that are truly needed by a large number of researchers. Therefore, the number of users is an important point in evaluation and continuing the project. In the 5th phase of NBRP Rice, we will engage in activities to encourage more and more researchers to use NBRP Rice resources. The title of the 5th phase of NBRP Rice is "Promote utilization of rice genetic resources through integrating resource traits and genomic information". Various traits and genomic information of the NBRP Rice resources, mainly wild *Oryza* resources at the NIG, will be provided from the NBRP Rice information website, Oryzabase (<https://shigen.nig.ac.jp/rice/oryzabase/>). We believe the information will be useful in deepening our understanding of the historic NBRP wild *Oryza* genetic resources. We hope many of you will use this site actively and expect research on rice, including wild *Oryza*, will become even more active.

This issue's column is contributed by Dr. Hiro-Yuki Hirano, Professor Emeritus at the University of Tokyo, who is working actively as a science writer. In the column, Dr. Hirano described the relationship between wild *Oryza* and his own research from 1988 to 1995, when he was working at the NIG, along with the photos of the NIG at that time. It is surprising to see large areas of fields and lawns, where buildings are standing now, and how much has changed since then. As a side note, my laboratory is located in the building where Dr. Hirano was working at that time. Although the building itself is the same as it was then, it is currently undergoing a large-scale renovation, and my laboratory will be renewed soon.

As introduced in Dr. Hirano's recent book, "Story of the History of Genetics" (Chuko Shinsho), it was Emerson and Beadle who systematically organized the strains of maize mutations and related information held by individual researchers, and made them available as "genetic resources" to those who wished to use them in the 1930s, and this was the origin of the project for biological genetic resources. The development of the maize research community since then is well known. The wild *Oryza* genetic resources have been preserved at NIG since the late 1950s, and the older ones have been maintained for over 60 years. During this time, genetics and its research methods have evolved greatly. The research environment has also changed dramatically (even comparing with the time when Dr. Hirano belonged to the NIG). Nevertheless, we have kept maintaining the same wild *Oryza* accessions since then, which remind us the importance and responsibility of the genetic resource project.



Transplanting seedlings of wild *Oryza* at the paddy field of Kashimadai Field in Tohoku University (author in the left)



Please use this QR code to visit Oryzabase.

