Review

Germplasm enhancement by developing advanced plant materials from diverse rice accessions

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Genetic dissection of a wide range of naturally occurring variations in rice has significantly progressed by means of quantitative trait locus analysis. This genetic dissection has resulted in molecular cloning of genes and loci with biological and agronomic interest. The success of these analyses depends strongly on the plant materials used. In the last decade, many kinds of plant materials, and particularly advanced backcross populations, have been developed for the genetic analysis of traits of interest. Some of those materials have been deposited in the public domain in order to facilitate further analyses of rice genetics and molecular biology. In this review, we describe how such plant materials, including chromosome segment substitution lines (CSSLs) and introgression lines, could be used in genetic analysis, as well as the kinds of plant materials that could be developed and that are now available to the rice research community. Furthermore, we introduce our current activities related to large-scale development of CSSLs using diverse Asian rice accessions as donors.

Key Words: Chromosome segment substitution lines, introgression lines, natural variations, genetic dissection, map-based cloning, rice breeding.

Introduction

Recent progress in plant genomics has enabled us to dissect naturally occurring variations and has contributed to our understanding of the genetic control of morphological and physiological traits in rice and other crop species. These achievements have depended strongly on the use of several types of DNA markers, including restriction-fragment length polymorphisms (RFLPs) and simple-sequence repeats (SSRs). Decoding the whole rice genome (IRGSP 2005) has further enhanced the potential for designing and developing such DNA markers. In addition to DNA markers, the plant materials used in genetic analysis have played a crucial role in this progress. Together with technical advances in genomics tools, the appropriate use of plant materials is a key point in the molecular cloning of genes that are under complicated control (Fukuoka *et al.* 2010, Yamamoto *et al.* 2009). Therefore, the development of plant materials has been an important task in research on the genetics and molecular genetics of naturally occurring variations in rice.

Despite the importance of plant materials, plant molecu-

lar biologists and physiologists find it difficult to develop advanced plant materials. This is mainly due to the labor-intensive and time-consuming aspects of the development process. Furthermore, naturally occurring variations often exhibit complex inheritance as a result of multigenic control and gene–environment interactions. Therefore, many of these variations have not been easily accessible to plant physiologists and molecular biologists. As a result, these researchers often prefer to use mutants with clear phenotypic differences in particular characteristics. Although such mutants have contributed greatly to our understanding of plant biology and crop improvement, the analysis of naturally occurring variations has also revealed interesting biological phenomena and has contributed greatly to crop improvement (Fukuoka *et al.* 2009, Miura *et al.* 2010, Neeraja *et al.* 2007, Xu *et al.* 2006). Therefore, we must still pay attention to naturally occurring variations and devote a certain amount of effort to the development of plant materials.

In this review, we describe how such plant materials, and particularly advanced backcross populations such as chromosome segment substitution lines (CSSLs) and introgression lines (ILs), could be used in genetic analysis, as well as the kinds of plant materials that could be developed and that are now available to the rice research community in genetics and molecular biology. Furthermore, we introduce our current

Communicated by H. Yasui

Received August 6, 2010. Accepted October 15, 2010.

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activities related to the large-scale development of CSSLs using diverse Asian rice accessions as donors. Although the terms “CSSL” and “IL” are used synonymously with other terms, such as single-segment substitution lines (SSSLs), we have tried to avoid confusion of the terminology by using the terms CSSL and IL differentially to mean accessions of *Oryza sativa* and of other species of *Oryza*, respectively, as the donor lines.

Novel plant materials contribute to the genetic dissection of natural variation

Quantitative trait locus (QTL) analyses have provided a powerful strategy to associate genomic regions with their phenotypic effects in rice (Yamamoto *et al.* 2009). Many QTL analyses have been conducted using F_2 populations and recombinant inbred lines (RILs) (Yonemaru *et al.* 2010). Genetic mapping populations such as F_2 and BC_1F_1 populations are most frequently used in genetic analysis, but have several disadvantages in terms of the reproducibility and reliability of the results. In such populations, the phenotype must be measured in individual plants, without replication. This sometimes compromises the reliability of genetic analyses such as the detection of QTLs. Instead, RILs could be used for QTL mapping; these lines allow replication, thereby providing more powerful tools for use in the genetic dissection of complex traits.

