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Toward the mapping of physiological and agronomic characters on a rice function map: QTL analysis and comparison between QTLs and expressed sequence tags

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Abstract We have constructed a rice function map by collating the results on quantitative trait loci (QTLs) for 23 important physiological and agronomic characters (including 13 newly measured traits) obtained using backcross inbred lines of *japonica* Nipponbare×*indica* Kasalath. Using these materials, The Rice Genome project (RGP) has developed a high-density genetic map. QTLs controlling yield did not overlap with those controlling the morphological and physiological traits supposed to relate to yield, such as photosynthetic ability. This result suggests that these traits do not influence yield, at least in this genetic background and environment. QTLs controlling yield also did not overlap with the structural genes controlling carbon metabolism (*rbcS*, cytosolic or plastidic *fructose-1,6-bisphosphate*, *R-enzyme*, and *sucrose synthase*). The combination of a function map and results from the RGP can be advantageous. The utility of this map is discussed.

Keywords EST · Rice · *Oryza sativa* L. · Quantitative trait loci (QTLs)

Introduction

The relationships among various traits (yield, yield components, photosynthetic ability, leaf area, plant height, plant type, and the length of the senescence period of the flag leaf) have been studied mainly by statistical methods. For example, a high correlation was observed between photosynthetic ability and grain yield (Cook and Evans 1983). However, analyses of photosynthetic ability have confirmed that large genetic variation exists between rice cultivars (Osada and Murata 1965; Takano and Tsunoda 1971), and new cultivars are not always better than old ones with respect to these traits (Takeda et al. 1983).

Recent progress in the generation of a molecular genetic map and markers has made it possible to map individual genes associated with complex traits, called quantitative trait loci (QTLs) (Tanksley 1993). Molecular tags labeling QTLs can provide information on the linkages between QTLs, making it possible to analyze the genetic basis of the association between traits. Analysis of linkage relationships among traits is feasible not only at the genetical level but also at the physiological level (Koornneef et al. 1997; Prioul et al. 1997). QTL analysis allows a comprehensive analysis of the genetic relationships among morphological and agronomic traits. In contrast, relatively little information has been so far reported on QTL analysis of physiological traits.

The next important step in QTL analysis is to clarify the relationships of QTLs to candidate genes from expressed sequenced tags (ESTs) in order to understand the effects of the QTLs in terms of the underlying molecular mechanisms. QTLs can be used to determine the most likely candidates among various genes that control a trait. Using such an analysis, Prioul et al. (1997) showed that the gene *Sh1* was more likely than other candidates to control the sucrose or hexose content of maize leaves.

Using backcross inbred lines of Nipponbare×Kasalath, the Rice Genome Project (RGP) has developed a high-density genetic map with 833 ESTs (Kurata et al. 1994; Harushima et al. 1998; Lin et al. 1998). RGP and the International Rice Genome Sequencing Project will determine the genome sequence of Nipponbare as a universal model for rice within the next decade (Sasaki 1998). A partial genome sequence is already available (http://rgp.dna.affrc.go.jp/).

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The aim of the study reported here was to make a function map, locating as many agronomic, morphological, and physiological traits as possible, to advance studies further than is possible by independent QTL analysis alone. With this map, we attempted to analyze the relationships among yield and various traits and to analyze ESTs of the structural genes concerned with carbon metabolism. We discuss the utility of this map.

Materials and methods

Plant material

A *japonica* rice variety, Nipponbare, was crossed with an *indica* variety, Kasalath. Ninety-eight BC_1 plants were obtained by crossing the F_1 offspring with Nipponbare, and there were subsequently inbred by single-seed descent. These lines had restriction fragment length polymorphism (245) RFLP markers (Lin et al. 1998). Ninety-eight BC_1F_7 lines and their two parental lines were sown on 6 May 1997. Forty seedlings per line were transplanted into two rice fields in Tsukuba, Japan (lat 38°N), on 5 June where they were grown under natural conditions in a randomized complete design with two replicates.

Traits

At 5 and 25 days after heading, flag leaves were harvested at around noon (about 7 h after sunrise) on a sunny day. Leaves were immediately frozen in liquid nitrogen and stored at –80°C before use. The frozen leaves were divided into several parts and used for several biochemical analyses, as described below.