Column

The National Institute of Genetics and Wild *Oryza* 35 Years Ago

HIRANO, Hiro-Yuki

Professor Emeritus at the University of Tokyo, Science Writer

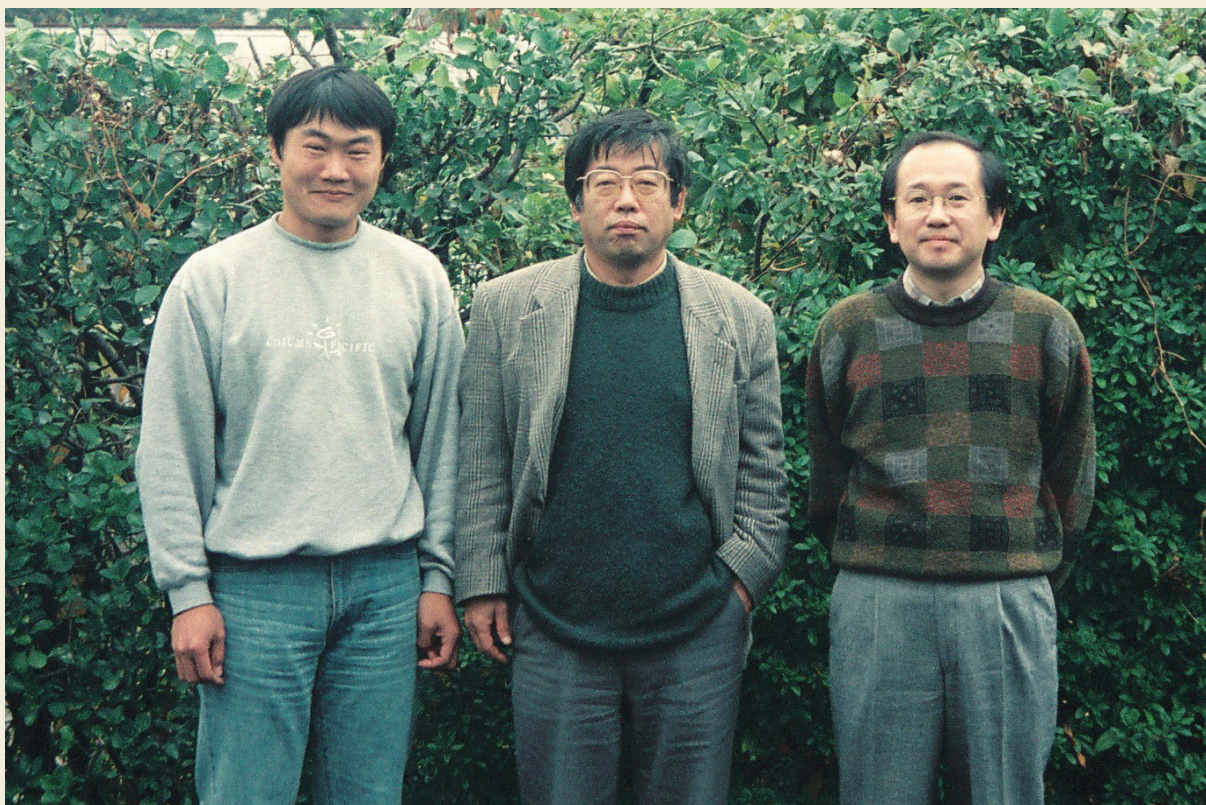
I worked at the National Institute of Genetics (NIG) for about seven and a half years from 1988, and spent most of my thirties at NIG. In this column, I would like to write about my memories of the plant research laboratories at that time and my research using wild *Oryza* at NIG.

Plant researchers

I was employed at the Plant Conservation Laboratory of the Genetic Stock Research Center. The Plant Conservation Laboratory was established in 1974 to conserve plant genetic resources and utilize them for research, and the first laboratory head was Dr. Taro Fujii. Unfortunately, Dr. Fujii passed away during his tenure, and the head was succeeded by Dr. Yoshio Sano, who later became a professor at Hokkaido University. When I started working at the lab, Dr. Sano showed me around the greenhouse of wild *Oryza* and other facilities, and he told me, "Our laboratory is responsible for collecting and preserving genetic resources of plants including wild *Oryza* collected from around the world, and distributing them to the researchers who wish to use them. However, I will take care of this task, so you can do any research

you like". I remember saying "yes" without realizing the generosity of his words. So, I was not a very good assistant. As I will describe later, my research was also based on the results of Dr. Sano's research on genetics. I was allowed to do research on anything I wanted, but when I looked back, I felt I was playing in the palm of Dr. Sano's hand like the Monkey King who was acting up in the palm of the Buddha's hand.

At that time, there were only five plant researchers at NIG, including Professor Hiroko Morishima and Assistant Professor Yoichiro Sato in the Division of Agricultural Genetics, and Assistant Professor Ikuo Nakamura in the Experimental Farm. All of us were working on rice. I still remember well how Dr. Morishima sometimes visited Dr. Sano's room for discussions. Dr. Hiko-Ichi Oka, a pioneer in wild *Oryza* research, came to NIG several days a week as an emeritus member to edit the Rice Genetics Newsletter. A few months before my arrival to NIG, Mr. Mitsugu Eiguchi had arrived as a technical staff member in NIG. He managed the fields and grew rice plants, and also supported Dr. Sano's research in his spare time. Mr. Eiguchi was the first technical staff member at NIG with



Dr. Yoshio Sano (center), Mr. Mitsugu Eiguchi (left), and the author (right)

bachelor's degree, and he helped me with his special skill in cell culture. Later, Dr. Sano and I moved to the Division of Agricultural Genetics in the organization, but we spent most of our time working in the conservation building. At that time, there were only few buildings in NIG. In front of the two-story conservation building, there was a lawn where the football club of NIG practiced during lunch break.



