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Integration of genomics into breeding in rice

Masahiro Yano*, Shuichi Fukuoka, Kaworu Ebana, Toshio Yamamoto

QTL Genomics Research Center, National Institute of Agrobiological Sciences,
Kannondai 2-1-2, Tsukuba, Ibaraki 305-8602, Japan
*Corresponding author: myano@nias. affrc.go.jp

ABSTRACT

Elucidation of the association between nucleotide and phenotypic changes has been a big challenge in plant molecular genetics. Toward this goal, we have been involved in the genetic dissection of natural phenotypic variations in rice and have identified several genes involved in complex traits, including heading date, shattering habit, pre-harvest sprouting, root morphology, disease resistance and eating quality. To enhance the power of genetic dissection of complex phenotypes, we are developing several mapping populations, such as recombinant inbred lines and chromosome segment substitution lines, which will be required to extract the useful alleles from natural variants. Marker assisted selection has been applied for the development of heading date and durable resistance. To facilitate allele mining using novel plant materials, we have also embarked on the genome-wide discovery of single nucleotide polymorphisms (SNPs). In particular, to overcome a shortage of SNPs among temperate japonica cultivars, we have attempted whole-genome sequencing of several cultivars using next-generation sequencing approaches. Although Japanese cultivars are closely related genetically, about 67,000 SNPs have been discovered between Nipponbare (reference) and Koshihikari by this approach. This SNP discovery has led to the development of an array-based SNP genotyping system in Japanese rice cultivars. Large-scale

genotyping of these SNPs has made it possible to visualize pedigree haplotypes for the Japanese landraces and modern cultivars. These new efforts in genomics have opened up new opportunities to accelerate not only the genetic dissection of complex traits, but also the improvement of rice cultivars.
Key words: Quantitative trait locus, Genetic mapping, Marker assisted selection, Single nucleotide polymorphism

INTRODUCTION

It is about 4 years since the whole rice genome was decoded (IRGSP 2005). The sequence information has provided new tools for genetics and has created a new paradigm of selection strategy in plant breeding. Many phenotypic traits of economic interest are controlled by multiple genes and often show complex and quantitative inheritance. Recent progress in rice genomics has had a great impact on the genetic dissection of such traits into single genetic factors or quantitative trait loci (QTLs) (Yamamoto *et al*., 2009). These resources have already contributed to both our understanding of biological phenomena in plants, and to the application of genomics tools to the development of new crop cultivars. Genes with agronomic value have been tagged by DNA markers and have been introduced into elite cultivars by marker-assisted selection (MAS). Technological innovations in large-scale sequencing and genotyping have opened new possibilities in rice genetics and breeding. This paper introduces our recent activity on the genetic dissection of complex traits, marker-assisted introgression, and pyramiding of agronomic traits, and future prospects in genomics-assisted breeding of rice.

Uncovering the Naturally Occurring Variations in Complex Traits

In the last decade, much effort has been paid to the genetic and molecular dissection of complex traits. An excellent example is the analysis of heading date in rice. Heading date is a key determinant for the adaptation of rice to different

cultivation areas and cropping seasons. Therefore, control of heading date is a leading objective in rice breeding. Many genetic studies have been conducted for QTL mapping of heading date using advanced backcross progeny (Yano *et al*., 2001). Fifteen QTLs, called *Heading date* (*Hd*)*1–Hd3a* and *Hd3b–Hd14*, have been detected by using several kinds of progeny from a cross between *japonica* cultivar 'Nipponbare' and *indica* cultivar Kasalath (Yano *et al*., 2001). Among them, nine QTLs—*Hd1*, *Hd2*, *Hd3a*, *Hd3b*, *Hd4*, *Hd5*, *Hd6*, *Hd8*, and *Hd9*—were mapped as single Mendelian factors (Yano *et al*., 2001; Lin *et al*., 2000; 2003). Detection of QTLs for heading date has allowed further genetic analyses, such as the development of nearly isogenic lines (NILs), analysis of epistatic interactions among QTLs, and map-based cloning. *Hd1* has been found to encode a protein with zinc finger and CCT motifs and to be an ortholog of *Arabidopsis CONSTANS* (Yano *et al*., 2000). *Hd6* and *Hd3a* were found to encode a casein kinase 2 alpha and an *Arabidopsis* FT-like protein (Takahashi *et al*., 2001; Kojima *et al*., 2002). A major QTL, *Early heading date 1* (*Ehd1*), for heading date was detected on chromosome 10 by using a BC_1F_1 population derived from a cross between cultivar T65 and an accession of another cultivated species, *Oryza glaberrima* (Doi *et al*., 1998). Further analysis revealed that *Ehd1* encodes a B-type response regulator (Doi *et al*., 2004). In all cases of QTL cloning for heading date, large-scale linkage mapping was required to narrow the candidate genomic region for the QTLs. These efforts led us to identify functional nucleotide polymorphisms in *Hd1*, *Hd6*, and *Ehd1*. To comprehensively dissect the genetic factors controlling naturally occurring variations in rice flowering, we have performed a QTL analysis using 12 populations derived from crosses of the *japonica* cultivar Koshihikari with cultivars and lines that originate from various regions in Asia. By QTL mapping, several QTLs were detected on the 12 rice chromosomes, some of which were shared among the different cross combinations. The chromosomal locations of these QTLs corresponded to those detected in Nipponbare and Kasalath (Yano *et al*., 2008). However, the allelic