The contents of ribulose bisphosphate decarboxylase (Rubisco), soluble protein, and chlorophyll (Chl.), and the ratio of Chl.-*a* to Chl.-*b* (Chl.*a:b*) were analyzed at 5 days after heading. The decreased Chl. content was analyzed from the differences between leaves at 5 and 25 days after heading. The amounts of soluble protein and Rubisco were determined according to Makino et al. (1986), with some modifications. Frozen leaves were ground in liquid nitrogen to a fine powder and suspended in a buffer containing 100 m*M* NaH_2PO_4 (pH 7.0), 1 m*M* phenylmethylsulfonyl fluoride, 1% (w/v) insoluble polyvinyl polypyrrolidone, and 1% (v/v) β-mercaptoethanol, and then centrifuged at 9,800 *g* for 5 min. The soluble protein concentration in the supernatant was determined by the method of Bradford (1976) with bovine serum albumin as a standard. Soluble proteins (2 µg) were subjected to SDS-PAGE using 12.5% (w/v) gels containing 0.1% (w/v) SDS, and the gels were stained with Coomassie brilliant blue. The intensity of the band corresponding to rubisco was measured with an image analyzer (Umax Technologies, Calif.). Chl. content was measured by the method of Arnon (1949).

Photosynthetic capacity was estimated from Rubisco content at 5 days after heading divided by leaf area, according to Makino et al. (1985). For carbon isotope analysis, grains were oven-dried at 80°C for 2 days, and δ-[^{13}C] values were determined according to the method of Samejima (1988) with a mass spectrometer (Finnegan MAT, San Jose, Calif.).

The main crop was harvested at maturity, and stems were cut to a 15-cm stubble height. Ratooning ability was determined at 30 days after harvest. Plant heights were measured twice, once at 50 days after planting for the middle of the growing phase (height 1) and again at 80 days to obtain the maximum length of the plants (height 2). At 80 days after planting, the height of the stem at the second leaf from the flag leaf (–2 leaf height) was measured, and stem numbers were counted. The area of the flag leaf was measured with a leaf area meter (Li-Cor, Neb.), and leaves were oven-dried at 80°C for 2 days and weighed. The specific leaf area (SLA) was calculated as leaf area/dry weight. From plant height, width, and stem numbers, the occupied space of plants (space) and the space per stem were calculated as follows: space=1/3 (width/2)^{2}plant height; space/stem=space/stem numbers. The means of ten replications per line were used in the data analysis for each trait. Means and descriptive statistics were generated with SAS (SAS Institute 1988).

QTL analysis

Chromosomal locations of putative QTLs were determined by single-factor analysis (one-way ANOVA) with the general linear model procedure of QGENE (Nelson 1997). In the inbred lines used, a probability level (*P*) of 0.01 was used because it is difficult to detect QTLs using a high threshold in smaller populations (Yano and Sasaki 1997; Lin et al. 1998). We used this threshold ($P \leq 0.01$) for comparisons among traits. Two-way ANOVAs for epistatic interaction were also carried out with QGENE at this threshold. The tests were done only with marker loci linked to QTLs with a single-factor *P* of ≤0.01 to reduce the likelihood of committing a type-I error (Davies et al. 1999). Lin et al. (1998) detected QTLs affecting seed dormancy and heading date. Kurata et al. (1994) reported the locations of ESTs (http://rgp.dna.affrc.go.jp/). Takahashi et al. (1998a, b) reported cytosolic or plastidic *fructose-1,6-bisphosphate* (*FBPase*). Nakamura et al. (1996) reported *R-enzyme*.

Results

Correlation between traits

There was a significant correlation between panicle number per plant and yield (r=0.303, P<0.001). There was a high correlation between plant height 1 (middle of growing phase) and plant height 2 (maximum growth phase) (r=0.760, P<0.0001). Among the physiological and morphological traits, there was a significant correlation between yield and decreased Chl. content (r=0.240, P<0.05), Chl.*a:b* (r=0.303, P<0.01), and δ-[^{13}C] (r=–0.270, P<0.01).

QTL detection

Eighty-seven QTLs controlling 23 traits, including existing agronomic traits and 13 newly measured traits, were placed on the function map (Fig. 1; Table 1). Only 1 QTL for photosynthetic ability was detected (Fig. 1). Six QTLs were detected for Chl. content and accounted for 0.534 (r^2) of the total phenotypic variation. Four QTLs for decreased Chl. content were found, with the Kasalath alleles increasing this trait for all detected QTLs. One QTL was mapped for the ratio of Rubisco to protein on chromosome 2. Three QTLs for the ratio of Rubisco to Chl. content were detected. Two QTLs were mapped for Chl.*a:b* and SLA. Six putative QTLs were found for δ-[^{13}C] and explained 0.628 (r^2) of the total phenotypic variation. None of the measured physiological traits was linked with the QTL for photosynthetic ability.

