NBRP-Rice 03

NATIONAL BIORESOURCE PROJECT RICE NEWSLETTER

Feb 2020

Contents

Various wild *Oryza* species grown in a paddy field in which short-day treatment can be performed. Many of them exhibit shattering, so their panicles need to be bagged for harvesting their seeds.

Reports on the third year of the NBRP-Rice 4th term

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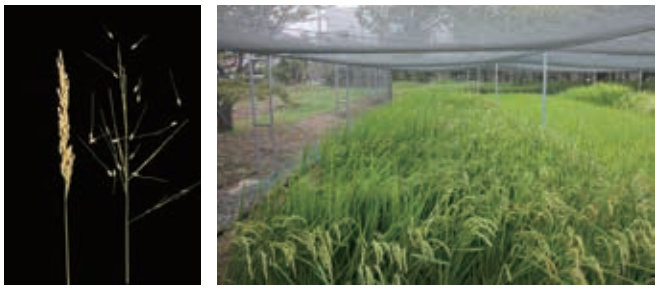
The 4th term of the National Bioresource Project (NBRP), launched in fiscal year 2017, is about to finish its third year. This is the third issue of our newsletter. This year was the year of midterm evaluation of the 5-year 4th-term projects. As you may know, the evaluation result of NBRP-Rice was not necessarily a satisfactory one. For stable continuation of the NBRP-Rice services, we need to raise the project's evaluation. The National Institute of Genetics (NIG) (Sato Lab and Nonomura Lab), the core facility, and Kyushu University (Yasui Lab and Kumamaru Lab), the sub-center of the project, will work together in NBRP-Rice activities to facilitate increased utilization of NBRP-Rice resources for basic research in the rice research community.

Here, I would like to share my knowledge with you about how NBRP evaluations are performed. In the 4th term of NBRP, 30 bio-resource development programs are in progress and core information centers are being established. Evaluation of these program activities focuses on how much the NBRP resources have been utilized by research communities using individual resources and how much they have contributed to the research publications. The evaluation seems to be made based on data such as, on an annual basis, the number of researchers using NBRP resources, the number of strains distributed and the number of publications based on NBRP resources, as well as on the operators' activities in promoting resource utilization. In the case of NBRP-Rice, NBRP-Rice resources have been used by only a small number of researchers, which seems to be the bottleneck. Meanwhile, the high quality of research publications based on NBRP-Rice resources seem to have appealed to the evaluators. I would like to appreciate our resource users in this regard and also feel proud and encouraged as a fellow rice researcher.

Regarding operators' activities, we introduce NBRP-Rice resources by issuing this newsletter and holding open field visits. We also continue collection of novel attractive resources, such as various chromosome segment substitution lines (CSSL) and mutant lines, so that more researchers will be interested in using our resources. Collection of novel resources is basically achieved through deposits from the research community. If you have any rice resources such as CSSL series or mutant lines that can be shared within the community after publishing your papers, please deposit them with NBRP-Rice. We will undertake distribution and long-term storage of your resources on your behalf.

We are also working on information disclosure of the existing NBRP resources, such as their trait or genome information, to increase the utilization of the resources themselves. The genome and trait information of individual strains are available at Oryzabase, so please visit https://shigen.nig.ac.jp/rice/oryzabase/. We are gradually enriching the database for genome information of wild rice resources. For example, we will release Illumina read data (about 15x coverage) for about 600 accessions of *Oryza rufipogon*, a species regarded as the direct progenitor of cultivated rice, that are maintained at NIG. The information should be applicable to various research areas, so please look out for it. At Oryzabase, we are also planning to release detailed information applicable to molecular genetics using wild rice, including transformation methods for immature embryos. According to our survey, these methods are applicable to many of the closely related wild species as well as some of the distantly related wild species, so please look forward to this also.

Late last year, NIG hosted a research meeting titled "Research Promotion for Genetic Diversity Using Genome Information on genus *Oryza*". Many young researchers participated in the seminar and there were interesting presentations and active discussions. Among the NBRP-Rice resources, most of the wild rice resources maintained by NIG were collected by Professor Oka and Professor Morishima from the 1950s to the 1980s. Although several decades have passed since their collection, they are still regarded as attractive research materials by many researchers. NBRP-Rice would like to support further development of the rice research community and active research activities utilizing these materials.