effects of each QTL varied among the parental combinations used, suggesting that a large proportion of the wide range of phenotypic variations in flowering time could be generated by the combination of different alleles of the corresponding QTLs. These genetic and molecular studies have definitely contributed to our understanding of heading date in rice (Izawa, 2007; Tsuji *et al*., 2008).

Much effort has also been paid to the analysis of other complex traits, such as grain size, shattering habit, disease resistance, and environmental stress tolerance. These activities are summarized in the "Gramene-QTL" database (Jaiswal *et al*. 2002). Around 8000 QTLs have been detected so far. Among them, several with major effects have already been cloned by a map-based strategy (Yamamoto *et al*., 2009).

Current Status of Marker-assisted Selection in Rice Breeding

Once genes controlling traits with economic and agricultural interest are cloned or mapped precisely on the respective chromosomes, it becomes possible to use information such as gene sequences and chromosomal locations in breeding programs. Since the paradigm of MAS emerged nearly 20 years ago, much effort has been invested in the practice of MAS. Several examples of the development of NILs with particular traits in elite cultivars have already been reported. Submergence by deep water causes severe stress to rice in Southeast Asia, where flooding occurs during the monsoon season. A major QTL, *Submergence 1* (*Sub1*), was detected near the centromere of chromosome 9 (Xu *et al*., 2000). The underlying gene was cloned (Xu *et al*., 2006), and the *Sub1A* allele was introgressed by MAS into an elite cultivar grown widely in Asia. The resultant lines showed promising performance in yield and other agronomic traits, as well as tolerance to submergence (Neeraja *et al*., 2007; Septiningsih *et al*., 2009). Four QTLs for rice heading date—*Hd6*, *Hd1*, *Hd4*, and *Hd5*—were introgressed from Kasalath into Koshihikari by MAS to enhance the cropping potential of Koshihikari, one of the leading cultivars in Japan (Takeuchi *et al*., 2006). As a

result, NILs of Koshihikari with early and late heading dates have been successfully developed. The size of the introgressed chromosomal segments in those lines was very small: 300 to 600 kb in NILs for *Hd1*, *Hd6*, and *Hd5*. Precise information on the chromosomal locations of the genes allowed breeders to minimize the length of the substituted chromosome segments containing the target QTLs. That study clearly demonstrated the potential power of MAS in rice breeding. Recently, cloning of a durable blast resistance gene, pi21, has been cloned and this achievement led us to solve a linkage drag of pi21, associating with high level of blast resistance and inferior eating quality. Clarification of precise location of pi21 allowed us to develop several markers, which could be used for the dissection between blast resistance and eating quality (Fukuoka e*t al*. 2009). MAS also offers a new concept in breeding: Once NILs with particular economic value are developed, gene pyramiding can be performed by simple crossing between them (Ashikari and Matsuoka, 2006). To develop a new line with lodging resistance and high yield, the combination of two genes controlling semi-dwarfing and grain number were successfully introduced into Koshihikari (Ashikari *et al*., 2005). This concept can also be applied to multiple genes controlling specific traits. Four QTLs controlling partial resistance to rice blast in upland rice have been successfully pyramided into lowland rice cultivars by MAS (Fukuoka and Saka, 2006; Saka *et al*., 2007).