Although F_2 populations and RILs are useful mapping populations for the genetic dissection of QTLs, detection power is often decreased by the nature of the primary mapping population. One major problem with RILs and F_2 populations is the variation that occurs in heading date. In general, progeny derived from a cross between diverse accessions often exhibit wide variation in heading date, particularly as a result of strong transgressive segregation. Several morphological and physiological traits of agronomic interest, such as yield potential (Ando *et al.* 2008), eating quality (Takeuchi *et al.* 2008), culm length (Hori *et al.* 2009), cold tolerance at the booting stage (Takeuchi *et al.* 2001), and source (photosynthetic) ability (Takai *et al.* 2009), are often affected by heading date. Therefore, it may be difficult to precisely evaluate such traits among segregants with a large variation in heading dates.

Another problem is that the segregation of QTLs with major effects often hides the presence of minor QTLs from statistical detection (Ebitani *et al.* 2005, Uga *et al.* 2007, Yano and Sasaki 1997). In the last decade, rice genetics and functional genomics using natural variation have focused on large allelic variation that is detectable even under conditions such as genetic noise in the expression of the traits. To more effectively extract relatively small allelic variations that nonetheless have agricultural value, it will be necessary to use mapping populations with a small range of variation in heading date and a uniform genetic background. Eshed and Zamir (1995) proposed the novel concept of designing plant materials for genetic dissection based on a series of

introgression lines (ILs) with single or few segment substitutions, in tomato, but similar mapping populations have been developed as ILs in *Brassica napus* (Howell *et al.* 1996), as chromosome-substitution lines in *Arabidopsis* (Koumproglou *et al.* 2002), and as CSSLs or ILs in rice (Ebitani *et al.* 2005, Kubo *et al.* 2002). In these lines, researchers substitute one or more chromosome segments from a donor line into the genetic background of the recurrent line. The substituted segments can potentially cover all chromosomes throughout a set of lines. The potential power of these materials in genetic dissection has also been demonstrated (Ebitani *et al.* 2005, Eshed and Zamir 1996, Koumproglou *et al.* 2002, Kubo *et al.* 2002, Zamir 2001).

CSSLs or ILs can be developed by means of marker-assisted backcross breeding. In rice, four or five backcrosses are usually required for the selection of foreground (target) regions and background (untargeted) regions. After these backcrosses, additional two or three self-pollinated generations are required to create lines that are homozygous for all target chromosomal regions. As a result, this process can require more than 6 years, even with the adoption of accelerated generations. When we use CSSLs and ILs with a certain level of variation in the heading date, the range of variation is generally smaller than that of a primary mapping population derived from the same cross combination (Ando *et al.* 2008, Ebitani *et al.* 2005, Uga *et al.* 2007). This may reduce the amount of noise caused by differences in heading date.

Table 1 summarizes the plant materials that have already been developed, such as RILs and CSSLs, and that are publicly available to researchers. These materials have already been or will soon be used in the analysis of traits of interest. They can be obtained through the Rice Genome Resource Center (<http://www.rgrc.dna.affrc.go.jp/index.html>) and the National BioResource Project resource database (<http://www.nbrp.jp/>) or the rice genome database “Oryzabase” (<http://www.shigen.nig.ac.jp/rice/oryzabase/top/top.jsp>). In this issue of *Breeding Science*, other authors describe the development of new CSSLs and ILs (Hirabayashi *et al.* 2010, Shim *et al.* 2010, Yasui *et al.* 2010, Yoshimura *et al.* 2010). Some of these materials can also be obtained from the National Institute of Crop Science (http://www.naro.affrc.go.jp/index_en.html) and the National BioResource Project.

