

BioResource Now!

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Research and Bioresources <NO.14>

Research on Hybrid Sterility in Rice by Using Chromosome Segment Substitution Lines

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Genetic Diversity of Rice and Its Use

Morphological differences are difficult to detect among paddy rice varieties in Japan, such as Koshihikari and Akitakomachi. In contrast, variations and characteristics of external morphologies are observed in rice cultivars around the world (Fig. 1).



Fig. 1. Variations in the seed morphology among rice cultivars

Wild rice species show a wide range of features. One species may be as tiny as a weed while another species may grow to heights more than that of a human being. The diversity of wild rice species is because of their adaptation to their immediate natural environment over time. In the case of cultivated species, artificial selection based on local culture has also induced adaptation.

Japanese rice has excellent taste. However, it presents minimal genetic variation and weak resistance to environmental stress. These disadvantages have been addressed by selective breeding by using various foreign genetic resources. However, this breeding effort has been technically difficult. In selective breeding, parents possessing different properties are hybridized, and descendants presenting superior traits are selected. This selection is repeated for several generations to obtain a new variety. However, the hybrid progeny does not always exhibit excellent performance, and undesirable traits such as sterility and malformation are simultaneously expressed. These biological disturbances are inevitable in breeding with remote hybridization. We have been studying cultivated rice (*Oryza sativa* L.), which is further classified into two subspecies, *O. sativa* L. spp. *japonica* and *O. sativa* L. spp. *indica*. When these two subspecies are hybridized, hybrid sterility is often observed (Fig. 2). This phenomenon is very important in understanding the evolution of rice and the mechanism underlying its reproduction and development. However, the mechanism of reproduction and development in rice has remained elusive.



Fig.2. Normal ears of rice (top) and ears of rice without ripe seeds due to hybrid sterility (bottom)

Chromosome Segment Substitution Lines

Several agronomic characteristics such as hybrid sterility, plant length, and spikelet number are quantitative traits that continuously vary. Several genes are responsible for the occurrence of quantitative traits. Because gene–gene and gene–environment interactions are involved, quantitative traits are generally complicated and their genetic analysis is often difficult to perform.

To analyze quantitative traits, we have created chromosome segment substitution lines (CSSLs), which involves the partial substitution of a chromosomal region of *O. sativa* L. spp. *japonica* with that of *O. sativa* L. spp. *indica* [1]. On the basis of the change in phenotype exhibited by each CSSL, the effects and loci of specific genes can be examined. For example, when chromosome 3 of *O. sativa* L. spp. *japonica* was partially substituted with that of *O. sativa* L. spp. *indica*, long and narrow grains were produced, which are characteristic of *O. sativa* L. spp. *indica*. Thus, the specific trait controlled by this specific gene could be established. Thus, GS3, a gene conferring grain length in rice, was identified [2]. By evaluating phenotypes of a series of CSSLs that cover the whole genome, all genes involved in natural variations could be detected. The CSSLs that we created consisted of two series. The first series was generated by substituting a segment of *indica* with that of *japonica* in *japonica* rice. The second series was produced by substituting a segment of *japonica* with that of *indica* in *indica* rice (Fig. 3). These two series are useful for analyzing gene–gene interactions. CSSLs were developed under the guidance of Professor Atsushi Yoshimura of Kyusyu University. CSSLs can be obtained from the Integrated Rice Science Database called Oryzabase, which is available at <http://www.shigen.nig.ac.jp/rice/oryzabaseV4/>.