Left photo: left, the panicle of a cultivated rice strain; right, the panicle of wild rice strain exhibiting heavy shattering
Right photo: wild rice derived chromosome segment substitution lines (CSSLs) in the field

Column

The ritual of Overseas Scientific Investigation

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Although my research materials were Indica and Japonica rice, I had been far from Overseas Scientific Investigation until 30 years ago, in December 1989, when I finally got the chance to participate in a trip to survey wild rice in Thailand and Bangladesh as a member of the Overseas Scientific Investigation group led by Professor Morishima of NIG. After that, I accompanied Professor Morishima several times to South Asia for wild rice field surveys. Although I was a complete beginner in field surveys of wild rice and Overseas Scientific Investigation, Professor Morishima kindly taught me the "rituals of Overseas Scientific Investigation", which I would like to share with you.

I remember her gently saying, "the most important thing in Overseas Scientific Investigation is to do enough preparation before you leave. You cannot get good results unless you are well prepared". Preparation means to gather literature and information from researchers of the target country and build a plan for where, what and how to conduct the survey. Research institutions in the target country, survey dates, survey sites, accompanying researchers, transportation and accommodation must also be arranged before departure. Thanks to the internet, we can now easily collect information or hold meetings with target country's research institutions, but not in those days; I hear that they sometimes spent several years for preparation. Letters or phone calls were the only ways to collect information and hold meetings with researchers in the destination country. It took at least a week for an air mail to reach the destination country, and not a few letters did not reach the destination at all. International phone calls could only be made through operators, and we often had to wait for an hour before our calls could get through. When the Professor asked me to make a hotel reservation, I booked a hotel by phone but was never sure whether my booking was accepted, which kept me worried until we actually reached the hotel.

Professor Morishima often fell asleep while we were driving in a car, but she sometimes woke up and shouted, "stop the car!" when she spotted wild rice colony in roadside ditches. I would recognize the wild rice only after the car had stopped. "When you see any wild rice, you should stop the car", she said to me, so thereafter I

At the Bangladesh Rice Seed and Genetic Resources Laboratory; extreme left, the author; second from the left, Professor Morishima

did so when I spotted wild rice, which mostly turned out to be cultivated rice or even weeds. It took me several surveys before I could identify wild rice from a moving car. To survey as wide an area as possible with limited research funds and short survey periods, we needed to find survey sites as we traveled. Identifying wild rice from a moving car was one of the "rituals" I had to learn.

Professor Morishima also told me that "in Overseas Scieitific Investigation, it is important that we gain understanding and cooperation of local people and share our results with them". The day after our arrival in Bangkok, Thailand, the Professor and the members of the research group visited a rice research institute she had contacted in advance. There, she explained the purpose and details of our survey and asked for their understanding and cooperation. Also, in Bangladesh, we visited the Rice Seed and Genetic Resources Laboratory to promote their understanding of our survey and to gain their approval for rice researchers to accompany our party, which allowed us to survey not only in the areas around Dacca but also in the northern and southern regions of the country. We shared the rice seeds and plants collected in our survey with local research institutions, which was also our "ritual". The field notebook we took on the survey trip contained the items to be surveyed for wild and cultivated rice, and several members worked together to record all the information obtained during our short stay at the survey site. Because GPS data were not easily available then, the most difficult thing was to record the location of the individual survey sites. It was the new member's duty to constantly take notes of the distance travelled indicated on the mileage meter of the car, and to record the distance and direction from bridges or large buildings. It was also our "ritual" to write everything in this notebook in English to ensure that information obtained from the survey could be shared with the accompanying local rice researchers and local research institutes.