Allele Mining for Rice Breeding

Many successes have been achieved in cloning and MAS of particular QTLs in rice. However, these successes have largely depended on allelic differences. In most cases, the allelic difference was relatively large, allowing reliable determination of the chromosomal location. One major QTL, *Grain number 1a*, was successfully isolated by a map-based strategy (Ashikari *et al*., 2005). This finding contributed both to our understanding of the genetic control of spikelet development in rice, and to MAS to improve grain number per panicle. However,

in general, one major QTL alone is not enough to acquire the level of phenotypic performance needed. To this end, it would be necessary to combine genes with major and minor effects. Owing to the statistical power of detection, it is usually very difficult to detect QTL with minor effects in QTL analysis using F_2 and recombinant inbred lines (RILs). To solve this problem, we developed chromosome segment substitution lines (CSSLs) (Ebitani *et al*., 2005; Takai *et al*., 2007; Ando *et al*., 2008). In these novel plant materials, a particular chromosomal segment from a donor line is substituted into the genetic background of the recurrent line. The substituted segments cover all chromosomes in a whole set of lines. The potential of CSSLs in QTL detection has been demonstrated in many ways. We have established a systematic research flow for exploration and cloning of useful genes (Fig. 1). For example, CSSLs can be used in genetic analysis to associate QTLs with particular chromosomal regions and to quickly develop NILs of target regions containing QTLs of interest. In general, when an association is detected between a chromosomal region and a trait, it is often difficult to validate the QTLs, especially those with very small genetic effects. In such a case, NILs are required in order to analyze genetic effects in detail (Miura *et al*., 2001; Sato *et al*., 2003; Ueda *et al*., 2004). Because CSSLs normally have one chromosomal region substituted, they can be used as NILs themselves or as starting material to develop NILs. Such NILs enable us to combine two or three QTLs in one genetic background in order to clarify epistatic interactions among them (Lin *et al*., 2000; 2003; Yamamoto *et al*., 2000). Furthermore, once we detect significant differences between the CSSLs and the recurrent parental line, comparison of the size of the substituted segments enables us to delineate candidate chromosomal regions of QTLs (substitution mapping). If a significant difference is found between a particular CSSL and the recurrent parent, a large mapping population can be easily produced by a simple crossing of the CSSL with the recurrent parent. Map-based cloning of the QTLs detected can be started quickly by using such plant materials. A simple survey of target traits on the CSSLs allows us to detect

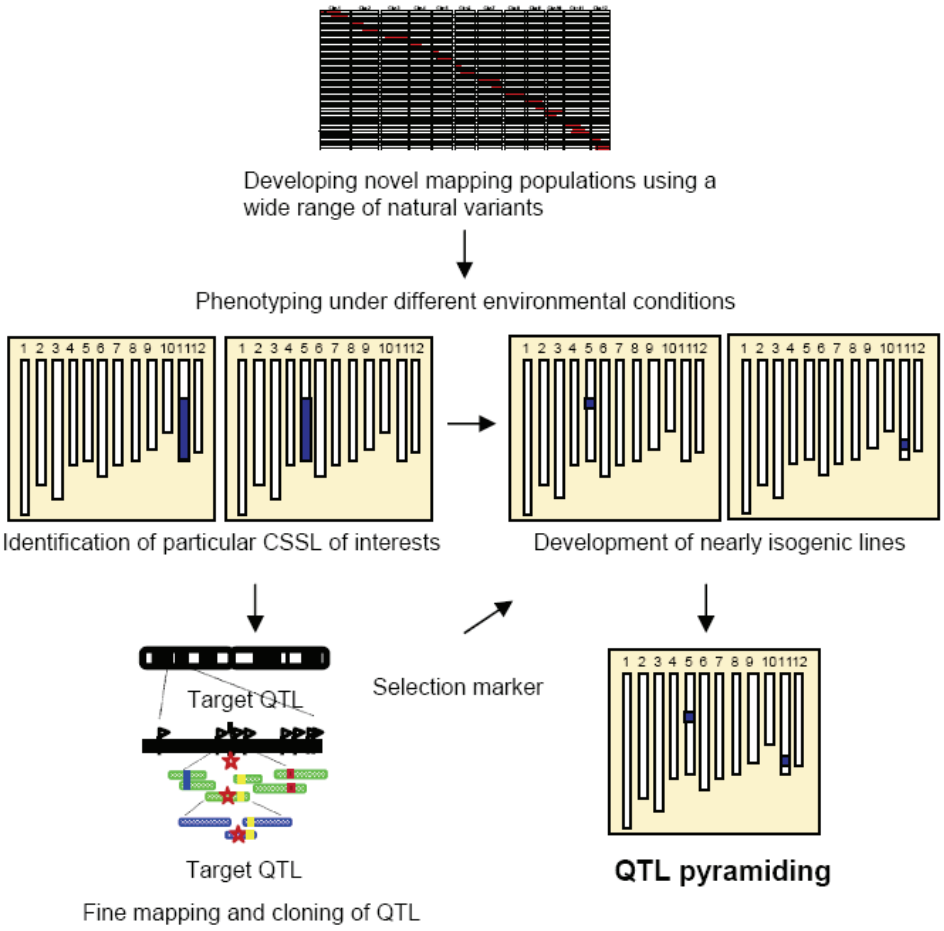

Fig.1 Systematic research flow for the exploration and utilization of natural variations in rice

