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Towards the Understanding of Complex Traits in Rice: Substantially or Superficially?

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Abstract

Completion of the genome analysis followed by extensive comprehensive studies on a variety of genes and gene families of rice (*Oryza sativa*) resulted in rapid accumulation of information concerning the presence of many complex traits that are governed by a number of genes of distinct functions in this most important crop cultivated worldwide. The genetic and molecular biological dissection of many important rice phenotypes has contributed to our understanding of the complex nature of the genetic control with respect to these phenotypes. However, in spite of the considerable advances made in the field, details of genetic control remain largely unsolved, thereby hampering our exploitation of this useful information in the breeding of new rice cultivars. To further strengthen the field application of the genome science data of rice obtained so far, we need to develop more powerful genomics-assisted methods for rice breeding based on information derived from various quantitative trait loci (QTL) and related analyses. In this review, we describe recent progresses and outcomes in rice QTL analyses, problems associated with the application of the technology to rice breeding and their implications for the genetic study of other crops along with future perspectives of the relevant fields.

Key words: QTL; near-isogenic lines; chromosome segment substitution lines; marker-assisted selection; map-based cloning

1. Introduction

Rice (*Oryza sativa* L.) is one of the most important food crops in the world. It is the staple of diet for heavily populated Asian countries as well as many African countries in which overpopulation is becoming a critical issue. In spite of the successful introduction of resistance to biotic/abiotic stresses into a variety of rice cultivars that resulted in improved crop yield, benefits of the 'green revolution'[1] will soon be exhausted due to the population pressure. Recent progresses in the genomics of rice plant seem promising to speed up its breeding to yield further improved varieties in a shorter period of time. A major challenge in this technology, however, resides in the development of methodology to associate the genomic data with phenotypes of economical significance.

The engine used for plant breeding is the selection of naturally occurring variants. Since the beginning of agriculture of rice initiated some 10 000 years ago, the traits of rice plant have been continuously improved through the introduction of naturally occurring beneficial alleles by spontaneous and/or artificial crossings. In the last century, systematic plant breeding theory based on modern Mendelian genetics was widely practiced. A limitation in this approach is that even though we can control the resultant phenotypes to a certain extent, mechanisms governing the genetic control of quantitative traits such as crop yield have been only poorly understood and exploited.

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Since the last decade, genetic dissection of 'quantitative trait loci' referred to as QTL has become a common approach, and has created a new paradigm in plant genetics. The notion emerged to understand the genetic basis of various 'quantitative traits' observed in cultivated plants such as crop yield that appeared to vary continuously among cultivars. Since such 'quantitative traits' are generally controlled by cooperatively acting genes of related functions, separate analysis of individual genes did not lead to successful characterization of the observed phenotypes. New methodology to categorically analyze the genetic loci involved in the phenotypes was thus necessary.

In conjunction with the completion of the rice genome sequencing project,[2] QTL analysis has become able to contribute to our understanding of natural variations in rice, thereby producing an enormous amount of information concerning their basis, such as chromosomal location of genes, allelic effects, epistatic interactions and so on. To date, however, such QTL information has not been fully exploited in rice breeding programs due largely to the nature of QTL analysis. Since it is based on statistical analysis, its reliability differs from one QTL analysis to another; in addition, information concerning the interactions with other genes and the effect of environmental conditions is hardly available. In this review, we examine recent progresses in rice QTL analysis, describe the outcomes and problems in its application to current rice breeding and discuss its possible implications toward the analysis of other crops as well as its future prospects.

2. Genetic and molecular dissection of complex traits in rice

2.1. *Brief overview of QTL information through 'Gramene QTL'*

Over the last decade, a large number of QTL have been generated using the linkage map constructed from DNA markers in the rice genome. The limited amount of information that anchors those different DNA marker used in different experiments makes it very difficult to compare the QTL generated by different researchers. In 2005, rice genome sequence has completely become available[2] and the physical positions of numerous QTL could be determined based on the sequence information of flanking markers. This has permitted the development of a more comprehensive QTL database in rice.

Gramene QTL[3] is the most popular rice QTL database owing to its comprehensive collection of QTL information, which can be accessed on a genome browser. In April 2008, *Gramene QTL* annotated 8646 QTL, which were extracted from 247 reports published between 1994 and 2006. These QTL are categorized into nine trait categories (TCs) encompassing 237 characters as subordinate categories. Of the 237 characters, the one representing the largest number of QTL is plant height (categorized into the TC of 'vigor') with 1011 QTL, followed by 'days to heading' with 618 QTL (TC of 'development'), 353 QTL for 'spikelet number' (TC of 'yield'), 330 QTL for 'spikelet fertility' (TC of 'sterility or fertility') and 253 QTL for 'panicle length' (TC of anatomy). Of a total of 8646 QTL, the physical position of 6293 QTL (73%) was determined on specific chromosomes (Table 1). The remaining 27% of QTL could be positioned on the linkage map but not on the physical map mainly due to the lack of physical locations of flanking DNA markers. Twenty-five percent of QTL that have a physical position have been mapped to an interval of >1 Mb, owing to the small number of markers used in the analysis. Nearly 32% of QTL with a physical position could be mapped to an interval of <1 kb on specific chromosomes. Some of these locations should be viewed with caution because in some cases only one linked marker has been described in associated papers; therefore, these positions have not been precisely determined.