Concerning surveys on genetic resources including wild rice, there are some international treaties such as the Convention on Biological Diversity and the Nagoya Protocol, enacted in 1993 and 2017, respectively. The purpose of these treaties is "the conservation of biological diversity, the sustainable use of biological diversity and the fair and equitable sharing of benefits arising from the utilization of genetic resources". By the conclusion of the Nagoya Protocol, procedures to be undertaken in provider and recipient countries of genetic resources were made explicit. It is recommended that the relevant research institutions sign a Memorandum of Understanding and, when transferring the collected materials, a Material Transport Agreement prior to the survey. They must also obtain international approval through an Internationally Recognized Certificate of Compliance. Although the Overseas Scientific Investigation of wild rice led by Professor Morishima was conducted before 1993, the year in which the Convention on Biological Diversity was enacted, her "rituals of Overseas Scientific Investigation" were in line with its purpose. I believe that her "rituals" also include facilitation of "fair and equitable sharing of benefits" with the provider countries of wild rice genetic resources we have collected and stored.

Children gathered around Professor Morishima taking survey photos

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

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Introduction

When a superhydrophobic leaf such as a rice leaf is submerged, an air layer called a gas film is formed around the leaf (Figure 1A). Upon submergence, the gas film confers flood tolerance to the plant by enhancing CO_2/O_2 gas exchange and enabling underwater photosynthesis/respiration (Figure 1B). Previous studies on leaf gas films have only focused on the physiological aspects of plants from which gas films were removed by surfactants, and not on the elucidation of genetic factors controlling it[1]. Rice leaves generally exhibit a high hydrophobicity, but the *dripping wet leaf7* (*drp7*) mutant (derived from Kinmaze) has a phenotype characterized by loss of leaf hydrophobicity[2]. Based on the hypothesis that the *drp7* mutant has defects in formation or retention of the gas film, we conducted physiological and molecular genetic analyses to reveal the relationship between the gas film and leaf hydrophobicity and to isolate the candidate gene.

Physiological analysis of Kinmaze (wild-type) and the *drp7* mutant

Although leaves from both rice plants formed gas films immediately after submergence, those from the *drp7* mutant lost their gas film, while the wild-type Kinmaze leaves retained it 1 day after submergence (Figure 1C). Assuming that the loss of gas film observed in the *drp7* mutant 1 day after submergence was caused by the defect of surface structure of the leaf blade, we performed scanning electron microscopy of the leaf surface. The leaf of Kinmaze had many wax crystals deposited on the surface and around the papillae, while the *drp* mutant leaf had significantly less deposition of wax crystals (Figure 1D), suggesting that wax crystals play an important role in gas film retention underwater. Continuously, underwater photosynthesis by the leaf blade was quantified using an oxygen electrode. Along with the post-submergence loss of gas film, a significant reduction in underwater photosynthesis from the pre-submergence level was observed in the *drp7* mutant (Figure 1E). When the *drp7* mutant was tested for survival in a paddy field, it died 3 weeks after transplanting (Figure 1F). Based on these results, it was concluded that long-term retention of gas film enhances underwater photosynthesis in rice plants, which contributes to flood tolerance.

Identification of genes controlling gas film retention

We conducted positional cloning using a 5,300 F_2 population derived from crosses between the *drp7* mutant (*Japonica*) and Kasalath (*Indica*). A single-base substitution was detected between the genomes of the *drp7* mutant and Kinmaze in the *OsHSD1* gene, which belongs to the Short chain Dehydrogenase/Reductase (SDR) family (Figure 2A). Since the *drp7* mutant was characterized by the loss of its gas film, we named the candidate gene *Leaf Gas Film1* (*LGF1*). We generated a complementation line overexpressing the Kinmaze *LGF1* gene in the *drp7* mutant background and confirmed that the phenotype observed in the vector control (*pUb*(VC)/*drp7*), i.e. loss of gas film 1 day after submergence, reduction of underwater photosynthesis and loss of wax crystals, was complemented in the overexpressing line (*pUb*::*LGF1*/*drp7*) (Figure 2B-2D). These results demonstrate that the *LGF1* gene controls gas film retention[3].

