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Conditional and unconditional mapping of quantitative trait loci underlying plant height and tiller number in rice (*Oryza sativa* L.) grown at two nitrogen levels

Hua Jiang [a,b], Liang Jiang [b], Longbiao Guo [b,*], Zhenyu Gao [b], Dali Zeng [b], Lihuang Zhu [c], Guohua Liang [a], Qian Qian [b]

[a] *State Key Laboratory of Rice Biology/Key Laboratory of Plant Functional Genomics, Ministry of Education of China, Yangzhou University, Yangzhou 225009, China*
[b] *State Key Laboratory of Rice Biology, China National Rice Research Institute, Hangzhou 310006, China*
[c] *Institute of Genetics, Chinese Academy of Sciences, Beijing 100101, China*

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Abstract

In this study, we used 127 double haploid (DH) lines to analyze agricultural traits of rice. The DH lines, derived from a ZYQ8 (*indica*)/JX17 (*japonica*) cross by anther culture, contained 160 RFLP and 83 SSR markers. Unconditional and conditional quantitative trait loci (QTL) mapping was conducted to analyze plant height (PH) and tillers per plant (TP) at five growth stages that were grown at two nitrogen levels. Fourteen PH and 13 TP unconditional QTL were identified in the different growth stages, including 19 QTL from high-nitrogen (HN) and 14 QTL from low-nitrogen (LN) conditions. The conditional QTL for 14 genomic regions under LN/HN conditions showed that there was a significant effect on PH and TP across the different stages. Only one conditional QTL, *ph2-3*, was unable to be detected in unconditional mapping. More QTL were detected in the first four rice growth stages than in the final stage. Furthermore, a line from the DH mapping population, DH78, was identified in extreme phenotypes of PH and TP that exhibited dwarfism and less-tiller (dft) characters. The gene *dft1* was mapped to chromosome 2 using a backcrossed population of DH78/JX17 through a map-based cloning strategy. The location of *dft1* coincided with the mapping region of the small-LOD peak, QTL *ph2* and *tp2*, which were identified in plants grown in low-nitrogen conditions. Further backcrossing and fine-mapping successfully delimited the *dft1* locus to a 91 kb region.

Keywords: *Oryza sativa*; Quantitative trait loci; Double haploid (DH) population; Developmental behavior; Nitrogen; Map-based cloning

1. Introduction

Agronomic traits are quantitatively inherited in crops, and display continuous variation in segregated populations that may be affected by the environment. High-density molecular linkage maps and the use of permanent segregating populations have enabled the discovery of a large number of quantitative trait loci (QTL) for agricultural traits of crop plants in different developmental stages from both single and multi-environments [1–3]. Previous comparative QTL studies of key agronomic traits have shown that QTL-sharing frequencies for 1000-grain weight range from 9.5% to 52.9% between two environments [4,5]. For all traits, approximately 30% QTL are shared between two environments. QTL-sharing frequencies decrease across three or more environments [4,5]. Xing et al. [3] reported that the interaction between genotype and environment

* Corresponding author. Tel.: +86 571 63370537; fax: +86 571 63370389.
E-mail address: guolongb@mail.hz.zj.cn (L. Guo).

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that is commonly observed in quantitative genetic studies had a significant effect on developmental and agronomic traits. It is well known that these interactions are responsible for genetic variation of different QTL or alleles at the same locus across diverse environments [6]. However, differences in expression do not result from QTL × environment (QE) interactions alone, but also from the reduced genetic effects of QTL and QE interactions. Xu [7] reported that plant growth decreases the relative contributions of nonadditive gene action and environmental factors to variation, while increasing the contribution of additive gene action. Genes are expressed selectively at different growth stages.

In general, there are three dynamic analysis and time-related mapping approaches that are used to understand genetic expression at different developmental stages. One approach, called effect-accumulation analysis or unconditional QTL mapping, is based on the analysis of trait values measured at each observation time [8–10], from which the accumulated effect of a QTL can be estimated [11]. A second approach, called effect-increment analysis or conditional QTL mapping, is to analyze trait-value increments at sequential time intervals, allowing the incremental or net effect of a QTL to be estimated [8,11]. The third approach is to look at a QTL over time by fitting the parameters of a plant growth curve [12]. Zhu [13] developed a conditional analysis method that considers the net genetic effects of genes that are preferentially expressed in stress environments, as opposed to non-stress environments. This method has been used to study the developmental behavior of cotton [13], rice [11,14,15], and mice [16]. This method has also been used to determine those genes that are only expressed in low-nitrogen stress environments [15].