With Dr. Oliver E. Nelson, a leader of maize genetics. From left, Dr. Yoshio Sano, Dr. Nelson, Dr. Ko Shimamoto (then at Plantech Research Institute, Mitsubishi Chemical Corporation), and the author. Dr. Nelson had a lot of achievements in the study of *Waxy* gene in maize.

Research on the *Waxy* gene in rice

Dr. Sano was conducting genetic research on various topics, such as evolution of genus *Oryza*, sterility, elongation capacity of floating rice plants, and regulation of amylose content in rice grains, using wild *Oryza* and mutant plants, and he asked me to study one of the topics at the molecular level. After receiving a series of lectures on his research topics, I decided to work on the *Waxy* gene, which was involved in the regulation of amylose content in rice grains and the only gene that could be cloned at the time. Dr. Sano's genetic research had shown that amylose content in grains differed between japonica and indica rice, and it determined the eating quality of both types of rice (moderate and less stickiness). Thus, I started my research to elucidate the molecular mechanism determining amylose content in rice grains. However, at that point, neither genomic information nor molecular tools for rice was available, so I started by constructing a genomic library. Moreover, since I planned to compare the *Waxy* genes of japonica and indica rice, I needed to create libraries for both genomes.

Although I succeeded in cloning the *Waxy* genes in a relatively short period of time and wrote several papers about the genetic cloning, it took many years to elucidate the different function of the *Waxy* genes in japonica and indica rice. The expression level of the *Waxy* gene detected by northern hybridization in indica was about

10 times higher than that in japonica, and I thought that this was due to differences in transcriptional regulation, however this idea was wrong. After some twists and turns, I found the splicing efficiency of the first intron upstream the coding region was low in japonica, and this induced the low expression of the *Waxy* gene. I hypothesized a single base mutation in the splicing site of *Waxy* gene in japonica greatly reduced the expression level of the gene. To test this hypothesis, I checked the nucleotide at the splicing site and the expression level of *Waxy* gene in several accessions of wild *Oryza*, including *Oryza rufipogon*. As a result, indica and all of the tested wild *Oryza* had the same nucleotide at the splicing site, and the expression level of *Waxy* gene in the wild *Oryza* were comparable with that in indica. These results supported the hypothesis that mutation occurred in japonica. In other words, a mutation in the splicing site of the first intron of the *Waxy* gene occurred during the microevolutionary process of japonica cultivation, which caused the lower amylose content (moderate stickiness) of japonica rice (Hirano et al. Mol. Biol. Evol. 1998).

I came up with the idea using wild *Oryza* for the final confirmation of my hypothesis since I was contacting wild *Oryza* everyday under Dr. Sano's supervision at that time. Now, this mutation in the *Waxy* gene is still widely used as a SNP that characterizes japonica.

Study of retrotransposon transposition

After some time cloning the *Waxy* gene, a research group led by Dr. Eiichi Ohtsubo and Dr. Hisako Ohtsubo at the Institute of Molecular and Cellular Biosciences, University of Tokyo, discovered the first small retrotransposon, SINE (Short Interspersed Element), in plants by



The Building of Genetic Research and Conservation of Experimental Organisms in the 1990s. There was a large lawn area near the entrance.



Participants in the Japan-China Joint Research Meeting "Verifying the Origin and Evolution of Rice" (organized by Dr. Morishima in 1995). Dr. Hiko-Ichi Oka (standing in the center of the second row), Dr. Hiroko Morishima (first row, far right), and Dr. Yoshio Sano (second row, far right).

examining the *Waxy* gene. Before I was assigned to NIG, I studied on repetitive DNA sequences, which include retrotransposons. A large number of copies of repetitive sequences in eukaryotic genomes was thought to have accumulated during evolution. At the time, copies of repetitive sequences were identified by the density and patterns of bands in southern hybridization. I came up with idea that it might be possible to determine when the retrotransposon at a particular locus transposed by using wild *Oryza* and PCR.

