

## イネの自然変異と多様性研究

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## NATURAL VARIATION AND THE STUDY FOR ENHANCING GENETIC DIVERSITY IN RICE

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### Introduction

Asian cultivated rice (*Oryza sativa* L.) has great morphological and physiological diversity. The beneficial traits found in germplasms have been introduced into other rice through cross breeding. Since agronomic traits are controlled by multiple loci, genetic improvement is time consuming, especially when introducing traits from exotic germplasms. Non-random distribution of the beneficial traits in the gene pool and the reproductive barriers observed in the progenies of the cross between genetically differentiated germplasms is also a factor associated with the efficiency of breeding. Therefore, information on the genetic controlling of the traits and structure of the gene pool is of interest to the rice breeders who need to determine the cross-combinations and establishing efficient procedures for germplasm enhancement.

Rice genome information can help improve strategies for enhancing rice germplasm. A high-density genetic linkage map (KURATA *et al.* 1994; HARUSIMA *et al.* 1998) is a powerful tool for targeting genes for agronomic traits and their selection (YANO and SASAKI 1997). DNA markers are also useful for depicting the structure of the gene pool and selecting a subset of the germplasm panel to maximize the use of genetic diversity in cultivated rice (KOJIMA *et al.* 2005). Complete genome sequence information (IRGSP 2005), a gene annotation database (ITO *et al.* 2005), full-length cDNA libraries (KIKUCHI *et al.* 2003) of a Japonica rice variety Nipponbare were developed as tools for identifying the structure and func-

tion of the genes controlling traits. New technologies based on rice genome information have been accelerating genetic studies for practical breeding, establishing the connection of genetic studies with other basic sciences.

The present paper focuses on a study of the genetic dissection of field resistance to rice blast, as an example study of an important agronomic trait under complicated genetic control, as well as a study revealing the genetic structure of cultivated rice. These studies present strategies for rice germplasm enhancement incorporated with rice genome information.

### Genetic dissection of field resistance to blast

Rice blast is a destructive disease of rice worldwide, and strengthening resistance to blast is an important breeding objective. Two types of resistance to blast, complete resistance and field (partial) resistance have been described in rice (EZUKA 1972). Complete resistance induces a hypersensitive reaction and is characterized by a resistant infection type. More than 20 loci for complete blast resistance have been identified (MCCOUCH *et al.*, 1994), and some were cloned in rice (WANG *et al.* 1999; BRYAN *et al.* 2000). Despite their significant effect against rice blast, complete resistance genes were overcome by compatible races of the pathogen several years after the varieties with this type of resistance were released. Field resistance is usually incomplete and characterized by a susceptible infection type. Field resistance limits the proportion of diseased leaf area and allows effective

control of the parasite under natural conditions. This type of resistance is considered to be durable when exposed to new races of the pathogen. Japanese upland rice has been used as a source of the genes conferring field resistance to rice blast in a Japanese breeding program that was initiated in the 1920's. However, field resistance in upland rice has not contributed much to the improvement of Japanese rice varieties (INOUE *et al.* 1983), possibly due to the close linkage between the genes for field resistance and certain undesirable characteristics. Some genetic studies reported that many genes with additive effects are responsible for the expression of field resistance (HIGASHI and KUSHIBUCHI 1978, HIGASHI and SAITO 1985). These results suggest that identification of the number of genes and their chromosomal locations are important for understanding field resistance and for enhancing its use.