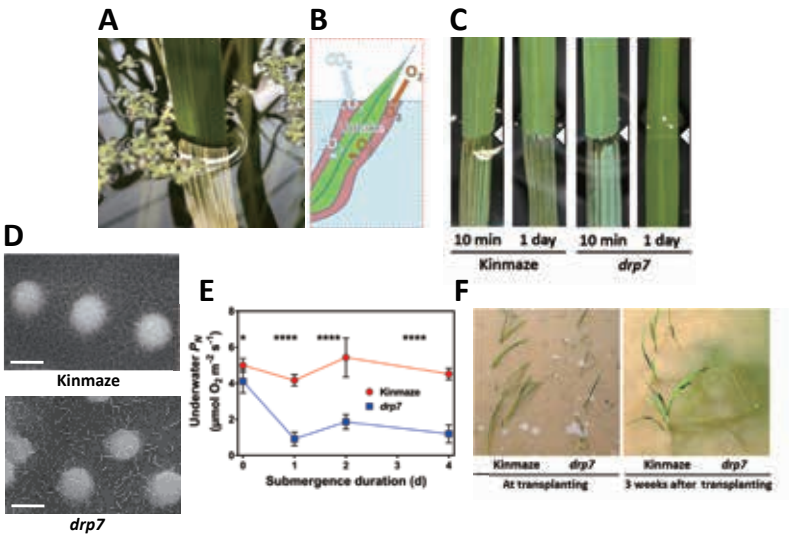

Figure 1. Comparison of gas film retention, leaf blade surface structure, underwater photosynthesis and survival in the paddy field between Kinmaze and *drp7* mutant

(A,B) An air layer (silver) formed around the submerged leaf blade is called a gas film (A), which enhances gas exchange of the underwater leaf (B).
(C) Gas films observed 10 minutes and 1 day after submergence. The arrowheads (white) indicate the water surface.
(D) Scanning electron microscopy of the surface of rice leaf blade. The white, dome-shaped structures are the papillae. Crystallized wax can also be seen (Bar=5μm).
(E) Measurement of underwater photosynthesis of rice leaf blade using an oxygen electrode
(F) Evaluation of survival immediately and 3 weeks after transplanting

Analysis of wax components in rice leaf blades

Wax crystals in rice leaf blades are known to consist of several components. In order to identify which of these components or which component ratios play important roles in gas film retention, we quantitatively compared the surface wax compositions in leaf blades of Kinmaze, the *drp7* mutant, *pUb*::*LGF1*/*drp7* and *pUb*(VC)/*drp7* (Figure 3A, 3B). The *drp7* mutant and *pUb*(VC)/*drp7* had lower levels of C (carbon number) 30:primary alcohol and higher levels of C30:aldehyde compared to Kinmaze (Figure 3). Meanwhile, the amounts of these components in *pUb*::*LGF1*/*drp7* were similar to those in Kinmaze (Figure 3), suggesting that LGF1 might be involved in the conversion of C30:aldehyde to C30:primary alcohol and that the balance between C30:primary alcohol and C30:aldehyde is extremely important for gas film retention in rice leaf blades. This was the first study to identify a gene controlling the gas film trait and elucidate part of its molecular mechanism.

Acknowledgements

The *drp7* mutant used in this study was kindly provided by Professor Toshihiro Kumamaru and Professor Atsushi Yoshimura of Kyushu University. I would like to thank Professor Ole Pederson of University of Copenhagen, Professor Timothy David Colmer and Dr. Al Imran Malik of University of Western Australia for their guidance in the gas film and underwater photosynthesis measurements, and Professor Hiroyuki Ohta, Associate Professor Mie Shimojima, Dr. Yuko Sasaki-Sekimoto and Mr. Kosuke Shimazaki of the Department of Life Science and Technology of Tokyo Institute of Technology for their cooperation in wax component analysis of rice leaf blades. I would also like to thank Associate Professor Junichi Ito and Ms. Saori Aiga of Breeding Science at the University of Tokyo for their kind support in the leaf hydrophobicity test. Finally, I would like to thank Professor Motoyuki Ashikari, Assistant Professor Keisuke Nagai, Dr. Takeshi Kuroha, Dr. Yosuke Toda, Dr. Phung Danh Huan, Mr. Huangqi Qu and Mr. Yoshinao Mori of the Laboratory of Plant Gene Function at Nagoya University for their contribution to the study.