To put my ideas into action, I examined the presence or absence of two SINEs at the *Waxy* locus using a large number of wild *Oryza* accessions. As a result, I found the SINE inserted in the first intron was present in all the wild *Oryza* and cultivated rice accessions, while the SINE inserted in the tenth intron was present only in *O. rufipogon* and cultivated rice accessions. These results suggested that the insertion of the former SINE occurred in the past and preceded the speciation of genus *Oryza*, while the latter SINE was inserted to the *Waxy* gene during the divergence of *O. rufipogon* from other wild *Oryza* species. I demonstrated that retrotransposons were transposed to a specific locus at a specific time in evolution for the first time through plants and animals (Hirano et al. J. Mol. Evol, 1994). This research was accomplished only because of the use of the wild *Oryza* genetic resources consist of closely related species.

Although the motivation and the procedure of this research may look reasonable at first glance, this research actually started from an accident. In those days, there were frequent power outages in the conservation building, and the freezer was left at room temperature for several days during the power outage when I was away on a business trip. Various enzymes, such as restriction

enzymes and ligases, were damaged. When I checked the activity of those damaged enzymes, the only enzyme that remained active was the *Taq* polymerase derived from thermophilic bacteria. I was quite disheartened because I could not immediately prepare all the enzymes again due to the lack of research funds, and I wondered if I could do some kind of research using this admirable *Taq* polymerase. In fact, this was the starting point of the research on detecting the timing of the SINE transposition. Facing the accidental loss of most of the enzymes, I was able to conduct research with the help of wild *Oryza*.

Up to graduate school, I majored in biochemistry. I started my career in genetics at NIG, and became more aware of the importance of genetic resources, such as wild *Oryza*, in my subsequent research activities. The Plant Conservation Laboratory, which I belonged to, has been reorganized several times since then, and continues to exist as the Plant Genetics Laboratory at present. Since Professor Yutaka Sato was assigned to the lab, the activities for the conservation and utilization of rice genetic resources seem to have become more and more active. However, at NIG, where researchers are required to conduct a world-leading research, it takes an extra effort to carry out one's own original research and manage genetic resources at the same time. I believe that rice researchers publishing their excellent research to the world using these genetic resources in an effective way will be the greatest support and encouragement to the researchers and technical staff who are engaged in the management of these genetic resources.

Mutations at three genetic loci promoted rice domestication - reducing seed shattering leading to an increase in yield

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Introduction

The rice (*Oryza sativa*), which we eat every day, is originated from weed of wild rice (*Oryza rufipogon* Griff.). It is thought that our ancestors, who were hunters and gatherers, selected wild rice that had characteristics suitable for cultivation, which led to the domestication of rice. Wild rice has a character of shattering behaviour to scatter their seeds as their reproductive strategy. However, to increase grain yield, ancient people desired to suppress seed shattering. Since the discovery of the *sh4* gene, which acts on the reduction of seed shattering, in 2006, people have recognised that mutation in this gene led the wild rice to domestication process. However, our previous study showed that *sh4* mutation alone was insufficient to reduce the seed-shattering degree, although it was involved in reducing seed shattering, suggesting that mutation in other loci might still be required (Ishikawa et al. 2020). In this study, we focused on the initial steps of rice domestication to clarify how seed shattering was suppressed to increase the yield. The research was collaborated with researchers in the fields of plant genetics, phytoarchaeology, and structural mechanics.