minor phenotypic differences with reference to the recurrent parent, because there is almost no effect of background genetic noise (i.e., allelic effect of other QTLs). Although the resolution of QTLs in terms of linkage mapping and the power to detect epistatic interactions are not comparable with those given by primary mapping populations such F_2s and RILs, the use of CSSLs can facilitate the discovery of valuable alleles from donor chromosome segments. To enhance the potential of CSSLs, we are now developing CSSLs from a wide range of cross combinations, with Koshihikari as the recurrent parent. Donor parental lines are *indica* and *japonica* cultivars, including some of our core collections (Kojima *et al*., 2005).

Genome-wide Discovery of SNPs and Development of Genotyping Arrays

The genetic dissection of natural variations requires novel and effective genotyping and phenotyping. New technology recently enabled us to perform massive analysis of sequences (Blow, 2007; Hutchison, 2007). This method, called next-generation sequencing technology, has opened new opportunities for polymorphism discovery (Lister *et al*., 2009). In Arabidopsis, this system has been applied to two natural accessions to perform genome-wide single-nucleotide polymorphism (SNP) and insertion/deletion (InDel) discovery (Ossowski *et al*., 2008). This method may provide a unique opportunity to identify nucleotide polymorphisms between genetically closely related strains, such as Japanese cultivars. In addition, a system of simultaneous genotyping of thousands of SNPs on the genome scale has been recently developed (Steemers and Gunderson, 2007). The combination of both new technologies has also facilitated the genetic dissection of complex traits by QTL mapping and whole-genome association studies (Nordborg and Weigel, 2008).

We have detected about 5000 SNPs from our core collection of Asian cultivated rice accessions (Ebana *et al*., 2007). In addition, we have sequenced the whole genome of Koshihikari by using a next-generation sequencing technology, and

have discovered a comprehensive set of SNPs and enhanced the potential power of allele mining among *japonica* cultivars (Nagasaki *et al*., 2008). An enormous amount of genomic sequences, equivalent to more than 20 genomes, has been obtained for Koshihikari, and a consensus sequence of Koshihikari established from the mapped sequences covers nearly 80% of the reference Nipponbare sequences. Comparison of these two sequences has provided more than 60 000 SNPs. These candidate SNPs were validated by an array-based SNP detection system. These SNPs will facilitate the genetic analysis of traits with economic interest among temperate *japonica* cultivars.

From Gene Selection to Genome Selection

The development of a genome-wide SNP typing system allows us to perform large-scale genotyping of recent breeds of rice. We have genotyped a large set of SNPs using landraces, old cultivars, and current leading cultivars (Yamamoto *et al*., 2008). By including their pedigrees, it has been possible to visualize the genotype of the whole genome (pedigree haplotype) of those successions. This analysis provided valuable information on current rice breeding. For example, we can visualize the proportion of the genome derived from Koshihikari, and which chromosomal regions are shared among current leading cultivars. In addition, recombination events that contributed to the improvement of cultivars can also be identified. Previously, MAS has targeted particular chromosomal regions or genes, because DNA markers are genotyped one by one. However, the simultaneous analysis of a large number of SNPs provides a new concept of selection criteria in breeding: It might be possible to perform selection based on a whole-genome genotype image and haplotype images of particular chromosomal regions.

Future Prospects

MAS, developed as an armchair theory about 20 years ago, has now been realized by the accumulation of information on precise chromosomal locations and tightly

linked DNA markers flanking genes with major effects. However, its application to rice breeding has been limited in the precision mapping of genes with minor effects and their introduction into elite cultivars. This limitation is not matter of genomics—the tools and information—but depends on phenotyping methods and plant materials; for example, it depends on how we discover useful genes from diverse germplasm and how we establish reliable phenotyping methods. Integration of all resources, such as tools, analytical methods, and plant materials, will be necessary to facilitate further dissection of complex traits with agricultural importance, such as yield performance, drought tolerance, and grain quality. These efforts will make genomic-assisted breeding more effectively and practically.

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