During the last three decades, nitrogen fertilization has been widely used to increase rice yield. However, application of nitrogen fertilizer must be optimized to reduce production costs and nitrate pollution. It remains critical to develop rice cultivars that have high-nitrogen-use efficiency that can be grown under low-nitrogen conditions. Few studies have investigated QTL for agronomic traits in nitrogen stress and non-stress environments [17], except for QTL × N level interactions in rice [18].

In this study, we examined specific gene expression for agronomic and developmental traits [19] in rice subjected to nitrogen stress. To our knowledge, no one has explored the net QTL expression of rice traits under two nitrogen levels. Thus, we combined statistical analysis of conditional genetic effects [13,20] with the composite interval mapping method [21,22] to dissect the net QTL expression for plant height (PH) and tillers per plant (TP) at two nitrogen levels. Our study sought to understand the mechanism of gene differential expression affecting PH and TP in different environments, and to provide an insight into the genetic basis of PH and TP. This can provide background information for genetically improving rice. A variant line from the DH population, DH78, with dwarf and less-tiller (*dft*) characters (Fig. 1), and its parent, JX17, were used to construct a backcrossing-population and for genotyping. Fine-mapping of the *dft1* gene was also conducted using a map-based strategy.

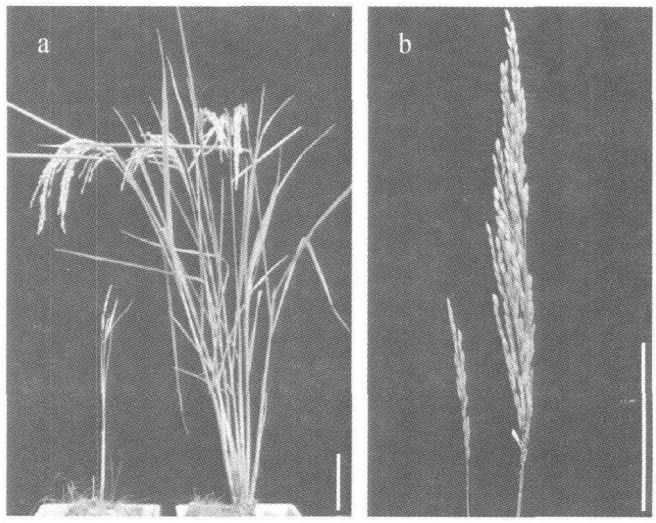

Fig. 1. Gross and panicle morphology of the DH78 and the parent JX17 lines. (a) Gross morphology in the mature stage of DH78 (left) and JX17 (right). (b) Panicle morphology in the mature stage of DH78 (left) and JX17 (right). Bar = 10 cm.

2. Materials and methods

2.1. Plant materials

We used a double haploid (DH) population of 127 lines derived by F_1 anther culture from a cross between a typical *indica* variety of rice, Zhaiyeqing 8 (ZYQ8), and a typical *japonica* rice, Jingxi 17 (JX17) [23]. A variant line from the DH population, DH78, showed dwarfism, few-tiller (dft), and short panicle characteristics (Fig. 1). It showed a similar response as parent lines to the two nitrogen environments (data not shown). DH78 derived from DH78/JX17 was used for primary mapping of the *dft1* gene, and its F_2 and backcrossed population, BC_2F_2, derived from DH78/JX17//JX17, was used for fine-mapping.