1. Mutation at the *sh4* locus alone did not alter seed shattering behavior in wild rice

Seed shattering is caused by a tissue called an abscission layer that is formed in the basal part of the grain. To examine the effect of the previously identified seed-shattering locus, *sh4*, in rice domestication, we crossed cultivated rice, *O. sativa* Nipponbare (Npb) with wild rice *O. rufipogon* (W630), and resulting F1 was backcrossed with W630 to develop an introgression line (IL) containing small chromosomal segment from *O. sativa* Npb covering the *sh4* locus in the genetic background of W630. This IL (*sh4*-Npb) showed complete abscission layer formation as observed in W630 (Ishii et al. 2013). This result

suggested that mutation at the *sh4* locus alone could not suppress seed shattering, and mutations at other loci might be involved in. Through further genetic analysis, we identified *qSH3* locus related to suppressing seed shattering. *qSH3* encodes *OsSh1*, a rice homolog of the *Sh1* gene involved in the control of seed shattering in sorghum, and a single nucleotide substitution at the gene was found to be responsible for reduced seed-shattering behaviour. Since the mutation at the *qSH3* locus was conserved in most of the cultivated rice, it was suggested that the mutation might have played an important role in the process of rice domestication. However, similar to *sh4*, *qSH3* locus alone could not suppress seed-shattering behaviour in wild rice. When combining mutations at both *sh4* and *qSH3* loci, seed shattering of wild rice was suppressed by partial inhibition of abscission layer formation (Figure 1).

2. Closed panicle formation assisted the inhibitory effect of *sh4* and *qSH3* loci on abscission layer formation

Mutations at the *sh4* and *qSH3* loci inhibited the formation of the abscission layer around the vascular bundles. However, with such a weak abscission layer inhibition, grains still might fall off easily, as in wild rice, under a natural environment, and it could be difficult to increase yields. Focusing on the panicle morphology of wild rice, we noticed wild rice has an open panicle structure and awn on the top of the grain, which promotes seed dispersal (Ishii et al. 2013). Since the closed panicle formation was a trait selected during rice domestication, we planned to compare the effect of mutations at the *sh4* and *qSH3* loci on abscission layer inhibition in open or closed panicles. Seven introgression lines with a genetic background of wild rice and different combinations of the mutations at the *sh4* and *qSH3* loci, and the *SPR3* locus, which is responsible for closed panicle structure, were generated by crossing. Using these seven ILs and wild rice, their seed gathering rates were examined in the paddy field. The results showed that the effect of mutation at each one of the three loci on the seed gathering rates was small. Although the seed gathering rates slightly increased by loss of open panicle structure, the rates were not significantly different from the wild rice in ILs with a single mutation at one locus or even in ILs with mutations at two loci. However, when mutations at three loci were combined, the seed gathering rate increased dramatically (Figure 2, Ishikawa et al. 2022). These results suggested that panicles, with the three

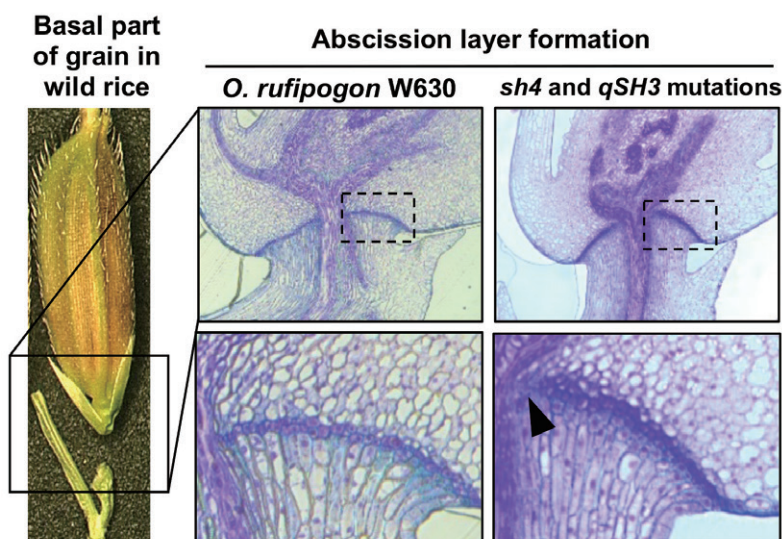


Figure 1. Combining *qSH3* and *sh4* mutations partially inhibited the abscission layer

Wild rice with the double mutation exhibited slight abscission layer inhibition around the vascular bundles (as indicated by the arrowhead) and it was determined that the seed was attached to the rachis. However even with this partial abscission layer junction, the seeds still tend to fall easily in a natural environment.

mutations, retaining more seeds might have attracted our ancestors and made them to select those plants.