### 1) Mapping of QTL for field resistance to rice blast

QTL analysis was carried out using 146 F<sub>4</sub> lines of a cross between the Japanese irrigated rice cultivar Nipponbare and the upland cultivar Owarihatamochi to determine the chromosomal regions involved in

field resistance to blast in Japanese upland rice (FUKUOKA and OKUNO 2001). Nipponbare and Owarihatamochi have no gene for complete resistance to rice blast, based on the test using Japanese differential blast races. The frequency distribution of the field resistance score based on the diseased leaf area (DLA) in the F<sub>4</sub> lines was continuous and ranged from 1 (highly resistant) to 10 (highly susceptible). The scores for Owarihatamochi and Nipponbare were 2.8 and 8.0, respectively. We used 116 DNA marker loci for QTL mapping and detected four QTLs located on three chromosomes (Fig. 1). The resistant alleles on chromosomes 4 and 12 come from Owarihatamochi, while that on chromosome 9 is derived from Nipponbare. The two QTLs on chromosome 4, close to marker loci *G271* (*qBR4-1*) and *G177* (*qBR4-2*), explained 45.7% and 29.4% of the phenotypic variation, respectively, while the QTLs on chromosome 9 (*qBR9-1*) and 12 (*qBR12-1*) explained 7.9% and 13.7% of the total phenotypic variation. All together, the four QTLs explained 66.3% of the total phenotypic variation.

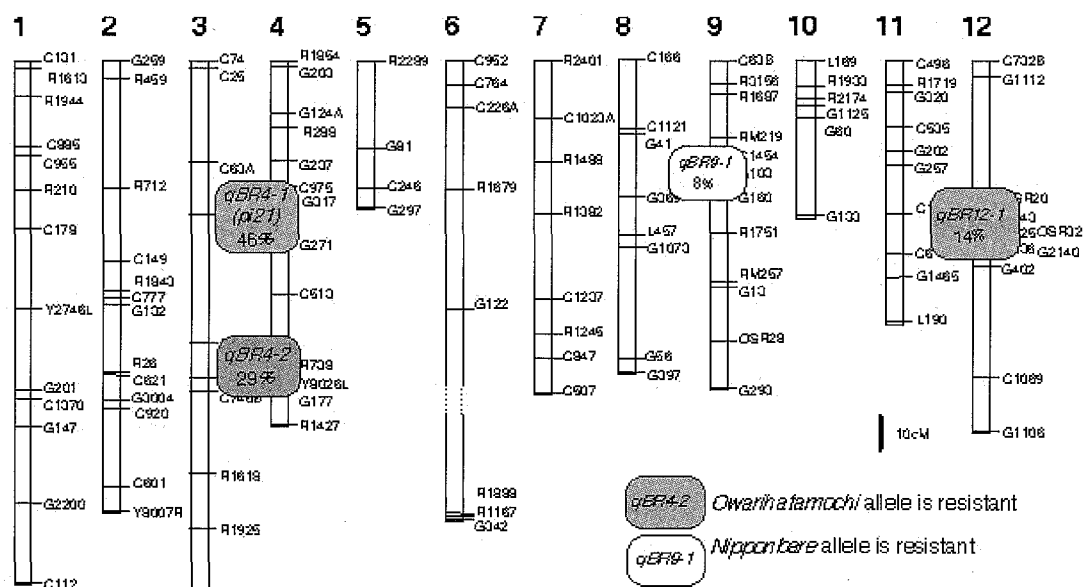


Fig. 1 Chromosomal location of the QTLs for rice blast field resistance in F<sub>4</sub> lines from the cross between irrigated rice Nipponbare and upland rice Owarihatamochi. (adapted from FUKUOKA and OKUNO, 2001)

## 2) Marker-assisted selection of field resistance in backcrossed lines

To evaluate the effect of each QTL, advanced backcrossed lines with putative QTLs of Owarihatamochi were developed by marker assisted selection using a highly susceptible lowland rice variety, Aichiasahi, as the recurrent parent. During backcross and selection, the proportion of upland rice chromosomes in these plants decreased to less than 6% based on the genotypes of DNA markers. We selected three BC<sub>2</sub>F<sub>1</sub> plants that contained just one out of three blast resistance alleles from Owarihatamochi. The field resistance in the 98, 44 and 46 BC<sub>2</sub>F<sub>3</sub> lines for *qBR4-1*, *qBR4-2* and *qBR12-1*, respectively was assessed and compared to the levels of field resistance among the lines with Owarihatamochi-homozygous alleles, heterozygous alleles and Aichiasahi-homozygous alleles. Genotypes at each QTL were estimated based on the genotype of DNA markers around the QTLs. The average DLA score of the lines with the Owarihatamochi-allele was significantly higher than those with the Aichiasahi-allele at all three QTLs (Table 1). The effect was largest at the *qBR4-1* and smallest at the *qBR12-1*, in good accordance with the result of the QTL analysis.