2.2. Estimation of plant height and tillers per plant at two nitrogen levels

The 127 lines and their parents, ZYQ8 and JX17, were evaluated at the experimental station of the National Rice Research Institute in Hangzhou, China. Field planting followed a randomized complete block design with two replications at two nitrogen fertilizer levels. The nitrogen treatments included no N fertilizer as top dressing for the low-nitrogen (LN) level and 225 kg hm^{-2} N fertilizer as top dressing for the high-nitrogen (HN) level [18]. The field soil had 24.2 g kg^{-1} organic materials, pH 6.2, 2.27 g kg^{-1} available nitrogen, 10 g kg^{-1} phosphorus, and 66 g kg^{-1} potassium [24]. Other cultivation conditions were consistent with optimum rice production in these regions. The

seeds were sown on May 23, 2003 and the seedlings were transplanted after 27 days. Two parents and 127 DH lines were planted in three rows of eight hills per row, at a spacing of 16 cm × 23.1 cm. Ten days following transplantation, plant height and tiller number per plant were measured. Measurements were taken at five different stages of rice growth from July 2 to September 22.

2.3. QTL analysis

A rice linkage-map containing 160 RFLP and 83 SSR markers that were evenly distributed on all 12 rice chromosomes was constructed using Mapmaker/EXP version 3.0, as described previously [5]. SSR markers that could only be mapped in the present population were denoted GAXX, CTXX, ATTXX, and TCTXX [19], while the others were named RMXX [25]. The unconditional QTL mapping for two traits was conducted as a separate phenotypic value in the different growth periods using composite interval mapping [21,22].

Conditional QTL mapping was also conducted on the phenotypic value at low-nitrogen based on the phenotypic mean at high-nitrogen [$y_{(LN/HN)}$] using composite interval mapping [21,22]. The conditional phenotypic means [$y_{(LN/HN)}$] in rice were obtained using the mixed-model approach [13]. The derived phenotypic value was partitioned as follows:

$$y_{(LN/HN)} = \mu_{(LN/HN)} + G_{(LN/HN)} + E_{(LN/HN)} + e_{(LN/HN)}$$

In which $y_{(LN/HN)}$ is the conditional phenotypic value, $\mu_{(LN/HN)}$ is the conditional population mean, $G_{(LN/HN)}$ is the conditional QTL effect, $E_{(LN/HN)}$ is the conditional effect for the environment, and $e_{(LN/HN)}$ is the conditional residual error. In the conditional analysis ($_{LN/HN}$) denotes the phenotypic value at low-nitrogen that was based on excluding the phenotypic value at high-nitrogen. Unconditional QTL revealed segregated genetic effects at the two nitrogen levels. Comparing LN and HN QTL showed net genetic effects of genes that were only expressed in low-nitrogen conditions relative to no-stress conditions.

The original and conditional values of PH and TP during different growth periods were used to analyze the QTL-linked molecular markers using QTL Cartographer v.1.1b [26]. The total phenotypic percentage variation of each component was estimated within the multiple models [13], in which all significant QTL fitted simultaneously. The threshold to declare QTL was set at a significance level of $P < 0.05$, which could be transformed with LOD or likelihood ratio (LR) values [27].

2.4. Chromosome location and fine-mapping of the dft1 gene

The F_2 population of DH78/JX17 was used as the primary mapping population. A backcrossed BC_2F_2 population derived from DH78/JX17, with JX17 as a recurrent parent, was used as the fine-mapping population. Total genomic DNA was extracted from fresh leaves of each selected individual of the same phenotype and parent line, DH78, and from the F_2 or BC_2F_2 populations. DNA was extracted using the CTAB method with some minor modifications [28]. Following the development of new molecular markers, PCR and linkage-map construction were performed as previously described [29].

3. Results

3.1. Variation within the parents and DH populations

The two parents differed significantly for all measured traits. Higher PH and TP values were found in JX17 and ZYQ8, respectively (Table 1). Higher PH and TP values at each stage were found at the high-nitrogen level than at the low-nitrogen level. Within the DH population, PH and TP values were continuously segregated, while the skew and kurtosis values were less than 1.0 during most measured stages except TP at the 2nd measuring stage. Thus, PH and TP segregation of the DH population fitted a normal distribution at most stages and was suitable for QTL analysis.