CSSLs and ILs have not been used frequently in the genetic dissection of traits in rice during the past decade (Yonemaru *et al.* 2010). This might be due to the lower availability of such advanced materials. Recently, several studies have involved the development of such materials, and the use of these materials in genetic analysis has increased. However, most studies have developed CSSLs and ILs with target chromosome segments that do not cover the whole genome. Researchers have also selected random substitution lines by screening for a particular phenotypic performance, such as drought tolerance or yield-related traits (Xu *et al.* 2005, Yoon *et al.* 2005). Unfortunately, these materials cannot be used in the genetic analysis of other traits. Xi *et al.* (2006) developed 217 single-segment substitution

Table 1. Mapping populations developed from diverse rice accessions and that are now publicly available to researchers

Cross combination ^a		Type of population ^b	Recurrent parent line	No. of lines	No. of markers	Type of marker ^c	Reference	Availability ^d
Nipponbare (J)	Kasalath (I)	BIL	Nipponbare	98	245	RFLP	Lin <i>et al.</i> (1998)	RGRC
Nipponbare (J)	Kasalath (I)	CSSL	Nipponbare	54	204	RFLP	RGRC	RGRC
Koshihikari (J)	Kasalath (I)	BIL	Koshihikari	182	162	RFLP	Ma <i>et al.</i> (2002)	RGRC
Koshihikari (J)	Kasalath (I)	CSSL	Koshihikari	39	130	RFLP	Ebitani <i>et al.</i> (2005)	RGRC
Sasanishiki (J)	Habataki (I)	BIL	Sasanishiki	85	236	RFLP	Nagata <i>et al.</i> (2002)	RGRC
Sasanishiki (J)	Habataki (I)	CSSL	Sasanishiki	39	166	SSR	Ando <i>et al.</i> (2008)	RGRC
Akiahikari (J)	Koshihikari (J)	DHL	–	210	169	RFLP	Takeuchi <i>et al.</i> (2001)	RGRC
Koshihikari (J)	Nona Bokra (TrJ)	CSSL	Koshihikari	44	45	RFLP	Takai <i>et al.</i> (2007)	RGRC
Nipponbare (J)	Koshihikari (J)	BIL	Nipponbare	79	111	SSR/SNP	Matsubara <i>et al.</i> (2008)	RGRC
Nipponbare (J)	Koshihikari (J)	BIL	Koshihikari	127	111	SSR/SNP	Matsubara <i>et al.</i> (2008)	RGRC
Nipponbare (J)	Koshihikari (J)	CSSL	Nipponbare	41	108	SSR/SNP	Hori <i>et al.</i> (2010)	RGRC
Nipponbare (J)	Koshihikari (J)	CSSL	Koshihikari	48	108	SSR/SNP	Hori <i>et al.</i> (2010)	RGRC
Asominori (J)	IR24 (I)	RIL	–	83	375	RFLP	Tsunematsu <i>et al.</i> (1996)	NBRP
Asominori (J)	IR24 (I)	CSSL	Asominori	98	116	RFLP	Kubo <i>et al.</i> (2002)	NBRP
Asominori (J)	IR24 (I)	CSSL	IR24	70	87	RFLP	Kubo <i>et al.</i> (2002)	NBRP
Kinmaze (J)	DV85 (I)	RIL	–	81	138	RFLP	–	NBRP
Taichung65 (J)	DV85 (I)	RIL	–	100	150	RFLP	Yasui <i>et al.</i> (2010)	NBRP
Taichung65 (J)	ARC10313 (I)	RIL	–	125	107	RFLP	Yasui <i>et al.</i> (2010)	NBRP
Taichung65 (J)	<i>Oryza glaberrima</i> (IRGC104038)	IL	Taichung65	41	71	RFLP	Doi <i>et al.</i> (1997)	NBRP

^a J, TrJ, and I indicate the cultivar groups *japonica*, *tropical japonica*, and *indica*, respectively.