Three QTLs with large effects on yield were identified, accounting for 0.546 (r^2) of the total phenotypic variation. On chromosome 3, the QTL for yield with the largest phenotypic effect was mapped to approximately the same location as the QTL for panicle

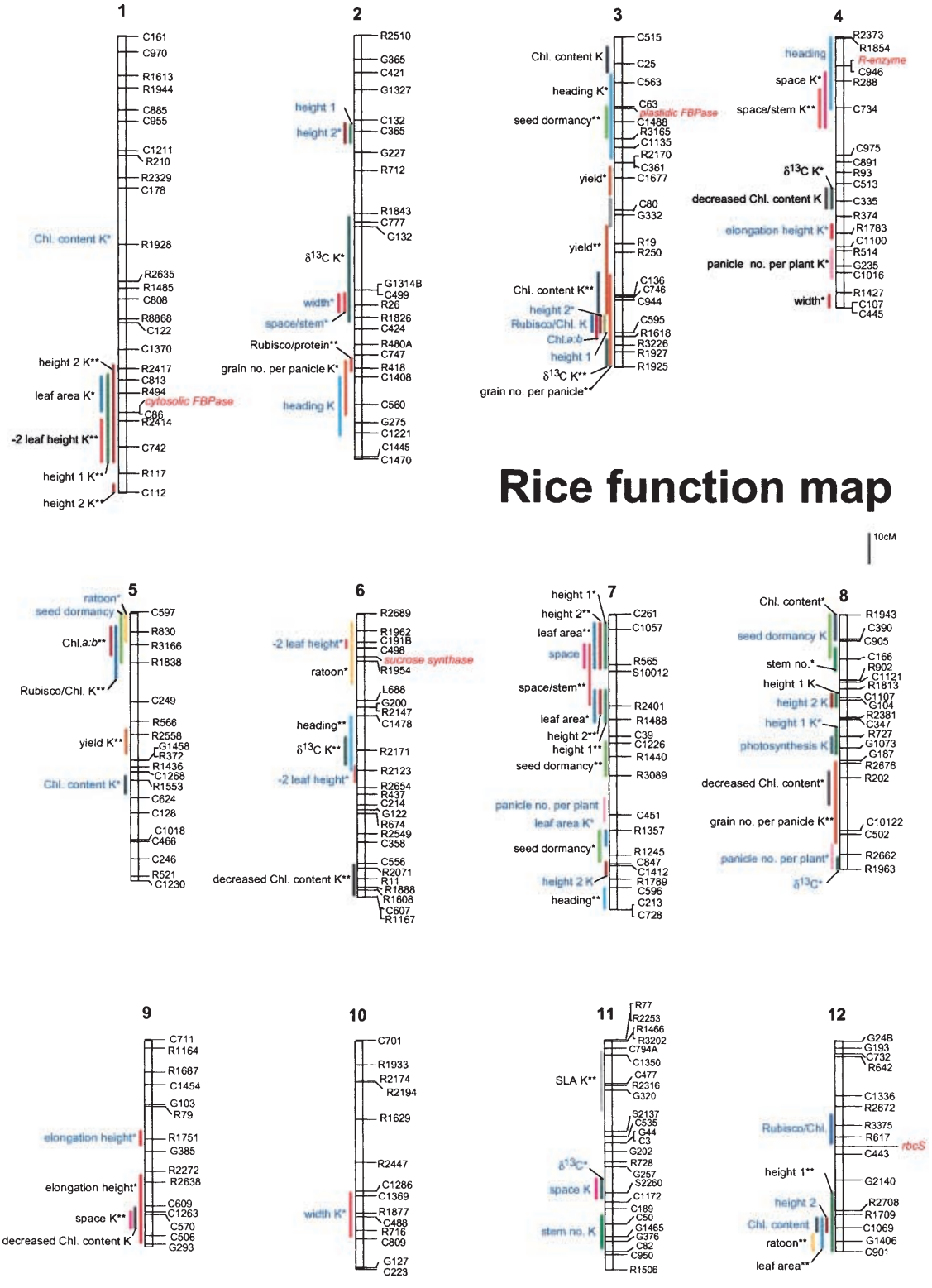

Fig. 1 A rice function map giving the positions of agronomic, morphological, and physiological traits. QTLs that were significant at the 0.01 probability level or higher (by ANOVA) are shown: * $P<0.005$, ** $P<0.001$. QTLs with LOD ≥2.0 are shown in *black*; those with LOD <2.0 in *blue*. *K* means that the Kasalath allele increased the value; a QTL *without K* means that the Nipponbare allele increased the value. ESTs concerned with carbon metabolism are shown in *red*. *Chl.* Chlorophyll content, *Chl.a:b* ratio of chlorophyll *a* content to chlorophyll *b* content, $\delta^{13}C$ δ-[^{13}C] value, *ear num* ear number per plant, *height 1* plant height at the middle of the growing phase, *height 2* plant height at the maximum length of the plant, *–2 leaf height* height of the 2nd leaf from the flag leaf, *Rubisco/Chl* ratio of content of ribulose-bisphosphate decarboxylase to chlorophyll content, *Rubisco/protein* ratio of Rubisco content to soluble protein content, *SLA* specific leaf area, *space* space occupied by plant, *space/stem* space per stem

Table 1 QTLs for traits of agronomic, physiological, and morphological traits on the rice function map. * means the first measured trait in QTL analysis of rice in this study. DPE; direction of phenotypic effect. N and K indicate that Nipponbare and Kasalath allele increased that value, respectively. R^2 indicates phenotypic variation explained by each QTL. QTLs for seed dormancy and heading date were reported by Lin et al. (1998)