A total of 4090 QTL in *Gramene QTL* are physically distributed unevenly on 12 chromosomes (Fig. 1). There is a tendency for a cluster of QTL to occur in particular chromosomal regions. For example, several hundred QTL including 95 QTL related to vigor has been mapped to specific chromosomal region of 40 Mb of chromosome 1. It is known that the *sd1* (*semi-dwarf 1*) gene is located in this region[4] and the variation of plant type originating from *sd1* allelic differences may be detected by several QTL analyses. Similarly, many QTL for yield in the region of distal end of chromosome 1 may be detected by the action of *Gn1a* (*Grain-number 1a*).[5] On the basis of the nature of QTL analysis, it is unavoidable at present to report QTL redundantly. Metric characters such as plant height can be readily measured and have been usually used for the characterization of characters in several QTL analyses. Therefore, it is often the case that different trait characters can be identified as QTL on the same region of a specific chromosome.

On the other hand, very few QTL has been positioned in centromeric regions and in the short arm of chromosome 10. This may be due to the limited number of trials (247 reports) and bias in plant cross-combination that has been used in the QTL analysis. In many different reports, the same populations of recombinant inbred lines (RILs) were used for the analysis of traits. This can generate QTL with unequal distribution on the chromosomes. Furthermore, some QTL have been putatively

Table 1. The number of *Gramene QTL* classified into each trait category

Interval (Mb)	Abiotic stress	Anatomy	Biochemistry	Biotic stress	Development	Quality	Sterility or fertility	Vigor	Yield	Total	Ratio
0.001–0.01	52	153	16	32	48	28	72	139	163	703	8%
0.01–0.1	3	8	4	2	8	4	11	22	19	81	1%
0.1–1	29	83	14	35	53	50	20	88	151	523	6%
1–10	136	313	67	91	195	223	62	343	493	1,923	22%
≧10	23	50	18	6	37	24	8	48	66	280	3%
Not interval[a]	250	420	16	74	326	157	208	618	714	2,783	32%
Not located[b]	592	725	47	236	603	260	311	1,194	1,168	5,136	59%
Total	835	1,332	166	402	944	589	484	1,834	2,060	8,646	
Ratio	10%	15%	2%	5%	11%	7%	6%	21%	24%		

[a]Most data were estimated from only one flanking marker.
[b]No data for the physical position.

determined as loci exhibiting epistatic interactions with other loci or environmental factors. The reliability of these QTL cannot be evaluated simply by information provided by each report. This issue is discussed in more detail later in this review.

In spite of the accumulated experience in QTL analysis and the relatively large number of QTL analyses performed so far, an overview of QTL information in the *Gramene QTL* database reveals that it may be premature to use some of that information in marker-assisted selection in rice breeding. In order to cross-reference QTL information in rice, researchers must pay much more attention to the designation of target traits, population size and the number of markers used in the analysis.

2.2. *Natural variations can be a target of molecular dissection*

In spite of the mixture of different levels of quality in QTL analyses, some of the QTL for morphological and physiological traits have been treated as a single Mendelian factor and cloned by using a map-based strategy (Table 2).[6] These studies have also revealed the kinds of nucleotide changes involved in determining phenotypic differences. The cloning of causal genes/QTL for natural variations has allowed us to understand the genetic control mechanisms of naturally occurring complex variations. The clearest example is the analysis of flowering time, for which five QTL have been cloned: *Hd1*, *Hd3a*, *Hd6*, *Ehd1* and *Ghd7*.[7–11] Recently, *Hd3a* was found to act as a flowering promoter, florigen, which had been predicted previously but had not been proved.[12] This series of studies has contributed to the description of a pathway of flowering time control in rice.[13,14] As a result, this kind of scenario can be applied to other natural variations of agronomical interest. Recent progress in cloning causal genes involving major phenotypic change within natural variation, such as seed shattering, seed size and growth habit, has further elucidated the rice domestication process.[11,15–19] For example, the *qSH1* gene, a major QTL of seed shattering in rice, encodes a BEL-type homeodomain and a single-nucleotide polymorphism (SNP) has been detected in this gene.[15] This SNP, lying in the 5′ regulatory region of *qSH1*, generates a phenotype characterized by the loss of seed shattering. Furthermore, based on the analysis of several old landraces of Chinese and Japanese rice, this SNP might have arisen during the domestication process. It was demonstrated that a deletion of ~1 kb has been a key event in changing from small to large seed during the *japonica* rice domestication process.[19] It was also demonstrated that gene duplication and divergence in the *Sub1* locus, which is involved in submergence tolerance, also have occurred both prior to and after rice domestication.[20] Consequently, the accumulation of several domestication signatures among current and old rice accessions permitted the establishment of a model of the *japonica* rice domestication process.[21]