^b RIL, recombinant inbred line; BIL, backcrossed inbred line; DHL, doubled haploid line; CSSL, chromosome segment substitution line; IL, introgression line

^c RFLP, restriction-fragment-length polymorphism; SSR, simple-sequence repeat; SNP, single-nucleotide polymorphism

^d Seeds are available from RGRC, the Rice Genome Resource Center (<http://www.rgrc.dna.affrc.go.jp/index.html>), or from NBRP, National BioResource Project resource database (<http://www.nbrp.jp/>) or the rice genome database “Oryzabase” (<http://www.shigen.nig.ac.jp/rice/oryzabase/top/top.jsp>).

lines (SSSLs) using two *indica* and four *japonica* cultivars as donors in the genetic background of an elite *indica* cultivar, Hua-Jing-Xian 74. However, the derivation of the chromosome segments from different donors was not even, and it was unclear whether coverage of all chromosomes from all donors was achieved in these plant materials. Tan *et al.* (2007) developed 76 ILs of *Oryza rufipogon* in the Teqing genetic background. This series of ILs may cover most of the genome of an *O. rufipogon* accession.

Large-scale development of CSSLs covering the diversity of Asian rice cultivars

In general, parental cultivars can be selected on their phenotype. A wide range of phenotypic variations have been observed among Asian rice accessions. Such variations are a potential source for the improvement of rice cultivars adapted to particular regions and environments. For example, several *indica* and *tropical japonica* cultivars have been selected in the past decade as donor parents for the development of new materials (Ando *et al.* 2008, Ebitani *et al.* 2005, Takai *et al.* 2007).

We selected 10 accessions from a rice core collection to comprehensively characterize the diversity of Asian cultivated rice (Kojima *et al.* 2005) (Fig. 1). We based our selection not on phenotypic differences but on the presence of

sequence variation detected by means of RFLPs and on the accessions' geographical distribution (Kojima *et al.* 2005). The accessions originated from different regions of Asia and belong to three different cultivar groups in three clusters: *japonica* (A), *aus* (B), and *indica* (C) (Fig. 1). Genetic divergence among these accessions has been demonstrated by using QTL analyses for heading date in F₂ populations. Each accession exhibited a different combination of alleles at several known QTLs (*Hd1*, *Hd2*, *RFT1*, *Hd6*, *Ghd7*, and *Hd5*) (Ebana and Yano, unpublished data). This result may support the potential utility of the CSSLs that are now under development. CSSLs are now under development, and most of the plant materials are now at the stage of advanced backcross generations, such as BC₄F₂ or BC₄F₃. A set of CSSLs from these crosses will soon be available for public use. Naturally occurring allelic variation could be systematically analyzed in all chromosome segments from a particular donor line used to generate CSSLs or for particular chromosomal regions obtained from diverse donor lines.

Although some diverse accessions have been used to develop CSSLs in the Koshihikari genetic background, it is obvious that 10 accessions cannot cover the full genetic diversity of rice populations. Unfortunately, dealing with a larger number of donor accessions during the development of the CSSLs would require an impractically large investment of labor and time. Furthermore, we cannot avoid redundancy

Fig. 1. Origins and genetic relationships among the donors used to develop chromosome segment substitution lines (CSSLs). (A) Map of the origin of 10 donors from a rice core collection (filled circles, Koshihikari background) and donors from outside the core collection that were selected on the basis of the presence of agriculturally interesting traits (gray circles, Koshihikari background; open circles, Hitomebore background; dotted circles, Sasanishiki background; cross-hatched circles, IR64 background; horizontally striped circles, Nipponbare background). The reciprocal CSSLs are available for the donors labeled with asterisks. The donors labeled with † are currently available, but others are under development. (B) A dendrogram of the genetic relationships among the 10 donors from the rice core collection. The RFLP data from Kojima *et al.* (2005) were used.

among the CSSLs while increasing the number of donor accessions on the basis on the whole-genome diversity of molecular markers. One practical solution may involve the selection of donors by focusing on chromosomal regions on the basis of haplotypes determined by using DNA markers, thereby maximizing the regional genetic variation among donors. This strategy may be workable because diverse sequence variations, proven by introgression, were frequently observed in relatively small chromosomal regions even among accessions belonging to the same cultivar group (Kojima *et al.* 2005, Shomura *et al.* 2008). Such regional variations in the rice genome may reflect functional variations of the genes located in these chromosomal regions.