Trait[a]	Number of QTLs detected	Chromosome number	Nearest marker locus to putative QTLs	LOD	DPE[b]	R^{2c}
Agronomic traits						
Grain number per panicle	3	2	C1408	2.0	K	0.075
		3	C136	4.3	N	0.206
		8	C10122	3.7	K	0.114
Panicle number per plant	3	4	R514	2.1	K	0.106
		7	C451	1.4	N	0.072
		8	R2662	1.8	N	0.088
Yield	3	3	C1677	4.0	N	0.192
			C136	6.3	N	0.222
		5	R2558	2.5	K	0.132
Ratoon*	3	5	C597	1.5	N	0.080
		6	R1962	2.5	N	0.099
		12	C901	3.1	N	0.136
Physiological traits						
Chl. content*	6	1	R1928	1.8	K	0.084
		3	C25	2.0	K	0.078
			C136	2.8	K	0.108
		5	R1553	1.6	K	0.088
		8	C390	2.5	N	0.098
		12	C1069	1.6	N	0.078
Decreased Chl. content*	4	4	C335	2.0	K	0.077
		6	C607	2.9	K	0.109
		8	R202	2.4	N	0.110
		9	C570	2.0	K	0.084
Photosynthetic ability*	1	8	G1073	1.5	K	0.075
Rubisco/protein*	1	2	R418	2.0	N	0.080
Rubisco/Chl.*	3	3	C595	1.4	K	0.083
		5	R1838	4.2	K	0.179
		12	R3375	1.7	N	0.080
Chl. *a:b**	2	3	R595	1.5	N	0.070
		5	R3166	2.9	N	0.129
SLA*	2	3	G332	1.7	N	0.088
		11	C1350	2.1	K	0.079
δ-[^{13}C] value*	6	2	R26	3.0	K	0.114
		3	R1925	3.0	K	0.115
		4	C335	2.1	K	0.090
		6	R2171	2.5	K	0.132
		8	R1963	1.7	N	0.092
		11	S2260	1.8	N	0.085
Morphological traits						
Plant height 1 (middle stage)	8	1	R2414	3.8	K	0.129
		2	C365	1.4	N	0.083
		3	C595	1.4	N	0.076
		7	R565	2.6	N	0.104
			R2401	2.7	N	0.113
		8	C1107	2.0	K	0.097
			G1073	1.8	K	0.075
		12	G1406	3.7	N	0.145
Plant height 2 (maximum)	9	1	R2414	5.7	K	0.192
			C112	3.0	K	0.129
		2	C365	1.5	N	0.079
		3	C595	1.5	N	0.086
		7	R565	3.0	N	0.121
			R2401	3.0	N	0.123
			C847	1.7	K	0.084
		8	C1107	2.2	K	0.103
		12	C1069	1.7	N	0.075
Elongation of plant height*	3	4	R1738	1.6	K	0.088
		9	R1751	1.7	N	0.085
			C506	2.7	N	0.106
–2 leaf height*	3	1	C742	3.6	K	0.161
		6	C191B	1.8	N	0.089
			R2123	1.9	N	0.100