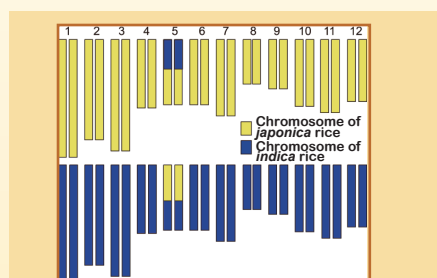


Fig. 3. Chromosome segment substitution lines showing different chromosomal compositions

Exhaustive Analysis of Genes Causing Hybrid Sterility

By investigating the CSSLs and sister lines in detail, we have identified 10 genes that were associated with hybrid sterility in hybrids of *indica* and *japonica*. Various phenotypes have been observed, such as male sterility, female sterility, and weak sterility. The results of our genetic analysis of hybrid sterility is summarized as follows:

- 1) An unexpectedly large number of genes are involved in each event of hybrid sterility, and each event is controlled by complex gene–gene interactions.
- 2) Hybrid sterility is caused by gene interactions in the gametes and zygotes.
- 3) Multiple genes that are associated with hybrid sterility occur as a genetic cluster.
- 4) No individual speciation gene responsible for reproductive isolation has been detected.

Our studies have shown that no single gene was responsible for hybrid sterility. Therefore, genetic variations might have accumulated in multiple genes over time, eventually resulting in the reproductive isolation of specific rice varieties [3]. In this study, by carefully examining CSSLs, we identified a gene network for hybrid sterility, which has remained undetectable using conventional analytical methods. Some of the parental alleles causing hybrid sterility have been predicted not to be involved with the loss of protein function. Our future research direction will involve the elucidation of the mechanism of hybrid sterility-related protein functional disorders.

Unlike microorganisms and animals, the function of various genes involved in plant development and growth remain unclear. Hybrid sterility is the product of the functional differentiation of genes that are involved in reproduction and development. To elucidate the mechanism of hybrid sterility, it is essential to understand the mechanisms behind reproduction and development. We anticipate that our research activities on reproduction and development would complement our findings on hybrid sterility.

Hybrid Sterility and Breeding

"Linkage drag" means that when a gene possessing a superior trait is introduced from another variety, a linked gene possessing an inferior trait is simultaneously introduced. We have also analyzed morphological and physiological traits observed in CSSLs. The analyzed traits were related to the differentiation between *indica* and *japonica* in terms of grain shape and heading time. Our results showed that several genes causing morphological and physiological traits were adjacent to genes causing hybrid sterility. To our knowledge, no study has investigated the relationship between the loci of adjacent genes that cause linkage drag in the whole genome.

Therefore, our findings may be potentially applied to future breeding schemes.

Recently, hybrid rice has been distributed across various Asian countries. Heterosis (the viability and fertility of a hybrid variety become stronger than those of the parents due to hybridization) has been used for the creation of hybrid rice. However, hybrid sterility remains an obstacle to these hybridizations. As previously mentioned, for various genetic resources of rice to be effectively used, hybrid sterility must be thoroughly understood. In the future, we will conduct investigations on the mechanism underlying hybrid sterility and develop technologies that are applicable to the improvement of various rice cultivars around the world.

References

- [1] Kubo, T., Aida Y., Nakamura K., Tsunematsu H., Doi K., and Yoshimura A. (2002) Reciprocal chromosome segment substitution series derived from Japonica and Indica cross of rice (*Oryza sativa* L.). *Breed. Science* 52: 319-325.
- [2] Takano-Kai N., Jiang H., Kubo T., Sweeney M., Matsumoto T., Kanamori H., Padhukasahasram B., Bustamante C., Yoshimura A., Doi K., and McCouch S. (2009) Evolutionary history of GS3, a gene conferring grain length in rice. *Genetics* 182:1323-1334.
- [3] Kubo T., Yoshimura A., and Kurata N. (2011) Hybrid male sterility in rice is due to epistatic interactions with a pollen killer locus. *Genetics* 189: 1083-1092.

Introducing Start Menu 8



While a year is about to pass since the initial release of Windows 8, there are still many users who are uncomfortable migrating to the new operating system. The primary reason for this may be the fear that the refreshed user interface has considerably changed the usability of the operating system. In particular, the absence of the conventional "Start Button" had caused a big stir during the initial release of Windows 8. There was an expectation that the Start Button will make a comeback with the release of Windows 8.1 in October this year, but we now know that this "Start Button" only redirects you to the Start Screen, and its functionality is therefore completely different from the Start Button in Windows 7 and older.