3.2. Unconditional QTL mapping

Twenty-seven QTLs responsible for PH and TP were identified on the 12 rice chromosomes at five growth stages under both nitrogen levels (Tables 2 and 3, and Fig. 2). These included 14 PH QTLs and 13 TP QTLs. For the two traits, 19 QTLs were observed at the high-nitrogen level and 14 QTLs at the low-nitrogen level.

Fourteen QTLs were detected for PH. Ten were detected at the high-nitrogen level, including 2, 3, 3, 2, and 2 at the five measured stages between July 2 and September 22. Eight QTLs were identified at the low-nitrogen level, including 3, 3, 4, 3, and 2 QTLs at the five measured stages. These QTLs were located on 9 of the 12 chromosomes, with the exceptions of chromosomes 3, 6, and 9. Four QTLs were detected on chromosomes 2, 4, 8, and 12 at both nitrogen levels. Thirteen QTLs were detected for TP. Nine QTLs were detected at the high-nitrogen level, including 2, 3, 3, 2, and 2 at the five measured stages. The QTLs were located on chromosomes 1, 2, 3, 4, 5, 6, 8, 9, 11, and 12. Six QTLs were observed at the low-nitrogen level, including 2, 3, 3, 2, and 3 at the five measured stages.

These findings suggest that more QTLs can be detected during the four earlier plant growth stages than at the final stage. Different QTLs may be detected at different stages or environments and the genes controlling PH and TP may be differentially expressed during the growth period. Thus, QTL expression for PH and TP may vary among growth stages and environmental conditions.

Table 1
Phenotypic value of plant height and tillers per plant for parents and DH population at five measured stages and two nitrogen levels.

Trait	Environment	Period	Parent					DH population		
			ZYQ8	JX17	*t*-Value	DH78	Mean	Range	Skewness	Kurtosis
PH	HN	7.2	45.67	60	−6.00**	30.33	48.82	30.33–64.67	0.05	0.07
		7.16	60.67	78	−4.61**	41.06	66.55	41.06–86.67	−0.06	0.11
		7.30	81.47	98.23	−4.72**	44.67	84.62	44.67–114.37	0.32	0.67
		8.14	102.76	109.67	−1.83	46.46	97.42	46.46–134.49	0.29	0.46
		Final[a]	97.06	106.63	−2.91*	55.80	97.73	55.80–136.91	0.31	0.19
	LN	7.2	39.33	47.67	−4.19**	27.33	43.64	27.33–56.67	0.13	0.38
		7.16	50.67	69.67	−5.70**	40.67	58.19	40.67–75.67	0.04	−0.22
		7.30	66.34	86.42	−6.23**	45.37	73.16	45.37–92.39	0.19	0.34
		8.14	84.17	96.73	−3.18**	46.68	85.79	46.68–121.13	−0.34	−0.29
		Final	81.54	89.02	−1.88	54.61	84.02	54.61–119.02	0.27	0.16
TP	HN	7.2	3.33	2.13	1.42	1.02	1.79	1.02–4.33	0.97	0.94
		7.16	15.43	7.27	5.44**	2.37	5.72	2.37–15.67	1.57	3.41
		7.30	20.17	14.67	2.80*	2.83	14.03	2.83–24.13	0.97	0.91
		8.14	17.67	13.13	1.85	2.97	14.67	2.97–25.42	0.84	0.79
		Final	12.36	8.76	1.99	2.41	12.57	2.41–17.26	0.73	0.64
	LN	7.2	2.33	1.07	6.32**	1.07	1.68	1.07–4.24	0.92	0.71
		7.16	5.03	3.67	1.75	2.01	4.13	2.01–9.33	1.25	1.69
		7.30	11.43	8.37	2.79*	2.13	9.12	2.13–14.76	0.74	0.91
		8.14	12.37	9.16	2.67*	2.33	15.12	2.33–16.33	0.91	0.75
		Final	9.36	5.79	2.54*	2.26	9.67	2.26–12.39	0.85	0.94

[a] Plant height in the final growth stage was measured from the surface of the soil to the tip of the panicle; others are all measured from the surface of the soil to the tip of the plant.
* Indicate significance at 0.05 level.
** Indicate significance at 0.01 level.