Table 1 Continued

Trait[a]	Number of QTLs detected	Chromosome number	Nearest marker locus to putative QTLs	LOD	DPE[b]	R^{2c}
Leaf area	5	1	C813	2.8	K	0.076
		7	R565	2.3	N	0.125
			R2401	2.9	N	0.098
			R1357	1.8	K	0.076
		12	G1406	3.3	N	0.123
Space*	4	4	C734	2.1	K	0.167
		7	R565	1.1	N	0.076
		9	C570	2.8	K	0.117
		11	S2260	1.6	K	0.083
Space/stem*	3	2	R26	1.6	N	0.093
		4	C734	2.3	K	0.128
		7	S10012	2.3	N	0.091
Stem number per plant	2	8	C166	2.0	N	0.092
		11	C50	1.9	N	0.080
Width	3	2	R26	1.6	N	0.092
		4	C445	2.1	N	0.099
		10	C1369	1.9	K	0.094
Seed dormancy	5	3	C1488	5.1	N	0.256
		5	R830	1.4	N	0.075
		7	R1440	2.5	N	0.112
			R1245	2.3	N	0.111
		8	C390	1.5	K	0.070
Heading date	5	2	C560	1.1	K	0.072
		3	C1488	1.4	K	0.094
		4	R2373	1.4	N	0.081
		6	R2171	5.1	N	0.276
		7	C728	3.5	N	0.169

[a] *, Indicates the first trait measured in the QTL analysis of rice in this study

[b] DPE, Direction of phentypic effect. N, K, The Nipponbare and Kakalath allele increased that value, respectively

[c] R^2, Phenotypic variation explained by each QTL, QTLs for seed dormancy and heading date have been reported by Lin et al. (1998)

number per plant; C136 was the nearest marker. The values of both traits were increased by the Nipponbare allele. Three QTLs for ratoon reproduction with a high LOD were detected for the first time.

Eight QTLs were found for plant height 1, accounting for 0.822 (r^2) of the total phenotypic variation. Nine QTLs were detected for plant height 2 and accounted for 0.992 (r^2) of the total phenotypic variation. Most QTLs were detected at both stages. The QTL for plant height 1 nearest to marker G1073 on chromosome 8 did not overlap with the QTL for plant height 2. On chromosome 12, a QTL for plant height 1 did not overlap any of those for plant height 2. There were two clusters of QTLs associated with traits associated with plant height 1 or 2 and leaf area on chromosomes 1 and 7. Kasalath alleles were associated with an increase on chromosome 1, and Nipponbare alleles on chromosome 7. Four QTLs for space and 3 QTLs for space per stem were detected. QTLs controlling morphological and most physiological traits detected in this study did not overlap those for yield (Fig. 1; Table 1).

Candidate gene

The structural genes controlling carbon metabolism (*rbcS*, cytosolic or plastidic *FBPase*, *R-enzyme*, and *sucrose synthase*) were mapped on the function map (Fig. 1). These genes did not overlap the QTLs controlling yield or photosynthetic ability.

Discussion

QTL detection

A complex trait (e.g. yield) is regulated by a number of elementary factors, but it is likely that the factors are not equally effective in determining the trait. Comparing the location of QTLs makes it possible to determine the genetic relations among traits and the genetic limiting factors for a complex trait. The QTLs controlling yield did not overlap with all of the QTLs for morphological and physiological traits expected for Chl. content (Fig. 1; Table 1). This result suggests that these traits do not influence or limit yield, at least in this genetic background and environment. On chromosome 3, the QTL for Chl. content was mapped near the QTL for yield, with the same nearest marker (C136). The regions of the genome that had effects on the multiple traits may have acted through the pleiotropic effects of a single gene or by the chance linkage of multiple QTLs. The cloning of QTLs on a higher resolution linkage map of this region is required to assess these two possibilities.