3. Questions in the genetic dissection of natural variation

3.1. *How do we quantitatively score phenotype?*

Some morphologically distinguishable quantitative traits, such as grain weight, days to heading or plant height, have been evaluated by QTL analysis, but it is sometimes difficult to characterize the phenotype of economically important traits. A typical example is the genetic analysis of yield potential, which is one of the most important traits in agriculture. Rice yield—the total amount of grain harvested—is broadly divided into two quantifiable components, source potential and sink size.[22] Both of these traits

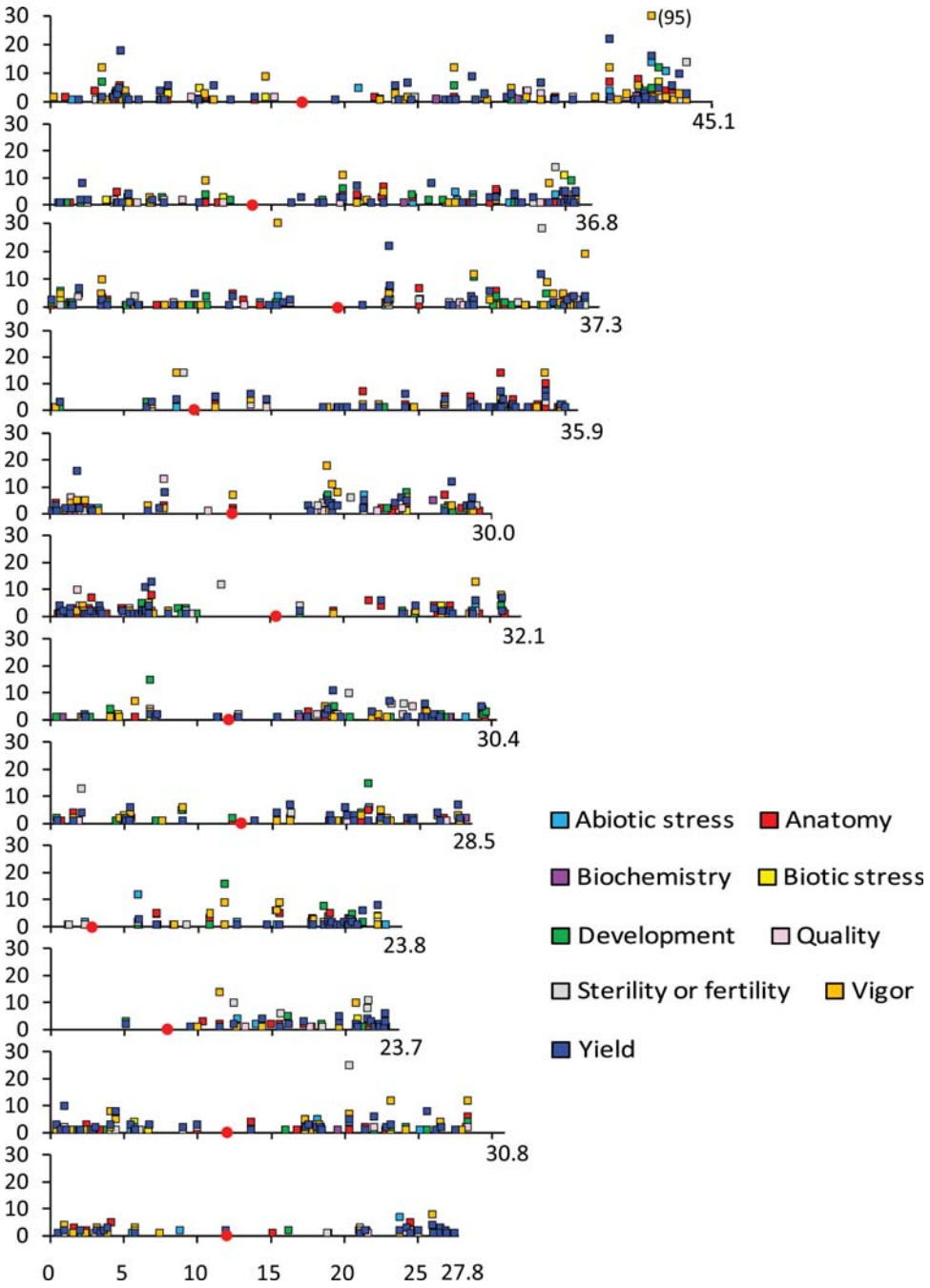

Figure 1. The location of *Gramene QTL* on rice chromosomes. In 6293 *Gramene QTL* with physical position of chromosome segment deduced from the sequences of corresponding DNA markers, 4090 QTL within the interval of 1 Mb or less were only plotted by using the central value of the interval in each TC. *X*-axis is QTL position (Mb) on each chromosome (1–12, from top to bottom). *Y*-axis is the number of QTL. Red circles indicate the position of the centromere on each chromosome. The 95 QTL of 'Vigor' at 40.9 Mb on chromosome 1 was off scale and the value is written in parentheses.

must be determined to characterize the yield potential, but progress on the genetic analysis of source potential is far behind that of sink size.[5,18,19,21] Since source potential, such as leaf photosynthesis or translocation, is physiological traits, scoring them is time-consuming, laborious and likely to be influenced by environmental conditions. Therefore, these traits are still not applicable to QTL analysis, which needs to score large numbers of segregated progeny at the same growth stage.