## 3) Map-based cloning of a field resistance gene *pi21*

Genetic analysis for field resistance was conducted in 82 BC<sub>1</sub>F<sub>3</sub> lines in which *qBR4-1*, a QTL with the largest effect was segregated. A resistance gene for this QTL was mapped as a single recessive gene *pi21* between the RFLP marker loci *G271* and

Table 1. Average score for diseased leaf area of the lines with different genotypes at QTLs

QTL	O-homozygous	Heterozygous	A-homozygous
<i>qBR4-1</i>	3.1±0.46 (10)	5.7±0.77 (55)	6.9±0.75 (100)
<i>qBR4-2</i>	6.7±0.66 (67)	7.3±0.51 (83)	8.2±0.29 (100)
<i>qBR12-1</i>	6.4±0.29 (80)	6.8±0.64 (93)	7.0±0.57 (100)

O: *Owarihatamochi*, A: *Aichiasahi*.

Percentages of the diseased leaf area compared with A-homozygous lines are indicated in parentheses.

*G317* at a distance of 5.0 cM and 8.5 cM, respectively (FUKUOKA and OKUNO 2001). An intensive DNA marker survey using the end-fragment DNA sequence of the P1 artificial chromosome (PAC) clones covering the *pi21* region identified eleven DNA markers around the *pi21* locus. We used two mapping populations consisting of a total of 3717 plants for fine genetic mapping of *pi21*. The analysis delimited the 1.7kb-genomic region where the segregation of DNA markers completely associated with the resistant/susceptible phenotypic difference. The search of the gene annotation database and Nipponbare cDNA clone identified that this region is within the coding region, and that the sequence variations between susceptible varieties Aichiasahi and Nipponbare, and resistance variety Owarihatamochi, cause length-polymorphisms at the amino acid level. Deletion of the Pi21 protein in Owarihatamochi was considered to result in an improvement in of field resistance. The molecular function of the candidate gene for *pi21* is unclear, and its DNA sequences have no similarity with previously reported disease resistance genes. The functional analysis of the gene is under progress.

## 4) Conclusions

DNA marker-based genetic dissection of a beneficial trait, field resistance to blast, efficiently identified chromosomal regions for the trait that enhances the use of natural variation in the gene pool. Further analysis using backcrossed lines enabled us to confirm the effect of each QTL for the trait, and the lines are useful as a material for pre-breeding as well as of one for basic research. Identification of the gene for the trait will help enable precise genotyping of the gene, and will contribute to further understanding of the mechanism of the expression of field resistance.

## The analysis of the genetic structure in Asian cultivated rice

The intraspecific variation of Asian cultivated rice has been investigated by many researchers. Distinct differentiation within this species was first reported

by KATO *et al.* (1928), and has been supported by many reports using various kind of methodologies, such as morphological, and physiological characteristics, isozyme and DNA markers (ex. OKA 1953; GLASZMANN 1987; ZHANG *et al.* 1992). When reviewing these studies in detail, there were some discrepancies in the results among methodologies, suggesting that the key traits or factors for each methodology represent different aspects of genetic variation in Asian rice, and also suggesting the genetic differentiation within each of the two major variety groups. Since adaptation to diverse environments and to different cultural practices can be factors in the genetic differentiation in rice, understanding the genetic structure is useful for identifying varietal groups and the chromosomal regions relevant to varietal differentiation. Such information will be helpful for screening potential germplasms from the huge number of collections, and for determining their use. DNA markers identified on the rice genome provide an efficient means for detecting genetic variation and the distribution of this variation among the rice germplasm. The markers that cover the rice genome are also useful for detecting genetic differentiation and the genetic variation associated with characteristics for adapting to different environments and cultural practices.