Thus, it is unlikely that a start button, as seen in Windows 7 and older, will ever be officially implemented in Windows 8. However, there are currently various paid and free software that can display a start button with very similar functionalities. I would like to introduce "Start Menu 8," one such software that is both free and highly configurable.

Let's try installing Start Menu 8

- 1 Access the official website at <http://www.iobit.com/iobitstartmenu8.php> and click on the "Free Download" button to download the setup file "startmenu-setup.exe" (Fig. 1).
- 2 Execute the downloaded file. The installer as shown in Fig. 2 will be launched. Follow the instructions on the screen to complete the installation.



Fig. 1. Official website

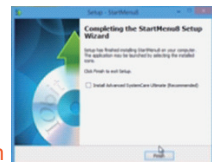


Fig.2. Installation screen

- 3 After the installation, you will be presented with an option to select a skin for the start button (Fig. 3). Choose a skin that you like from this list and click on [OK].

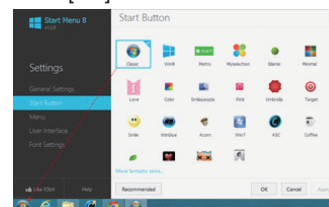


Fig. 3. Screen to select the start button skin

Setup is then completed. A start button should appear on the left side of the menu bar (Fig. 4).



Fig. 4. The start button

Right-click on the start button and select [Settings] to display the screen viewed above during installation. You can also make further customizations including the color, font size, and display language of the menu.

Although the innovative user interface of Windows 8 often appears peculiar, over time, users may learn to appreciate various qualities of the operating system including its high performance and good design that functions well with the touch interface. Why not add the start button that you are familiar with and start using Windows 8 in a convenient manner?

(Kyohei Matsuno)

Database of This Month

National BioResource Project "Pathogenic microbes"



DB name : Pathogenic microbes
 URL : <http://pathogenic.lab.nig.ac.jp/>
 Language : Japanese, English
 Contents :

- Strain names, species names, isolation sources, origins, danger levels, serotypes, and pathogenesis factors of pathogenic microbes (bacteria, actinomycetes, fungi, and protozoa)
- Database of bacterial toxins
- MICRON helps a family understand microbes (animation)

Features :

- Search across strains maintained at Chiba Univ., Osaka Univ., Gifu Univ., Nagasaki Univ., RIKEN, and the Institute of Medical Science, The Univ. of Tokyo.
- Items shown in the strain list can be selected.
- Guidelines involving the use of a material transfer agreement.

Cooperative DB : RRC (Research Resource Circulation)
 DB construction group : NBRP Pathogenic microbes, NBRP Information
 Management organization : Genetic Resource Center, NIG
 Year of DB publication : 2004 Year of last DB update: 2013

- Number of bacterial and actinomycetes strains: 10,595
- Number of fungal strains: 17,849
- Number of protozoan strains: 357 (as of September 2013)

Comment from a practicing developer : Comment from a practicing developer: The contents of this database can be updated by an officer-in-charge from each institution; this individual should be directly involved with the current list of pathogenic strains of his/her institution. The database has been continuously open to the public for nearly 10 years without large-scale modifications. Recently, we have reviewed the bacteria-related items displayed in this database. We have also developed a new database site, which includes the advanced search function. Please visit our new database, The Fungus and Actinomycetes Gallery in the Medical Mycology Research Center, Chiba University, which can be accessed from Gallery on our website, shows a collection of superb images. You can also enjoy watching a masterpiece animation on our website.

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Editor's Note

This month, Assistant Prof. Kubo has extensively discussed his research, for which he has received the Encouragement Award from the Japan Society of Breeding during its 123th Meeting. I believe this newsletter presents Assistant Prof. Kubo's tenacious efforts in elucidating the mechanism underlying hybrid sterility, which is a complicated biological phenomenon involving multiple genes, as well as the uses of CSSLs as experimental lines. The creation of CSSLs involves extensive time and effort. At present, because of the continuous efforts of Assistant Prof. Kubo and other researchers, CSSLs can be distributed to various research institutions. Rice can be quite mysterious merely by the sight of their heavy ears when ripe (Y. Y.).

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