As another example, general growth habits such as culm length, panicle number, fertility ratio or dry matter weight each measured under drought stress conditions are often used as an index of the degree of drought resistance.[23–27] Chromosomal locations of these QTL occasionally overlap with the previously

Table 2. QTLs with major effects on agronomic traits in rice

Trait	QTL	Chr	Protein and function	Population size[a] (no. of plants)	Candidate region[b] (kb)	FNP[c]	Reference
Heading date	*Hd1*	6	Zn-finger domain and CCT motif	1505	12	Premature stop codon	Yano et al.[7]
Heading date	*Hd3a*	6	FT-like protein	2207	20	Not determined	Kojima et al.[8]
Heading date	*Hd6*	3	Casein kinase 2alpha	2807	26	Premature stop codon	Takahashi et al.[9]
Heading date	*Ehd1*	10	B-type response regulator	2500	16	Amino acid substitution	Doi et al.[10]
Heading date	*Ghd7*	7	CCT motif	1882	2284	38.3 kb deletion	Xue et al.[11]
Submergence tolerance	*Sub1A*	9	Putative ethylene response factor	4022	182	Gene deletion	Xu et al.[101]
Seed number	*Gn1a*	1	Cytokinin oxidase/dehydrogenase	13 000	6.3	Not determined	Ashikari et al.[5]
UVB resistance	*qUVR10*	10	CPD photolyase	1850	27	Amino acid substitution	Ueda et al.[102]
Salt tolerance	*SKC1*	1	HKT-type transporter	2973	7.4	Amino acid substitution	Ren et al.[103]
Seed shattering	*qSH1*	1	BEL-type homeodomain protein	10 388	0.61	Single-nucleotide substitution in regulatory region	Konishi et al.[15]
Seed shattering	*Sh4*	4	Myb3 DNA binding domain and NLS	12 000	1.7	Amino acid substitution?	Li et al.[104]
Growth habit	*PGOG1*	7	Zn-finger domain	3051	14	Amino acid substitution	Tan et al.,[16] Jin et al.[17]
Seed length	*GS3*	3	BEBP-like domain, TNFR/NGFR family cysteine-rich domain, YWFC module	1384	7.9	Premature stop codon	Fan et al.[105]
Seed width	*GW2*	7	RING-type protein with E3 ubiqutin ligase activity	6013	8	Premature stop codon	Song et al.[18]
Seed witdth	*qSW5*	5	Unknown	4501	2.2	1212 bp deletion	Shomura et al.[19]
Low temperature germinablity	*qLTG3-1*	3	Unknown, GRP and LTP domains	3200	4.8	71 bp deletion	Fujino et al.[71]
Regeneration ability	*PSR1*	1	Ferredoxin-nitrate reductase	3800	50.8	Not determined	Nishimura et al.[106]

[a]No. of plants used in large-scale linkage mapping.
[b]Physical distance of candidate genomic region defined by mapping.
[c]Functional nucleotide polymorphism causing alleleic difference between parental lines.

reported QTL for growth habits such as 'days to heading' (flowering time) or 'culm length' examined under normal conditions. We may detect only a QTL of drought avoidance even though we hope to find a QTL involving drought tolerance. So far, crop physiologists and geneticists have attempted to evaluate drought tolerance by measuring root morphology,[28,29] but this might be a part of key factors in the trait in preventing drought stress. Scoring the phenotypic differences of non-morphological traits is a crucial issue for QTL analysis. Recent progress in plant physiology and biochemistry concurrently with the development of novel analytical tools will facilitate the investigation of target traits (see section 4.3).

3.2. *QTL that have minor effects remain poorly investigated*

QTL associated with complex traits have been identified and have already delivered crucial information to aid our understanding of natural variations. In general, a large number of QTL have been detected mainly by using primary mapping populations such as F_2, RILs or backcross inbred lines (BILs). One of the major questions in QTL analysis is whether we can detect all QTL including those with minor effects. On the basis of the statistical nature of QTL analysis, some bias in the results are expected due to 'noise' inherent in the complex biological and environmental systems involved. We must consider that QTL associated with minor effects might not be detected in the analysis using primary mapping populations (false negatives). In addition, we must also recognize and define false positives.

The QTL analysis of 'heading date' is a good example of the difficulty of identifying QTL with minor effects. In primary QTL analysis using F_2 populations between two varieties, 'Nipponbare' and 'Kasalath', five QTL have been detected.[30] Further analysis using backcrossed progeny derived from the same cross-combination revealed at least 10 additional QTL.[31] Some of these QTL are found to be masked by the QTL with relatively large phenotypic effects or an epistatic interaction.[32,33] The trait of 'spikelet number per panicle' is another example. Nagata et al.[34] estimated that 4 QTL controlled the number of 'spikelet per panicle' by using BILs derived from the cross between 'Sasanishiki' as a recurrent and 'Habataki' as a donor. Since then, Ando et al.[35] have developed chromosomal segment substitution lines (CSSLs), which are a series of lines covering entire chromosomal regions of 'Habataki' dividing it into small segments but on the genetic background of 'Sasanishiki'. They identified not only the same 4 QTL but also an additional 12 QTL with relatively small phenotypic effect. One of the common QTL located on chromosome 1 may correspond with *Gn1a*,[5] but this did not fully explain the phenotypic difference between the parents. This example clearly demonstrates the importance of finding QTL with small effect to understand the genetic basis of spikelet number.