### 1) Genetic structure of Asian cultivated rice based on RFLP

A total of 332 accessions originating from 23 countries, and including 281 landraces and 51 modern varieties were selected from the accessions maintained at the NIAS Genebank and used for the analysis using 179 RFLP marker loci on the high-density genetic linkage map of rice (KURATA *et al.* 1994; HARUSHIMA *et al.* 1998). Based on principal coordinate analysis of the RFLP data, the accessions were classified into three major groups (Fig.2)(KOJIMA *et al. in press*). Based on the proportion of alleles shared with the Indica and Japonica reference varieties, one group was assigned to Japonica, and the other two groups to Indica. However, each accession harbored both Indica- and Japonica- specific alleles to some extent (Fig. 3),

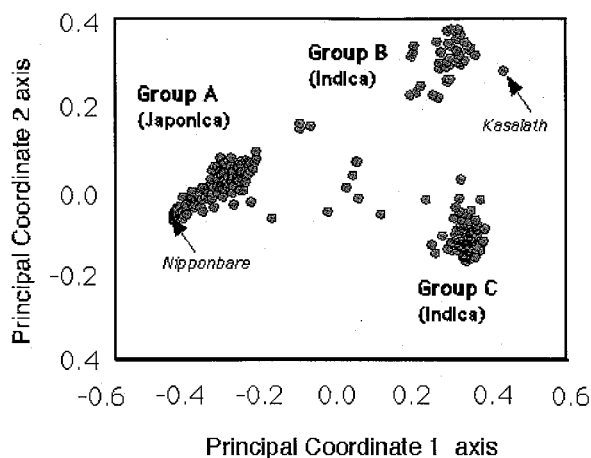


Fig 2. Principal coordinate plots of 332 accessions of rice based on DNA polymorphism at 179 RFLP loci. (adapted from KOJIMA *et al.*, 2005)

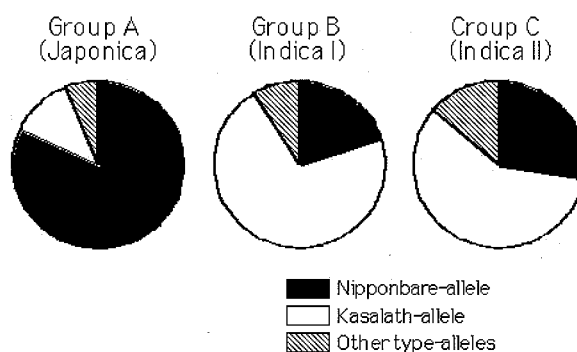


Fig 3. Characterization of the accession groups detected by the principal coordinate analysis (adapted from KOJIMA *et al.*, 2005)

and each chromosomal region showed mosaic structures with a combination of Indica- and Japonica-specific alleles. Hybridization and separation by the reproductive barrier among the rice accessions may account for the complicated mosaic structure of the chromosomes.

### 2) Core collection as a rice diversity research set

The core collection of rice was chosen as a rice diversity research set based on a genome-wide RFLP survey of 332 accessions of Asian cultivated rice (KOJIMA *et al.* 2005). The RFLP data on the 332 accessions were subjected to cluster analysis and 67 groups were recognized at a similarity index of 0.915. A single accession from each of the 67 groups was se-

lected. These 67 accessions retained 91% of the alleles detected in the original 332 accessions, and covered the variation of the initial set of accessions in terms of several agro-morphological traits. The 69 accessions, including varieties from 19 countries and the reference varieties, Nipponbare and Kasalath, were selected as a rice diversity research set. This collection, which is presently well characterized at the molecular level will be used for detailed genetic studies and rice improvement. This set is distributed by the NIAS Genebank (contact address is genebank@nias.affrc.go.jp). Accumulated data on the various traits and on DNA polymorphism in the collection will be provided as feedback to users, which should enhance the efficient use of rice genetic resources.