3.3. Conditional QTL mapping

Given the phenotypic values observed in rice grown under high-nitrogen, the conditional genetic effects observed at low-nitrogen levels suggest that the net effects of gene expression at low-nitrogen levels relative to high-nitrogen levels are independent of the casual effects. As a result, the conditional QTL for LN/HN revealed gene expression in only the low-nitrogen environment relative to the no-stress environment. The analysis of conditional genetic effects by composite interval mapping provided 14 genomic regions, indicating that conditional QTL for LN/HN significantly affected the PH and TP during the five growth stages (Tables 2 and 3). These 14 regions were mapped to 9 of the 12 rice chromosomes (Fig. 2).

Seven conditional QTL that affected the total PH were detected for (LN/HN) during the five growth stages, indicating that some genes were only expressed at the low-nitrogen level relative to the no-stress environment. *ph2-3* and *ph5* were both detected in three stages while the rest were detected in only one stage. The QTL genetic effects of (LN/HN) during the five stages were 3.45, 4.11, 6.61, 5.44, and 6.01 cm, respectively. *tp3* was detected at three stages, *tp6* and *tp9* were detected at two stages, and the others were detected at only one stage. The genetic effects of the QTL for TP at the five measured stages were 0.60, 1.36, 1.64, 1.77, and 2.58 for (LN/HN).

Gene expression for the PH and TP traits may differ among environments and growth stages. In 10 of the 14 genomic locations, QTLs detected using unconditional mapping at low-nitrogen were as follows: 6 QTLs at high-nitrogen only and 4 QTLs at both nitrogen levels. Only one conditional QTL, *ph2-3*, was not detected using unconditional mapping. Most chromosomal regions showed a significant conditional QTL at only one or several stages, indicating that the gene in a specific genomic region was only expressed during a specific period of plant growth. Ten conditional QTLs for PH and TP were mapped to the same region as shown using unconditional mapping of the same stages under low-nitrogen. These included *tp2-2*, *tp3*, and *tp9* and *ph1-3*, *ph2-2*, *ph2-3*, and *ph5*. Six conditional PH and TP QTLs were mapped to the same region as shown using unconditional mapping under high-nitrogen, however expression was significantly different at each stage.

3.4. Dft1 mapped to the same region as ph2-3 and tp2-2

The F_2 population of DH78/JX17 was used as a primary mapping population. Total DNA from DH78 and JX17 was extracted and amplified using published SSR primers. For linkage analysis, we selected 162 SSR markers showing polymorphisms between the parents that were uniformly distributed on the 12 rice chromosomes. For rough mapping, we used 87 individuals from F_2 populations possessing similar morphologies to the mutant parent DH78. Using RM5897 and RM5340, the closest SSR markers spanning the *dft1* region, seven informative plants harboring recombinant events were identified (Fig. 3a). In this experiment, we successfully located the *dft1* gene at the *C132* region on chromosome 2. This region harbored two

Table 2
Putative QTLs affecting plant height in the DH population and the additive effect based on unconditional and conditional composite interval mapping at five measured stages and two nitrogen levels.

Chr.	QTL	Marker interval	10D			24D			38D			52D			Final		
			LN	HN	LN/HN	LN	HN	LN/HN	LN	HN	LN/HN	LN	HN	LN/HN	LN	HN	LN/HN
1	*ph1-1*	CT380A-GA594					−2.64										
1	*ph1-2*	RG345-CT461											−3.37				
1	*ph1-3*	C49-CT372							−2.43								−3.04
2	*ph2-1*	CT87-G1234	−1.53	−1.98		−1.44											
2	*ph2-2*	CT565-RG171							−2.72			−2.83		−2.67			
2	*ph2-3*	C132-G357			1.32			1.86			1.71						
4	*ph4-1*	C975-RG449								−3.49							
4	*ph4-2*	CT500-C513	−2.36		−2.13							−3.83	−3.46				
5	*ph5*	CA41-CA257				−2.07			−2.38		−2.46	−2.85		−2.77			−2.97
7	*ph7*	TCT122-RG769								4.12							
8	*ph8*	RZ617-G2132				−3.16									−4.30	−4.68	
10	*ph10*	G2155-C16					1.73	2.25		1.96						3.73	
11	*ph11*	L190-RZ536B													−2.74		
12	*ph12-1*	RG181-G1106	−1.69	−1.51					−2.25		−2.44						
12	*ph12-2*	G2140-RG457					−1.74										
Variability (%)			29.74%	25.61	23.76	34.69	31.62	29.73	48.27	41.62	37.6	34.27	32.47	26.31	30.72	33.46	24.76
QTL No.			3	2	2	3	3	2	4	3	3	3	2	2	2	2	2