3.3. *Epistatic and environmental interactions: easy to estimate but hard to confirm*

To thoroughly understand the genetic control of complex traits, the important issues to be clarified are the epistatic interaction among the genes involved [genotype by genotype ($G \times G$) interaction] and the interaction between gene expression and environmental conditions [environmental ($G \times E$) interaction]. Statistical analyses such as two-way ANOVA and multiple regressions have been often used to reveal $G \times G$ interactions mainly on yield-related traits including heterosis, by using primary mapping populations.[28,36–46] These analyses undoubtedly have provided a clue to understand the complex nature of genetic control mechanisms. Note that very few of the indications of such interactions have been proved by further analysis. It has been sometimes reported that a large number of epistatic interaction pairs distributed whole genome were observed. For example, 195 and 328 digenic interactions were detected in the *indica/japonica* and *indica/indica* combinations, respectively.[47] Although some of these interactions may be important, it is possible that many false positives are also being included. In general, 100–300 plants or lines have been used in most of the QTL analyses reported. So is the presence of false positives caused by the bias inherent in a small population size, by experimental errors in phenotyping or by some other cause? How do we define a real digenic interaction? This may be particularly true in the case of interaction involving more than three loci. A large primary mapping population (e.g. >500) may be required to avoid the generation of false positives. Again, note that it is very hard to distinguish between real interactions and experimental noise in statistical analysis.

Meanwhile, estimation of the $G \times E$ interaction needs to be clarified in order to use in a breeding program significant alleles revealed by QTL analysis. $G \times E$ is frequently evaluated by year–year and/or location–location variations using mapping populations that are available for replication, such as doubled haploid lines (DHLs), RILs or BILs. Since an indirect methodology of QTL mapping with a 'QTL by environment' (QE) interaction was proposed,[48] many examples of chromosomal regions involving $G \times E$ have been reported for rice.[43,49,50] The *Gramene QTL* database[3] handles all locations detected

with interaction effects as independent QTL. The largest entry contains 611 QTL information sources,[43] which may imply that some epistatic pairs are possibly false positives. To minimize the risk of such false positives, rigorous statistical conditions using repetitive experiments in one environment should be employed in the experimental design.[51]

3.4. *A proposal: confirmation is more important than estimation*

Above we have pointed out that there are three problems that must be solved for a better understanding of the genetic dissection of natural variation in rice: (i) trait evaluation; (ii) detection of minor QTL and (iii) G × G and G × E interactions. On the basis of the nature of the analytical methods including the statistics used in QTL mapping of primary populations, it might be difficult to solve these problems. One practical solution is to design and develop plant materials with a uniform genetic background for validation of the results obtained in the primary analysis. Basically, advanced backcross progeny would be used to prove the genetic effect of major QTL.[52] This type of validation of a genetic effect can be performed on QTL with minor effects. The use of CSSLs, single-segment substitution lines (SSSLs) or introgression lines (ILs) will be a practical solution for trait evaluation and detection of minor QTL.[35,53–57] As described in section 3.2, Ando et al.[35] demonstrated the power of CSSLs to find QTL that have a minor effect. However, CSSLs cannot estimate candidate pairs of G × G interactions because they do not have enough genotype combinations for any two loci. To overcome this disadvantage, the combined use of a primary population (F_2s, RILs, DHLs) and a backcrossed population (CSSLs, SSSLs, ILs) is recommended. Successful proof should be obtained by evaluation of the phenotypic difference of both near-isogenic lines (NILs) of the QTL and their combined line after estimations of G × G by primary population.[58,59] Because CSSLs or ILs normally have one chromosomal region substituted in the recurrent genetic background, they can be used as NILs themselves or as starting material to develop NILs. These types of plant materials are particularly useful to discriminate between real QTL and false positives, and to prove G × E interactions.[60–62]

Figure 2 illustrates the roles of a primary population, such as F_2 or RIL, and a backcrossed population, such as CSSL or NIL. Putative QTL, G × E and G × G interactions are statistically estimated from a primary population. These QTL can be validated based on phenotypic differences between the recurrent parent and the CSSL whose substitution segment corresponds with the detected QTL region in the primary analysis. Moreover, G × E or G × G interactions can be validated by evaluation of phenotypic performance of the CSSLs. Finally, the information integrated from these analyses allows us to proceed to further studies, such as map-based cloning for biological characteristics or marker-assisted breeding. We are developing several kinds of CSSLs on the *japonica* rice genetic background; we select donor cultivars based on their geographical distribution in the world. Although the process of development is laborious and time-consuming, the resulting CSSLs will contribute to exploration of new economically important alleles as well as clarifying genetic control of natural variation.