**3) Association study in rice**

Association analysis, or linkage disequilibrium (LD) mapping, has been extensively used in animals to dissect quantitative traits. This approach has recently been extended to plants such as maize and *Arabidopsis* (FLINT-GARCIA *et al.* 2003). Association analysis is potentially advantageous in the resolution,

speed and allelic range compared with F<sub>2</sub>-based QTL mapping. LD, caused by the linkage and population structures resulting from the history of the population, plays a central role in association analysis and the extent of LD determines the applicability of association study. The knowledge of LD in rice is limited, and therefore it is important to determine the extent of LD to perform an association analysis in rice. We are collecting polymorphism information to study is genome-wide LD in rice.

**4) Detection of the chromosomal regions associated with cultural type differentiation among germplasms in northern Vietnam**

A total of 129 rice landraces from northern Vietnam, 89 accessions of upland rice and 40 of lowland rice, were analyzed by using 169 RFLP marker loci (FUKUOKA *et al.* 1993). Based on principle coordinate analysis of the RFLP data, Vietnamese landraces were classified into three groups; one of Indica, the other two of Japonica. The two groups in the Japonica rice accession were respectively corresponded with upland and lowland rice, implying the genetic differentiation in the Japonica can be the result of adapta-

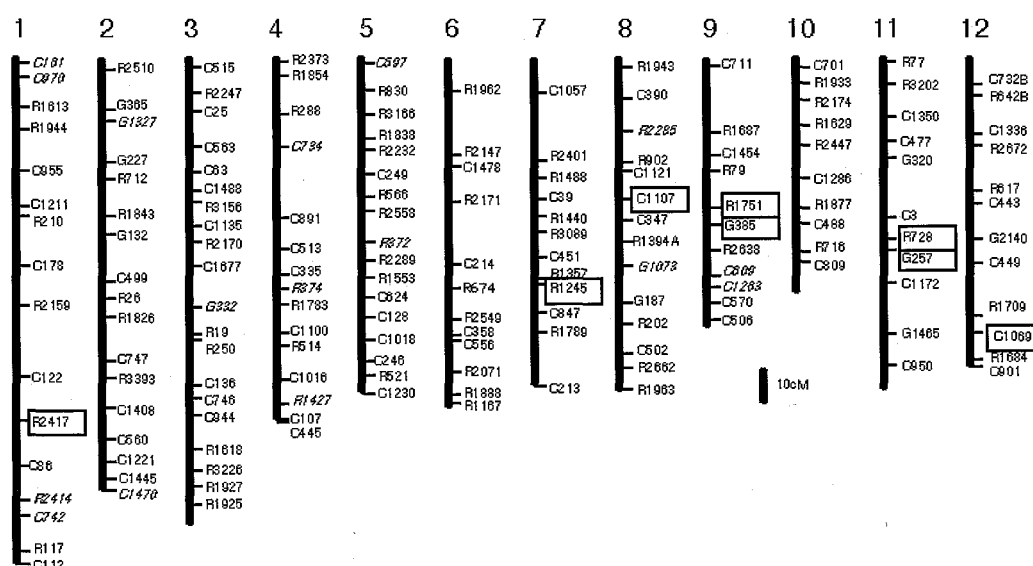


Fig.4. Chromosomal locations of RFLP marker loci and their association with genetic differentiation among Vietnamese landraces. Marker loci diagnostic to upland-lowland cultural type differentiation in Japonica rice were indicated in boxes. (adapted from FUKUOKA *et al.*, 2003)

tion to different cultural practices. In Indica accessions, out of 115 polymorphic marker loci, no marker loci were associated with cultural types differentiation. In Japonica rice, eight marker loci out of 81 polymorphic loci were associated with cultural type differentiation (Fig. 4). Some of the loci associated with upland-lowland differentiation were close to the QTLs associated with root morphology and seedling-vigor (YADAV *et al.* 1997; ZHANG *et al.* 2001; CUI *et al.* 2002). Such QTLs may possibly be related to the adaptation of rice to upland condition.