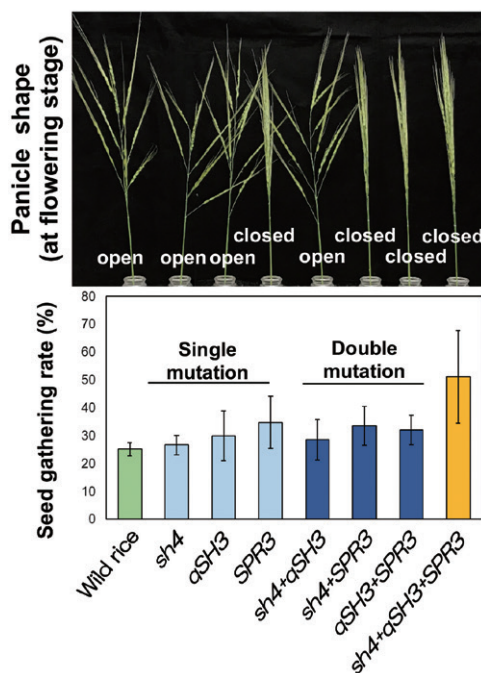


Figure 2. Seed gathering rate results for rice plants with a combination of three genetic mutations (in *sh4*, *qSH3* and *SPR3*)

The panicle shapes of 8 plants (above) and the seed gathering rate for each plant when grown on cultivated land (below).

3. Loss of open panicle structure and abscission layer inhibition were mutually complementary in structural mechanics

Although the change in “reduced seed-shattering behaviour” caused by mutations at the *sh4* and *qSH3* loci and the change in “closing panicle” caused by mutation at the *SPR3* locus were completely different traits, structural mechanics analysis revealed that their relationship was complementary to each other. When the wild rice with an open panicle and an awn at the top of the grain were exposed to wind and rain in the natural environment, seed shattering was facilitated because the abscission layer was under high-load of gravity. On the other hand, in the closed panicle, the load of gravity on the abscission layer at the basal part of the grain were reduced compared to that of open panicle, resulting in a lower bending moment. In the inhibition of abscission layer formation, the inhibition area around the vascular bundle expanded outward, and the ratio of inhibition area to total area increased in cultivated rice. This could be likened to the relationship of increasing strength with increasing wall thickness of the pipe wall, resulting in an increase in the section modulus. The force (bending stress) on the basal part of the grain decreased dramatically as the bending moment decreased and the

section modulus increased (Ishikawa et al. 2022). In the initial step of rice domestication, the changes in both traits complemented each other, and the seeds were more likely to be retained on the panicle, and such plants might have been selected for cultivation (Figure 3).

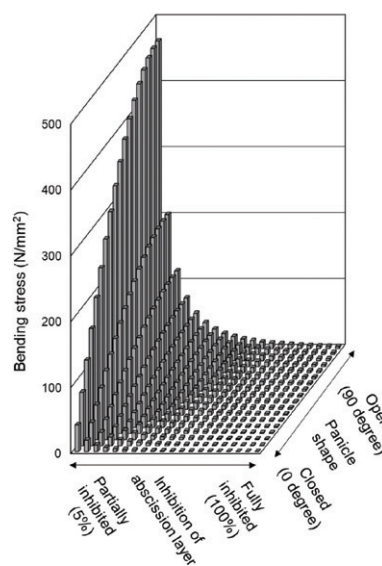


Figure 3. Structural mechanics analysis of panicle shape and abscission layer inhibition related to the initial loss of seed shattering.

Simulation of the bending stress exerted on the spikelet base depending on panicle shape and abscission layer inhibition. A higher stress was experienced in the open panicle with less inhibited abscission.

Conclusions

In this study, we showed that suppression of seed shattering, which was necessary for rice to get on the track of domestication, was not achieved through a process that can be explained by a simple genetic mutation, but achieved through a complex process involving multiple genetic mutations. The reduction in seed shattering acquired through the accumulation of mutations at three loci with small effects in rice cultivation may have acted as an important trigger for rice to take its course as a crop.