We propose that the best route forward is to identify QTL at the molecular level and to check their expression in each QTL combination. In the case of flowering time, which is one of the most successfully advanced traits in rice quantitative genetics, knowledge of the identified QTL is being integrated in order to construct the relevant gene network.[13,14] Map-based cloning of the QTL is currently becoming one of the standard strategies as a first step of this kind of work. Application of the developed CSSLs will provide a route to accelerate this process.

4. Rice QTL for practical use

4.1. *Rice varieties developed by marker assisted selection*

Once the genes controlling traits with economical and agricultural interest have been identified, there will be opportunities to utilize the resulting information such as the DNA sequence and chromosomal location of these genes in breeding programs. Since the paradigm of marker-assisted-selection (MAS) has emerged, a great deal of effort has been invested in the practice of MAS. Several examples have already been reported for the development of NILs with particular traits in elite rice varieties. For example, submergence by deep water causes severe stress to rice in south-east Asia, where flooding occurs during the monsoon season. A major QTL, *Submergence 1* (*Sub1*), was detected near the centromere of chromosome 9 and was eventually cloned (Table 2).[63,64] When the *Sub1A* allele was introgressed by MAS into an elite cultivar widely grown in Asia, the resultant lines showed promising agronomic performance in yield and other agronomic traits, as well as tolerance to submergence.[65,66] Heading date is an example where a series of NILs with different heading dates were developed in the genetic background of an elite cultivar, 'Koshihikari', in Japan.[67] In these cases, relatively small chromosome segments (~600 kb) were introduced by MAS. A further example is insect