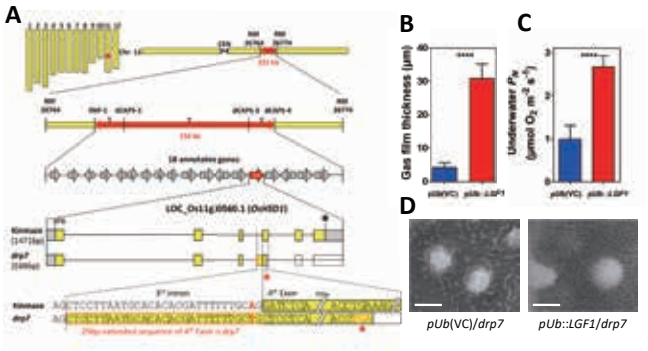

Figure 2. Isolation of the *drp7* candidate gene by positional cloning and identification of the causal gene by complementation test

(A) Results of gene mapping using an F_2 population derived from crosses between the *drp7* mutant and Kasalath
(B, C) Measurement of gas film thickness (B) on the leaf blade and underwater photosynthesis (C) in the complementation line
(D) Surface structure of the leaf blade of the complementation line (Bar=5μm)

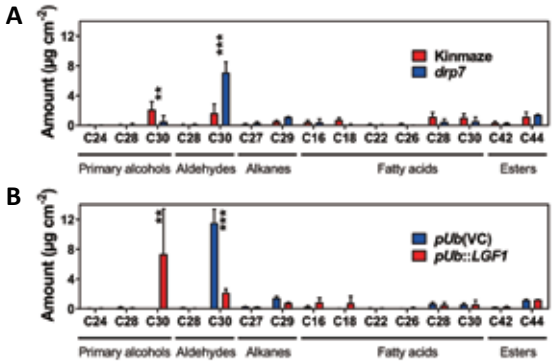

Figure 3. Comparison of wax component compositions among Kinmaze, the *drp7* mutant, the *LGF1*-overexpressing line (*pUb*::*LGF1*/*drp7*) and the vector control (*pUb*(VC)/*drp7*)

(A, B) Comparison of the amounts of various wax components in rice leaf blades, i.e. very-long-chain primary alcohols, aldehydes, alkanes, fatty acids and wax esters, between Kinmaze and the *drp7* mutant (A) and between *pUb*(VC)/*drp7* and *pUb*::*LGF1*/*drp7* (B)

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Technical Tips

Basics of wild rice cultivation: heading and day length

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1. A great barrier to wild rice cultivation in Japan: heading

It is not uncommon for wild rice plants to fail to head in Japan, under environmental conditions different from those in their natural habitats. Such failure can be attributed to the difficulty of controlling day length, which affects flower bud differentiation, in open field environments. Here, I would like to introduce my experience in day length control that affects heading, which is critical for harvesting seeds in wild rice cultivation.

2. Short-day treatment

Wild species of rice are basically categorized as short-day plants, initiating flower bud differentiation in response to shortened day length. At NIG, the length of light period adopted in a standard short-day treatment is 11 hours 30 minutes, which is significantly shorter than the day length in the summer season in Japan. Thus, an artificial short-day treatment is required to make wild rice plants head in the summer. NIG has 8 paddy fields equipped with shading systems (Figure), which are in full operation every year. They can create short-day conditions by automatically moving steel boxes (positioned on the right in the picture) on rails according to predetermined schedules to shade the paddy fields. They practically act as short-day treatment devices, so we call them “short-day paddy fields”. Most wild rice plants start heading 30-40 days after the short-day treatment. When about 20 strains of African cultivated species *O. glaberrima* were transplanted in short-day paddy fields and open paddy fields on the same day, 100% of the plants grown in the short-day paddy fields headed around 30 days after the treatment, but the plants grown in the open paddy fields did not head, even in the harvest period in October. Based on this result, we learned that, although they were categorized as cultivated rice, these strains needed a short-day treatment to start their heading.

There are some cases where the short-day treatment must be done manually. I myself had such experience when we ran out of short-day paddy fields; it was quite tough work to open and close the shading curtains every day at a predetermined time without causing any leakage of sunlight. When short-day treatment needs to be done manually, it may be wise to check the minimum number of days required beforehand. In order to find out the minimum number of days required for short-day treatment, we tested various durations of treatment using an *O. rufipogon* accession and found out that 1 week of treatment was sufficiently effective. I hope this information will be helpful.