Table 2 Physiological traits used as indicators of complex characters

Traits	Indicator	References
Decreased Chl. content	Senescence of leaf	Thomas and Stoddart 1980
Rubisco/Chl.	Balance between the activities of Calvin cycle enzymes and electron carriers in the light reactions	Hikosaka 1996
Chl.*a:b*	Relative amount of photosystem I or II	Wessels 1977
SLA	CO_2 absorption by mesophyll tissue	Tsunoda 1993
δ-[^{13}C] value	Intercellular CO_2 concentration	Ehleringer 1989

A decreased Chl. content indicates senescence (Thomas and Stoddart 1980). The flag leaf is the most important site of photosynthesis for supplying carbon to grains (Cook and Evans 1983), and the degree of senescence of this leaf is statistically related to yield (Kikuchi 1993). In this study, we found a significant correlation between decreased Chl. content and yield, and between 2 other traits and yield, but no overlap of their QTLs. The QTL for yield was detected near that for Chl. content, but there was no significant correlation between them. Simko et al. (1997) reported similar results for abscisic acid content of potato tubers and dormancy. It seems that statistical correlation does not necessarily reflect genetic relations. Therefore, we need to clarify the genetic relations among traits to find targets for genetic improvement of yield.

In addition to the importance of the traits themselves, physiological traits are widely used as indicators of complex characters (Table 2). QTLs for these traits could be related to other complex traits. The QTL for photosynthetic ability was not linked with other related physiological traits. This result suggests that photosynthetic ability is not influenced by complex characters (the balance between the activities of Calvin cycle enzymes and electron carriers in the light reactions, and the intercellular CO_2 concentration), at least in this genetic background and environment. It is time for physiologists to begin using QTL analysis (Prioul et al. 1997), although the difficulty in preparing materials still restricts this; this point is discussed below.

Synthesis of QTLs in cross combinations

QTLs affecting plant height or panicle number per plant were analyzed using various cross combinations of rice. One QTL for plant height has been detected in similar regions of chromosome 8 in four studies using cross combinations of cultivars and cultivar types: *indica*×*japonica* (Lu et al. 1996; Xiao et al. 1996; this study) and *indica*×*indica* (Zhuang et al. 1997). In three cross combinations of *indica*×*japonica* (Xiao et al. 1996; this study) and *indica*×*indica* (Lin et al. 1996), common QTLs for panicle number per plant were detected in similar regions of chromosome 4. We suggest that these common QTLs might be valid across rice cultivars and cultivar types. A QTL with high synthesis is regarded as a target for the cloning of a gene that functions broadly across various cultivars. However, the use of different molecular markers in each of the studies cited makes it impossible to compare exactly the synthesis of detected QTLs for the same traits. All molecular markers used in this study are available from our institute. By mapping these markers on each genetic map, it is possible to compare the position of a QTL for the same trait on the function map and to analyze synthesis among QTLs accurately across the cross combinations.

Candidate-gene approach

There are two reports of the map locations of a QTL and a major gene affecting the same trait (Edwards et al. 1992; Veldboom and Lee 1994). These findings support Robertson's hypothesis (1985) that QTLs allelic with major genes affect the same trait. In addition to facilitating a comparison of QTLs, the mapping of candidate genes can show their genetic relationship with QTLs already detected on our function map. The structural genes controlling carbon metabolism (*rbcS*, cytosolic or plastidic *FBPase*, *R-enzyme*, and *sucrose synthase*) did not overlap with the QTLs controlling yield. The fact that a QTL for yield is not detected in a particular chromosome region does not imply that a gene represented by an EST supposed to influence yield is not important for yield. The lack of a QTL in a specific chromosome region containing the structural gene coding for an enzyme could be due to the absence of functional polymorphism between the parental alleles of the gene controlling the level of that particular metabolic enzyme, whose role may nevertheless be important in determining yield. Furthermore, if the level of a specific enzyme is controlled by a transcriptional factor segregating independently from the affected structural gene, and the two parental lines have functionally different alleles at the locus encoding for the transcriptional factor, the support interval of the QTL will include the locus encoding for the transcriptional factor and not that of the structural gene. Therefore, in this case, the map position of the EST of a structural gene may not coincide with that of the QTL controlling its level of expression.

Utility of the function map

There are obvious similarities in the locations of QTLs for the same or corresponding traits in rice, maize, oats,

and barley (Briggs 1998; Timberlake 1998). Therefore, the rice function map could be used as the basic map for other cereal crops.

We welcome the cooperation of any interested scientists and offer them the use of the function map. Seed materials of all inbred lines and the most recent rice function map are available from the National Institute of Agrobiological Resources, Tsukuba, Ibaraki, Japan (http://rgp.dna.affrc.go.jp/). Scientists who identify a QTL will be able to identify QTLs for other characters. Already, 833 ESTs have been detected, and their genetic relationships have been clearly and accurately determined. The more QTLs that are detected on the function map, the more useful the function map will be in genetic and physiological studies. The map will be continually updated as new information becomes available; this point is the most important feature of the function map.

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