### 5) Conclusions

Asian cultivated rice is clearly differentiated into two major groups, as detected based on the RFLP survey. The genetic structure within these two groups is complicated, possibly due to the result of a natural hybridization event in the past. The rice core collection was chosen as a rice diversity research set based on genome-wide RFLP data to represent the genetic diversity of this species in a small number of accessions. This collection is useful for further intensive polymorphism surveying and characterization of rice germplasm. An association study is a potential approach for dissecting natural variation in a species, and needs further evaluation to determine the optimal procedure in rice

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## イネの自然変異と多様性研究

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アジア栽培イネは様々な環境で栽培され、多様な変異を保持している。しかしながら、それらの形質変異の多くは遺伝様式が複雑であるため、効率的に利用することは困難であった。イネゲノム研究が生み出した塩基配列情報やDNAマーカーなどの研究資源は、遺伝的多様性、とりわけ形質変異の解明に役立ち、多様性研究を大きく発展させる。

いもち病圃場抵抗性は、いもち病菌レース変動に対して安定した効果を示す有用な形質である。陸稲品種の優れた圃場抵抗性は古くより知られるが、複雑な遺伝様式を持つため、その育種利用は進まなかった。DNAマーカーを用いた遺伝解析によって、陸稲品種オワリハタモチの圃場抵抗性の主要なQTLが3カ所の染色体領域にあることを明らかにし、最も作用力の大きいQTL、*pi21*の座乗位置を決定した。さらに、戻し交雑集団を用いた解析によって、圃場抵抗性に関わる新たなQTLを明らかにした。また、*pi21*のマップベースクローニングを試み、遺伝子予測やcDNAクローンのデータベースを用いて候補遺伝子を特定し、圃場抵抗性に関わる変異を遺伝子レベルで明らかにした。既報の真性抵抗性遺伝子とは異なる構造を持つ*pi21*遺伝子の機能解析によって圃場抵抗性の機構解明が可能となる。このように、ゲノム情報を利用した有用形質の遺伝学的解析によって、自然変異を解明し効率的な利用技術を開発に役立つ。

自然変異の育種利用をはかる上で、集団構造の

解明は重要な研究課題である。アジア栽培イネにおける日本型イネ・インド型イネという亜種レベルの分化は、多数の遺伝子座の変異に基づいており、個々の要因を特定することは困難である。ゲノム上に散在するDNAマーカーを用いて品種間変異を調べると、イネの遺伝的多様性は日本型・インド型という基本骨格の上に、品種群内外からの遺伝子移入と新たに生じた変異が積み重なり構成されることがわかった。日本型・インド型イネ以外にも、水陸稲等の栽培様式や感光性等による生態型の分化があり、ベトナム北西部の在来品種のDNA変異を調査したところ、水稲・陸稲の栽培様式と相関のある染色体領域を検出できた。このような領域の中には、根の形態に関与するQTLが報告されており、品種間変異の解析によって品種群や生態型に特徴的な変異の検索が可能である。そこで、原産地情報およびDNA多型情報に基づき、アジア栽培イネ品種の変異を効率的に調査できる品種セット(コアコレクション)を選定した。今後、コアコレクションに対してDNA多型情報と形質変異情報の集積を行うことで、他殖性生物で利用されている連関(association)解析や連鎖不平衡(LD)解析を自殖性作物のイネに適用するための基礎的な情報を得ることができる。これによって、遺伝子の進化的意義を解明、選択圧となる環境要因の特定など、多様性解明に役立つ情報の発掘が期待できる。