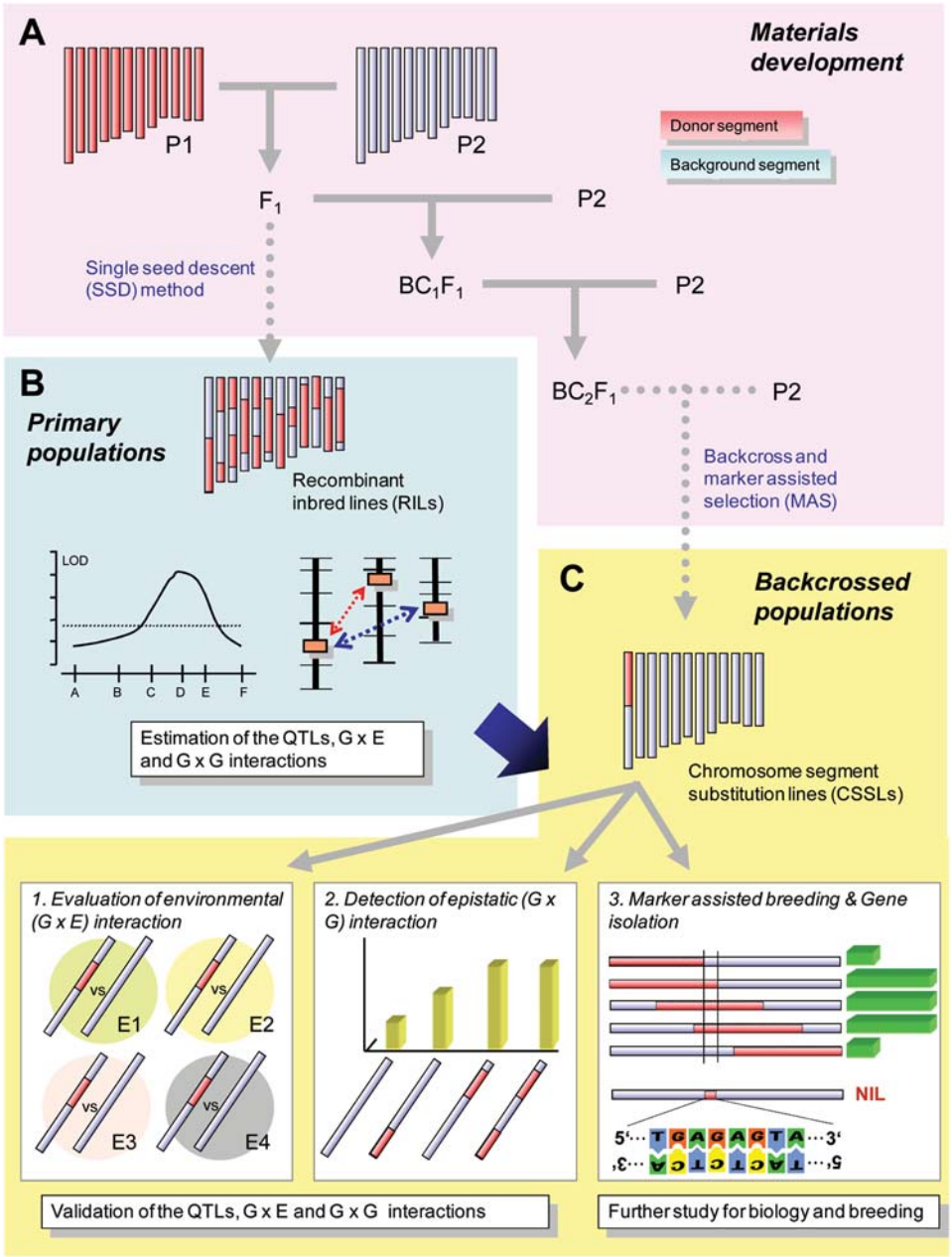

Figure 2. Scheme for the use of primary and backcrossed populations to investigate genetically complex traits in rice. (A) Two kinds of experimental populations—primary populations such as RILs and backcrossed population such as CSSLs—can be developed by crossing between a donor parent (P1) with trait of interest and a recurrent parent (P2) such as a representative cultivar. (B) Putative QTL, genotype by environmental interaction (G × E) or genotype by genotype (epistatic) interaction (G × G) are statistically estimated by QTL analysis of primary population. (C) A series of CSSL contribute to validation of the estimated QTL, G × E interaction (C1), G × G interaction (C2) and to fine mapping of the QTL followed by marker-assisted breeding and gene isolation (C3).

resistance, one of QTL controlling brown plant hopper resistance, which has been identified in a wild relative of rice, *Oryza officinalis*. This QTL has been introduced into the elite Japanese cultivar, 'Hinohikari', by MAS (Hirabayashi et al., unpublished data). In these MAS, it is necessary not only to perform background selection by DNA markers distributed in non-target chromosomal regions, but the size of the introgressed chromosome segment should be minimized to avoid unfavorable trait association (linkage drag). To accelerate the effectiveness of MAS, the precise map location of target QTL must be determined and several flanking markers developed.

MAS also offers a new concept in plant breeding. Once NILs representing a particular trait of economic value are independently developed, gene pyramiding can be performed by simple crossing between such NILs.[68] In order to develop a new line with lodging

resistance and high yield, the combination of two genes controlling the characteristics, 'semi-dwarf' and 'number of grain' was successfully introduced into 'Koshihikari'.[5] This concept can also be applied to multiple genes controlling specific traits. Four QTL derived from upland rice, control a partial resistance to rice blast and have been successfully pyramidized into lowland rice cultivars by MAS (Fukuoka et al., unpublished data).

4.2. *Genetic dissection of natural variations observed among closely related cultivars*

In general, performance in QTL analysis and map-based cloning is strongly dependent on sequence difference between two parental lines. In fact, most of the QTL listed in Table 2 have been detected and cloned by using distant crosses, such as *indica* and *japonica*. On the other hand, there is considerable phenotypic variation between *japonica* cultivars.[69,70] These variations between closely related cultivars have often been major targets for selection in rice breeding programs. This is particularly true in the breeding of cultivars adapted to regional environmental conditions. As a result, these natural variations are important targets for genetic and molecular dissection. Given this, it has been difficult to detect and clone QTL controlling variations among closely related cultivars, owing to the lack of informative DNA markers. The completion of whole-genome sequencing has dramatically changed this situation. It has allowed us to identify effectively microsatellite motifs and convert them to simple sequence repeats (SSR) markers.[2] This situation has allowed us to perform mapping of QTL using progeny derived from a cross between genetically closely related cultivars. Fujino et al.[71] cloned the QTL *qLTG3-1* for low temperature germinabilty using progeny between temperate *japonica* crosses. This is the first example of the molecular cloning of genes identified between genetically related cultivars. Recently, the use of SSR markers facilitated the detection of QTL with complex inheritance. For example, QTL controlling grain quality[72,73] and eating quality (preference)[74–76] among temperate *japonica* rice have been effectively detected by the combination of SSR genotyping and phenotyping of their RILs. Again, these phenotypic differences are current targets in rice breeding in Japan. Therefore, these techniques of genetic dissection and eventually molecular dissection will contribute not only to the understanding of genetic control of those traits, but will also facilitate MAS in rice breeding.

4.3. *Impact of technological innovation*

In the genetic dissection of natural variations, novel and effective genotyping and phenotyping need to be employed. For genotyping, recent innovations in sequencing technology have enabled us to perform sequence analysis on a massive scale.[77,78] This so-called next-generation sequencing technology has opened new opportunities for polymorphism discovery in the rice genome.[79] In Arabidopsis, this system has been applied to two natural accessions to perform genome-wide SNP and InDel discovery.[80] This method is expected to provide unique opportunities to identify nucleotide polymorphisms between genetically closely related strains, such as Japanese cultivars. Furthermore, the simultaneous genotyping thousands of SNPs on a genome-wide scale has also been recently developed.[81] The combination of these two technologies has facilitated genetic dissection of complex traits by QTL mapping and whole-genome association studies. So far, most QTL that have been identified are morphological traits, such as heading date, plant height, seed size, grain number and so on, which can be scored easily. Identification of novel QTL depends on the application of novel phenotyping methods. To further mine natural variations in rice, phenotypic performance should be measured by different and unique methods. Recently, several studies revealed that allelic differences at QTL are the result of subtle variations in the activity of plant hormones such as gibberellic acid and cytokinin in particular tissues. These variations affect plant height and seed number, which contribute to yield performance[4,5] Of particular interest will be an understanding of the genetic factors involved in causing these variations in the levels of plant hormones. Recently, simultaneous surveys of different plant hormones and their derivatives have been established.[82] Therefore, quantitative variations in a series of plant hormones can be a direct target for QTL analysis. In addition, recent progress with a high throughput monitoring system allowed us to reveal naturally occurring variations in metabolite profiles in plant and this approach could also be a target for QTL mapping in Arabidopsis and tomato.[83,84] In rice, a core collection has been subjected to metabolite profiling: metabolite levels in seed between accessions varies considerably.[85,86] Therefore, genetic dissection by QTL analysis can be applied to the rice metabolite profiles. This approach will provide a unique opportunity to understand more thoroughly naturally occurring variation and to improve rice cultivars.