3. How effective is natural day length?

Is it completely impossible to make wild rice plants head without any short-day treatment during the long summer days in Japan? Not necessarily. Heading under natural summer day length in Japan can be achieved in some *O. rufipogon* accessions; it is even normal for *O. punctata* (2X) and GG genome species.

If you grow wild rice plants in a facility such as a greenhouse that can be heated during winter, they can head and their seeds can be collected when the natural day length becomes sufficiently short. We conducted a 5-year survey on the heading date of 74 bucket-grown *O. rufipogon* accessions in a plastic greenhouse warmed by a hot spring at the Ibusuki Experimental Farm of Kyushu University Faculty of Agriculture, located in Ibusuki City, Kagoshima Prefecture. Of the 74 accessions tested, 50% headed in mid-to late November, and 70% headed from mid-October to early December. The day length in Ibusuki reaches 11 hours 30 minutes in mid-October, suggesting that many of the strains headed in response to the sufficiently shortened natural day length. (Mr. Koichi Ohta, who made a great contribution to the collection of these data as a member of the technical staff at the experimental field passed away in January 2019. I would like to express my deepest gratitude for his support in our wild rice cultivation management. I pray that his soul may rest in peace.)

4. Conclusion

Short-day treatment is important but not always necessary for making wild rice plants head in Japan. Whether or not heading occurs may vary between years or even among individuals within the same accession or vegetative clones. Involvement of various conditions during vegetative growth is also suspected, which may complicate the control of heading of wild rice plants. I hear that wild rice plants exhibit some variation in their heading dates even in their natural habitats. Considering their “wild” nature, it may be natural for them to have difficulty in adjusting their heading date to suit our convenience. We need the spirit of Don Quixote to address the difficult challenge of heading control in wild rice.

Figure: An excellent system for short-day treatment: a paddy field equipped with a shading device (short-day paddy field).
At NIG, 8 “short-day paddy fields” are in full operation every year for wild rice cultivation. The steel box positioned on the right automatically opens and closes on a predetermined schedule to create short-day conditions.

Activity Reports 2019

Open Fields (Study tour of wild rice genetic resources)

This year, we held 'open fields' (study tours of wild rice genetic resources) at NIG in September and at Kyushu University during August to October. The open fields were scheduled according to participants' needs, and many people from Japan and other countries came to observe wild rice and experimental lines derived from wild rice.

Open Fields at Kyushu University

Open Fields at NIG

A study tour of wild rice genetic resources for representatives of the CGIAR Genebank

On October 10, 2019, Dr. Charlotte Lusty (Crop Trust), Dr. Thomas Payne (CIMMYT) and Dr. Noelle Anglin (CIP) representing the CGIAR Genebank visited NIG to observe our wild rice genetic resources. They exchanged views with us on future application of wild rice genetic resources.

Representatives of the CGIAR Genebank visited NIG

NIG meeting "Research Promotion for Genetic Diversity Using Genome Information on genus *Oryza*"

On December 13, 2019, NIG hosted a seminar in which researchers engaged in genetics using related wild species in *Oryza* gathered and discussed future directions and prospects for wild rice research. At the end of the seminar, young working group members of NBRP-Rice collected participants' requests on NBRP resources.

Survey of requests on NBRP resources by young working group members

Public Relations at Academic Societies

Rice Genetics and Molecular Biology Workshop 2019 (at Tsukuba Business-Academia Cooperation Support Center in July 2019)

42nd Annual Meeting of the Molecular Biology Society of Japan (at Marine Messe Fukuoka in December 2019)

61st Annual Meeting of The Japanese Society of Plant Physiologists (at Osaka University in March 2020 (scheduled))

NBRP Rice Advisory Committee

The annual committee was held at ROIS on 11th December 2019.

Events 2020

Open Fields (Study tour of wild rice genetic resources)	In 2020, we will hold regular 'Open Fields' tours at NIG and Kyushu University. Additionally, we also plan to hold international 'Open Fields' tour at Myanmar in November 2020. If you are interested in any particular NBRP strains 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 and Japanese versions of this Newsletter are available on Oryzabase (https://shigen.nig.ac.jp/rice/oryzabase/).

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