Notes: Positive values of additive effects indicate that the favorable allele was from JX17; negative values of additive effects indicate that the positive allele was from ZYQ8.

QTLs, *tp2-2* and *ph2-3*, which were detected by QTL mapping in this study.

3.5. Delimitation of *dft1* to a 91 kb interval

Segregated multiple genetic factors cannot be used simultaneously to precisely map one of multiple genes [30]. To easily identify the morphology of plant progeny and decrease segregation affection by other QTLs, a backcrossed population derived from DH78/JX17 was constructed using JX17 as a recurrent parent. During construction of the BC population, we used several molecular markers associated with the identified QTL, RMXX, and RZXX, to rule out interference caused by segregation of the other QTLs using molecular assisted selection (MAS). The progenies' backgrounds were detected using 32 SSR markers distributed on 12 chromosomes from the linkage map and the previous polymorphic SSR markers between the parents (data not shown), with the exception of the *dft1* region.

Of the two backcrossed generations and one inbred generation, 780 individuals possessing similar morphology to DH78 were selected from the BC_2F_2 generation for fine-mapping of the *dft1* gene. To further map the *dft1* locus, BLASTN (http://www.ncbi.nlm.nih.gov/BLAST/) was used to search for sequences that matched RM5897 and RM5340 in the rice nucleotide database. Sequences matching RM5897 and RM5340 were identified in AP005797 and AP004070, respectively. Using the BAC/PAC clone sequences, another six overlapping BAC/PAC clones covering the *dft1* locus region were identified (Fig. 3b). Polymorphisms were detected in 9 of the 60 newly developed STS makers (Table 4). These primers were subsequently used for 780 individual genotypes, which mapped the *dft1* locus between STSD and STSI at a distance of 91 kb, with 14 ORFs in the corresponding region according to the TIGR (http://www.tigr.org) (Fig. 3c).

4. Discussion

We assessed differential gene expression at five growth stages of rice cultivated at two nitrogen levels using conditional and unconditional QTL mapping methods. Previous reports illustrated dynamic gene expression for developmental traits and causal gene expression for yield [11,13,15,20]. Our results indicated that the QTL responsible for PH and TP at one nitrogen level or developmental stage was undetectable in others. In addition, the differential gene expression in different environments suggested that the conditional QTL mapping approach may result in more information or special analysis of QTL for complex traits. For example, *ph2-3, ph1-3, ph5, tp2-2*, and *tp5* were only expressed when grown under low-nitrogen conditions. This method may be used to determine which genes are expressed in other stress environments such as low water or high salt.

TP and PH are key agronomic traits for grain production, and development requires many TP or PH QTLs on most of the 12 rice chromosomes [11,20,29,31,32]. We found 13 TP QTLs in the five growth stages of rice under both nitrogen levels, including 13 unconditional and 7 conditional TP QTLs. Nine QTLs were observed at the high-nitrogen level, six at the low-nitrogen level, and two under both nitrogen lev-

Table 3
Putative QTLs affecting tillers per plant in the DH population and their additive effects at five measured stages and two nitrogen levels based on unconditional and conditional composite interval mapping