5. How rice QTL contribute to functional analysis of genes in other cereals

Conservation and colinearity in genome structure in cereals have been recognized for nearly 20 years.

Using genetic markers, such as restriction fragment length polymorphism markers, syntenic correspondence of chromosomal regions between wheat, barley and rice has been identified.[87–89] Devos and Gale[90] established the cereal genome circle, which clearly illustrates the ancestral genome and the evolutional relationship of chromosomal regions between cereals. In this regard, the genome sequence of rice has contributed greatly to the structural and functional analysis of the genomes of other cereals. As the amount of information on the genes and QTL associated with natural variations has increased for rice, a critical question is whether this information can be transferred to the analysis of gene function in other cereals. Since the map location of many QTL has been determined in rice and other cereals, several studies have predicted orthologous relationships among corresponding genes in other species.[91,92] However, few proofs of these relationships that have been published so far. One case in particular is the analysis of flowering time in cereals. Comparative QTL studies in cereals have advanced using the knowledge we have accumulated in rice and in the highly studied Arabidopsis plant.[93–95] QTL cloning of rice has enhanced our capacity to find candidate ortholog genes in other cereals. Comparative QTL studies for forage grasses will present technical challenges because of their large genome size and the difficulty of molecular dissection due to their allogamous nature. The QTL for flowering time, disease resistance and sterility could be targets of comparative QTL studies.[96,97] As yet, there is no clear evidence that such a scenario of gene discovery can be extended to traits with agricultural importance, such as yield performance, environmental stress tolerance and other morphological characteristics of agronomical interest. Despite a large number of studies on complex traits of agronomical importance, very limited numbers of genes have been identified at the molecular level. Once we have the sequence of target QTL in rice, then this information can be transferred to other cereals by identifying and cloning their orthologs. Then comparative studies, such as linkage mapping of those genes and confirmation of co-localization of putative QTL of interest in other cereals, can be performed. The existing approximate chromosomal location of rice QTL can provide a pointer toward a syntenic relationship with corresponding target traits in other species, but further study cannot be done until each relevant QTL is mapped and cloned. In addition, phenotypic traits of agronomical importance such as yield performance and environmental stress tolerance are controlled by a complex network of multiple genes. Therefore, it is not necessary to share the same genes to generate natural variations. In fact, since *Hd1*, the rice ortholog of the Arabidopsis *CONSTANS* (*CO*) gene, has been isolated as a major QTL controlling heading date,[7] several studies have been performed to characterize the *CO*/*Hd1* ortholog relationship in other cereals such as wheat, barley and maize.[98–100] These studies demonstrate that the ortholog of *CO* in these cereals might be involved in genetic control of photoperiodic flowering. Interestingly, no clear evidence has been demonstrated to prove that such orthologs are involved in natural variations in flowering time in these species. This may imply that conservation in genetic control mechanisms of particular traits does not necessarily imply conservation in the contribution of orthologs to generate natural variation between cereals.

Although there is a strong potential contribution, it may be premature to apply rice QTL information to the comparative analysis of gene function of cereals. To make comparative analysis most effective, more comprehensive understanding of rice complex traits and the identification of genes involved in natural variations of agronomical importance must be achieved.

6. Conclusions

Remarkable progress has been achieved in the genetic and molecular dissection of naturally occurring variations in the last decade. Several QTL with major effects have been introgressed into modern rice cultivars by MAS. In addition, some of the QTL involved in sink size and plant statue have been already cloned; therefore, it is expected that these findings will increase the probability of modulating and enhancing cloned genes at the molecular level, thereby increasing yield potential. However, natural variations generated by these major QTLs have already often been recognized and properly utilized by breeders in conventional breeding. It will be necessary to discover new alleles for the improvement of current rice cultivars. In general, traits with relevance to rice breeding, such as yield performance and stress tolerance, have been found to be controlled by minor QTL. We propose that the major research focus in this area should be in the unused and unrecognized variations that have occurred after domestication. QTL with minor effects are often hidden by a large phenotypic variation of major QTL. This may be due not only to the complexity of genetic control but also to the reliability of the evaluation of phenotype. In order to overcome these difficulties, it will be necessary to design and develop appropriate plant materials and to apply phenotyping methods with high reliability. Furthermore, genome-wide genotyping using SNP typing arrays should provide a

more robust analytical method to analyze such complex traits. Therefore, the integration of all resources, such as the tools and analytical methods already discussed and plant materials, will be necessary to facilitate further dissection of the complex traits of agricultural importance. We have made considerable progress already, but it is essentially the starting point for a deeper understanding of naturally occurring variations and their utilization in rice breeding.

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