Acknowledgements

In this study, we used wild *Oryza* accessions that were provided from the National Institute of Genetics (NIG). We thank Dr. Cristina Castillo and Prof. Dorian Fuller of University College London, Prof. Robin Allaby of Warwick University, Associate Prof. Ken-Ichi Nonomura of NIG, Prof. Takashige Ishii and Prof. Kazuya Inoue of Graduate School of Agricultural Science, Kobe University, and students who worked with us in the Laboratory of Plant Breeding for their kind support. I would also like to express my deepest gratitude to Mr. Mitsugu Eiguchi, a former technical staff of NIG, who taught me the breeding techniques of rice and how to handle wild *Oryza* plants while I was working at NIG as a postdoctoral researcher.

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Activity Reports 2022

Workshop “The attraction of genus *Oryza*”

On September 23rd, 2022, a workshop titled “The attraction of genus *Oryza*” was held at Obihiro University of Agriculture and Veterinary Medicine during the 142nd Meeting of the Japanese Society of Breeding. Five speakers presented their studies based on ecological surveys of wild *Oryza* and utilizing wild *Oryza* accessions of NBRP Rice. Many people, including students and junior researchers, joined the workshop, and it ended on a high note.

Open Fields (Study tour of wild *Oryza* genetic resources)

This year, ‘Open Fields’, study tours of wild *Oryza* genetic resources, were held for visitors individually. Through observing the living plants of wild *Oryza*, the visitors realized the diversity of morphology among wild *Oryza*.

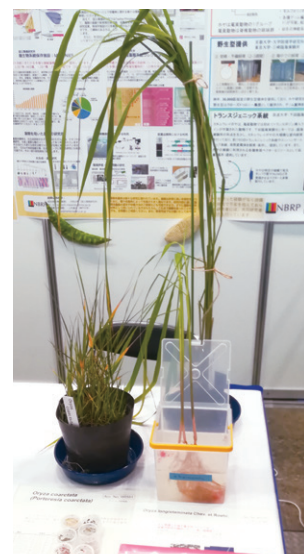


Visitors observing wild *Oryza*

Public Relations at Academic Societies

We restarted the NBRP Rice exhibition at on-site conference this year. In the exhibition, we will display real plants and seeds of wild *Oryza*. If you are interested, please join us.

- The 86th Annual Meeting of the Botanical Society of Japan (at Kyoto Prefectural University in September 2022)
- The 142nd Meeting of the Japanese Society of Breeding (at Obihiro University of Agriculture and Veterinary Medicine in September 2022)
- The 19th ISRFG (at Phuket, Thailand in November 2022)
- The 45th Annual Meeting of the Molecular Biology Society of Japan (at Makuhari Messe in November 2022)
- The 64th Annual Meeting of the Japanese Society of Plant Physiologists (at Tohoku University in March 2023 (scheduled))



NBRP-Rice Exhibition (The 45th Annual Meeting of MBSJ)

NBRP Rice Advisory Committee

The annual committee was held as web meeting on 15th December 2022.

Announcements

Open Fields (Study tour of wild *Oryza* genetic resources)

In 2023, ‘Open Fields’, study tours of wild *Oryza* genetic resources will be held for visitors individually. If you are interested in any particular NBRP accessions and would like to see or examine them in our field, please contact us by email (nig_openfield@nig.ac.jp). We would like to fulfill your requests as much as possible.

NBRP Rice Public Relations

Exhibitions will be held at several academic societies. You are welcome to join us.

Past issues

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



Vol. 5

- Column: Preservation of local varieties that supported traditional rice farming in Indo-China countries
- New Findings from Studies utilizing NBRP-Rice Genetic Resources: Initiation mechanism of internode elongation elucidated by research on deepwater rice that overcomes flooding
- Technical Tips: Mutant screening on the Internet ~Utilization of the NBRP Mutant Library~

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NBRP



March 2023

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