Among diverse germplasms, there may be several allelic variants based on differences in the nucleotide sequence that arose during the history of rice domestication and differentiation. Such variants may have functional genetic differences. It is important to discover new alleles with altered function, but it is sometimes difficult to learn the functional differences among alleles from their nucleotide sequences, particularly for single amino acid substitutions and in-frame deletions or insertions. In this case, a CSSL in a particular chromosomal region from diverse accessions would be crucial material for defining phenotypic variation linked to specific sequence polymorphisms. This potential was clearly demonstrated in alleles of the *pi21* rice blast resistance gene: Fukuoka *et al.* (2009) identified 12 variants (haplotypes A to L) in a set of cultivars that represented the range of genetic variation within cultivated rice on the basis of insertion-deletion polymorphisms at three positions in a proline-rich region. A series of CSSLs, each of which possessed one of

the *pi21* haplotypes in the genetic background of a susceptible cultivar, has been developed and their blast resistance has been surveyed. Only the line carrying haplotype L showed improved resistance to blast.

Recent progress in genome-wide discovery of single-nucleotide polymorphisms (SNPs) and large-scale SNP typing have made it feasible to define the haplotype compositions of particular chromosomal regions (Yamamoto *et al.* 2010, Zhao *et al.* 2010). It would be useful to develop a set of CSSLs that would let us compare the phenotypic effects of genes located in these regions.

Advantages and disadvantages of using CSSLs in genetic dissection of complex traits

The potential utility of CSSLs in genetic detection has been demonstrated in many ways. For example, we have established a systematic research flow for the exploration and cloning of useful genes (Fig. 2).

Many studies have been performed to detect genetic factors related to heading date (Yano *et al.* 2001), panicle number (Obara *et al.* 2004), Cd accumulation (Ishikawa *et al.* 2010), root length (Obara *et al.* 2010), leaf photosynthesis (Takai *et al.* 2009), and leaf bronzing induced by saline soil conditions (Takehisa *et al.* 2006). In these studies, QTLs have been detected by using primary mapping populations, such as backcross inbred lines (BILs). The chromosomal locations of the QTLs that were detected have also been precisely determined and their genetic effects have been confirmed by using CSSLs without the need for additional backcrosses.

Fig. 2. Schematic representation of a research flow for determining the utility of plant materials in the genetic dissection of naturally occurring variations. RIL, recombinant inbred line; BIL, backcrossed inbred line; CSSL, chromosome segment substitution line; IL, introgression line; NIL, near-isogenic line.

Genetic interactions are sometimes crucial factors that determine interesting phenomena. CSSLs could be used to obtain proof of these interactions. For example, digenic interactions in hybrid breakdown and leaf bronzing under salt stress have been elegantly demonstrated using CSSLs (Matsubara *et al.* 2007, Takehisa *et al.* 2006). Matsubara *et al.* (2007) performed QTL analysis on culm length and panicle number using BILs from a Sasanishiki $\times$ Habataki cross. Their results suggested that two major factors involved in poor growth are located on chromosomes 2 and 11. Moreover, they speculated that the digenic interaction resulted in poor growth when a plant was homozygous for the Habataki allele at the QTL on chromosome 2 and homozygous for the Sasanishiki allele at the QTL on chromosome 11. To test this hypothesis, they performed genetic analyses using a CSSL in which part of chromosome 2 from Habataki was substituted into the genetic background of Sasanishiki. Eventually, they confirmed the presence of two complementary genes on chromosomes 2 and 11; more recently, the two genes have been identified by using a map-based strategy with plant

materials derived from a cross between Koshihikari and Habataki (Yamamoto *et al.* 2010). This is a clear example of how advanced plant materials such as CSSLs can be effectively used in the genetic and molecular analysis of biological phenomena in rice.

Although genotyping using DNA markers, such as SSR and SNP markers, now requires less labor and time than used to be required, phenotyping of traits often remains labor-intensive. Usually, the primary mapping population is composed of more than 100 plants or lines. This population size sometimes prevents researchers from proper phenotyping of traits. The use of a relatively small number of CSSLs (fewer than 50) offers an advantage in this situation and makes it feasible to conduct more detailed phenotype assays for traits of interest.

The power to detect QTLs with a small effect in CSSLs is higher than that in primary mapping populations, such as F_2 populations and RILs, as mentioned above. However, the mapping resolution in CSSLs may be lower than that in primary mapping populations, because it depends on the size of

the substituted chromosome segments in the CSSLs. However, this disadvantage can be easily overcome by fine mapping of putative QTLs using the CSSLs as base materials. In a primary mapping population, developing NILs for target QTLs is required in order to map QTLs precisely as single Mendelian factors. In contrast, the uniformity of the genetic background of each CSSL enables rapid progress in linkage mapping of the target QTLs.

In addition, the use of CSSLs in genetic analysis may provide new opportunities to enhance rice breeding. In some CSSLs, elite Japanese cultivars such as Koshihikari and Hitomebore were used as the background genotype. Therefore, once a gene with agricultural value is identified by genetic analysis, further breeding, such as the elimination of linkage drag (Fukuoka *et al.* 2009) and the use of gene pyramiding (Ando *et al.* 2008), can be performed immediately using a particular CSSL (Fig. 2).

There are several disadvantages to detecting QTLs in CSSLs rather than in primary mapping populations. First, in contrast to QTL analysis based on a primary mapping population such as F_2 and RIL, QTLs detected in the analysis of CSSLs may contain a high degree of statistical noise, such as false positives or negatives. In ordinary QTL analysis using primary mapping populations, genetic parameters can be estimated on the basis of observed data, and then the parameters can be tested for significance by using a simple likelihood-ratio test. Therefore, type I statistical errors (i.e., false positives) can be more easily excluded. However, the detection of QTL effects in CSSLs must be determined by differences in mean values between a particular CSSL and an isogenic control line by using ANOVA or *t*-tests. This comparison is likely to be more sensitive to environmental noise. Therefore, type I errors may be included to a greater extent. Second, one of the potential risks with CSSLs is that unidentified introgression of small, untargeted chromosomal segments sometimes generates experimental noise that makes it more difficult to detect QTL effects in particular chromosome regions. Even though introgressed segments from donor lines could be identified by genotyping of DNA markers such as SSRs or SNPs that are distributed across all chromosomes, small donor segments are sometimes difficult to identify in the intervals between the DNA markers that are chosen. In such a case, phenotypic differences in the target traits might arise as a result of the unrecognized donor segments. This may occur most frequently in CSSLs developed without using marker-assisted selection during backcross procedures. Progeny testing using subsequent backcrossed populations or using a prior generation of the line in which the region containing the putative QTL is segregating will allow rapid validation and delimiting of the QTL. Third, it may be difficult to detect phenotypic differences generated by a combination of two or more donor alleles in different chromosomal regions. In this case, we recommend adopting a strategy to detect such phenotypic differences by using RILs or BILs first, followed by validation of relevant QTLs by means of crosses between CSSLs or NILs that contain the

respective putative QTLs. The mapping resolution of the respective QTLs can be improved by fine mapping to develop NILs using the CSSLs as base materials.

Integration of advanced plant materials and sequence information

Recently, we developed reciprocal BILs and CSSLs between Nipponbare and Koshihikari, which are closely related *japonica* cultivars. Using these materials, we have uncovered several variations. First, we used BILs to detect QTLs related to heading date (Matsubara *et al.* 2008), culm length (Hori *et al.* 2009), preharvest sprouting (Hori *et al.* 2010), and eating quality (Takeuchi *et al.* 2008). In all cases, we successfully identified novel QTLs with major effects and several QTLs with minor effects. Furthermore, we quickly verified the existence of the QTLs detected in the BILs and their genetic effects using the full set of CSSLs or particular CSSLs.

In these studies, reciprocal materials allowed us to verify the genetic effects of QTL alleles. For example, the QTLs for various components of eating quality were detected at the distal end of the short arm of chromosome 3, and were commonly identified in two BILs of both backgrounds (Takeuchi *et al.* 2008). The Koshihikari alleles at this QTL increased the eating quality. Their genetic effect was also confirmed by developing and analyzing a CSSL in which a relatively large Koshihikari segment from the short arm of chromosome 3 was substituted into the genetic background of Nipponbare, thereby improving its eating quality. In addition, through recent progress in genome sequencing, we have been given a new opportunity to assess naturally occurring variation by means of reverse genetics. More than 80% of the Koshihikari genomic sequences have been decoded by means of next-generation sequencing technology (Yamamoto *et al.* 2010). Simple comparisons of the genomic sequences of Nipponbare and Koshihikari allowed embossing of functional alterations in the genes in particular chromosome regions. Once a candidate chromosome region for a QTL of interest is determined by using a map-based strategy, we can focus on several genes in the region in which functional polymorphisms, such as deletions and amino acid substitutions, have occurred.

Functional identification of the genes can be performed by using reverse approach, from sequence polymorphisms to phenotypic differences. Hence, comparison of the Nipponbare and Koshihikari genome sequences may let us identify target genes characterized by the occurrence of functional polymorphisms, such as insertion/deletions and amino acid substitutions. Once we find a target gene of interest, we can infer the gene's function from existing gene annotation information (Rice Annotation Project 2007) and its expression profile, including tissue and development stage specificity (Jung *et al.* 2008). To clarify phenotypic effects caused by functional sequence changes, we may be able to compare the morphological and physiological phenotypes between the

recurrent line and particular CSSLs in which the target chromosomal region has been replaced with a segment from a donor line. In addition, we may be able to develop overexpression lines (Nakamura *et al.* 2007) or RNAi lines to clarify the function of the target gene. Alternatively, we could select gene-disruptant lines of the target gene using mutant panels with *Tos17* (Hirochika 2010) or T-DNA (An *et al.* 2005, Guiderdoni *et al.* 2007). As many kinds of plant materials have been developed for genetic mapping, and more will be developed, the genomic sequences of several donor accessions can be quickly analyzed using next-generation sequencing technology. This strategy can be applied to other diverse germplasms, not only to combinations of Nipponbare and Koshihikari lines.

Conclusions

Although the development of advanced plant materials such as CSSLs or ILs can require more than 5 years and a large amount of labor, the resulting accessions are crucial tools for identifying the relationships between phenotypic differences and naturally occurring changes in nucleotide sequences. Therefore, we need to pay more attention to developing new materials. Even though we are currently developing a diverse array of CSSLs and ILs, we must continue to develop new types of mapping populations based on new accessions. The advent of new technology that can be used to assay the phenotypic performance of rice plants will let us focus on new accessions with characteristics of interest.

In this regard, several wild relatives of cultivated rice may be interesting gene sources for use in future genetic analysis. Thus far, several populations of ILs have been developed using donor accessions such as *O. rufipogon* (Hirabayashi *et al.* 2010, Tan *et al.* 2007), *O. meridionalis* and *O. glumaepatula* (Yoshimura *et al.* 2010), and *O. glaberrima* (Angeles-Shim *et al.* 2010). To facilitate the development of new materials, generation acceleration systems will be important tools for facilitating the development of new materials. Furthermore, a rapid genotyping system will also be required to minimize the labor involved in the selection of appropriate plant materials. In this regard, a recently developed SNP discovery and typing system should be introduced to facilitate development of these materials (McNally *et al.* 2009, Yamamoto *et al.* 2010). We must also pay more attention to establishing a plant materials center that will be responsible for the maintenance and distribution of these materials.

Acknowledgments

We are grateful for the assistance of all members of the QTL Genomics Research Center (QTL-GRC) and Mrs. N. Ono, Institute of the Society for Techno-innovation of Agriculture, Forestry and Fisheries, who were involved in the development of the plant materials described in this paper. We also thank them for their technical assistance in activities

such as cross-hybridization and genotyping. We are also grateful to the Field Management Division of the National Institute of Agrobiological Sciences for their management of the rice fields. Work at the QTL-GRC was supported by funding (Integrated Research Project for Plant, Insect and Animals Using Genome Technology, QT-1005; Genomics for Agricultural Innovation, NVR-0001) to M. Yano from the Ministry of Agriculture, Forestry and Fisheries, Japan.

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