Chr.	QTL	Marker interval	10D			24D			38D			52D			Final		
			LN	HN	LN/HN	LN	HN	LN/HN	LN	HN	LN/HN	LN	HN	LN/HN	LN	HN	LN/HN
1	*tp1-3*	GA273-GA330	0.24						0.72	−0.67		0.87					
2	*tp2-1*	C424-G45		−0.22													
2	*tp2-2*	C132-G357													0.97		1.62
2	*tp2-3*	CT87-G1234					−0.41			0.86							
3	*tp3*	C746-GA505	−0.23		−0.26	−0.36		−0.43				−1.02		−1.13			
4	*tp4-1*	RG809-CT563		0.21			0.32				0.67						
4	*tp4-2*	CT404-CT500											1.22				
5	*tp5*	CT481-RG776B						0.47					−0.76		−1.06	−0.94	
6	*tp6*	CT380B-G294				0.39		0.46	0.42		0.38						0.96
8	*tp8*	RG598-RG418B					0.53										
9	*tp9*	B39A-B39B				0.48			0.56		0.59			0.64	−1.12		
11	*tp11*	PTA818-RG2			0.34					0.59							
12	*tp12*	Y12817R-RG457														0.87	
Variability (%)			27.36	26.24	28.91	35.64	33.56	31.27	45.36	43.29	39.27	37.61	39.81	34.74	32.69	34.62	29.37
QTL No.			2	2	2	3	3	3	3	3	3	2	2	2	3	2	2

Notes: Positive values of additive effects indicate that the favorable allele was from JX17; negative values of additive effects indicate that the positive allele was from ZYQ8.

els. Fourteen QTLs were detected for PH in 10 environments, including 13 unconditional and 7 conditional QTLs. Eight were observed at the high-nitrogen level and 8 at the low-nitrogen level. The genetic effects of the PH and TP QTLs varied among the five stages measured at both nitrogen levels as a result of the expression of new genes at different stages. More QTLs were detected in the five stages than for the final phenotype at both nitrogen levels, which only detected three QTLs for both PH and TP. These results indicated that there is little consistency of QTL expression among the different stage/environment combinations, and differential expression is observed at different stages.

In general, environments conceal quantitative variance from genetic factors. Austin and Lee [17] suggested that different QTLs or alleles at the same locus are responsible for genetic variance under diverse environmental conditions. Multi-environment QTL and conditional mapping are helpful to identify more QTLs than that detected for a final trait phenotype, as a result of differential expression and variance at different stages. Similar results were reported in previous studies [3,11,20,32]. However, most genes were expressed at early stages and not expressed during the final stage. For example, *tp3*, which shows continuous expression during the first four stages, may be used to control tiller number. For breeding, however, we should select a variety with the same high tiller number as the productive tiller number, so as not to waste energy [33]. *ph5* was expressed during the middle three stages and could be used to control plant height, as its expression corresponded to the elongation growth stage.

Similar to previous studies, some putative QTLs controlling PH and TP were located on the same loci or at close intervals in near-iso-environments or heterogeneous environments [12,31,32]. We detected four and six QTLs for PH, with similar QTL in the *RG634* region on chromosome 2. Yan et al. [11] mapped two similar PH QTLs on the *RG95* region of chromosome 2 and the *RG910* of chromosome 3. Miyamoto et al. [34] detected three TP QTLs on similar regions of the *C424* of chromosome 2, the *R2147* of chromosome 6, and the *R2662* of chromosome 8. Some PH and TP QTLs were located on the same or similar regions in different populations or environments; however, the differences between the detected QTLs for agronomic traits were highly significant. Our results suggest that different sets of alleles, possibly at different loci, are being expressed under different environmental conditions and in different genetic populations.

Rice geneticists have widely applied linkage maps to map genes for qualitative and quantitative inherited traits of economic importance [35]. Differential expression analysis could aid special environment selection during breeding. Clarifying the molecular mechanisms of differential expression at the two nitrogen levels is essential for improving rice cultivars, increasing nitrogen-use efficiency, using marginal land for rice production, and for environmental protection. Differential analysis of molecular markers may directly use QTL with consistent effects under low-nitrogen to develop rice cultivars by MAS or positional cloning [36–40]. However, *ph2-3*, *ph1-3*, *ph5*, *tp2-2*, *tp5*, and *dft1* that were only detected in low-N conditions may be associated with nitrogen efficiency. Conditional mapping showed that the PH and TP QTLs that were only expressed at low-N levels may have been related to high absorption efficiency and nitrogen utilization. Further studies are needed to elucidate the mechanisms of nitrogen efficiency.

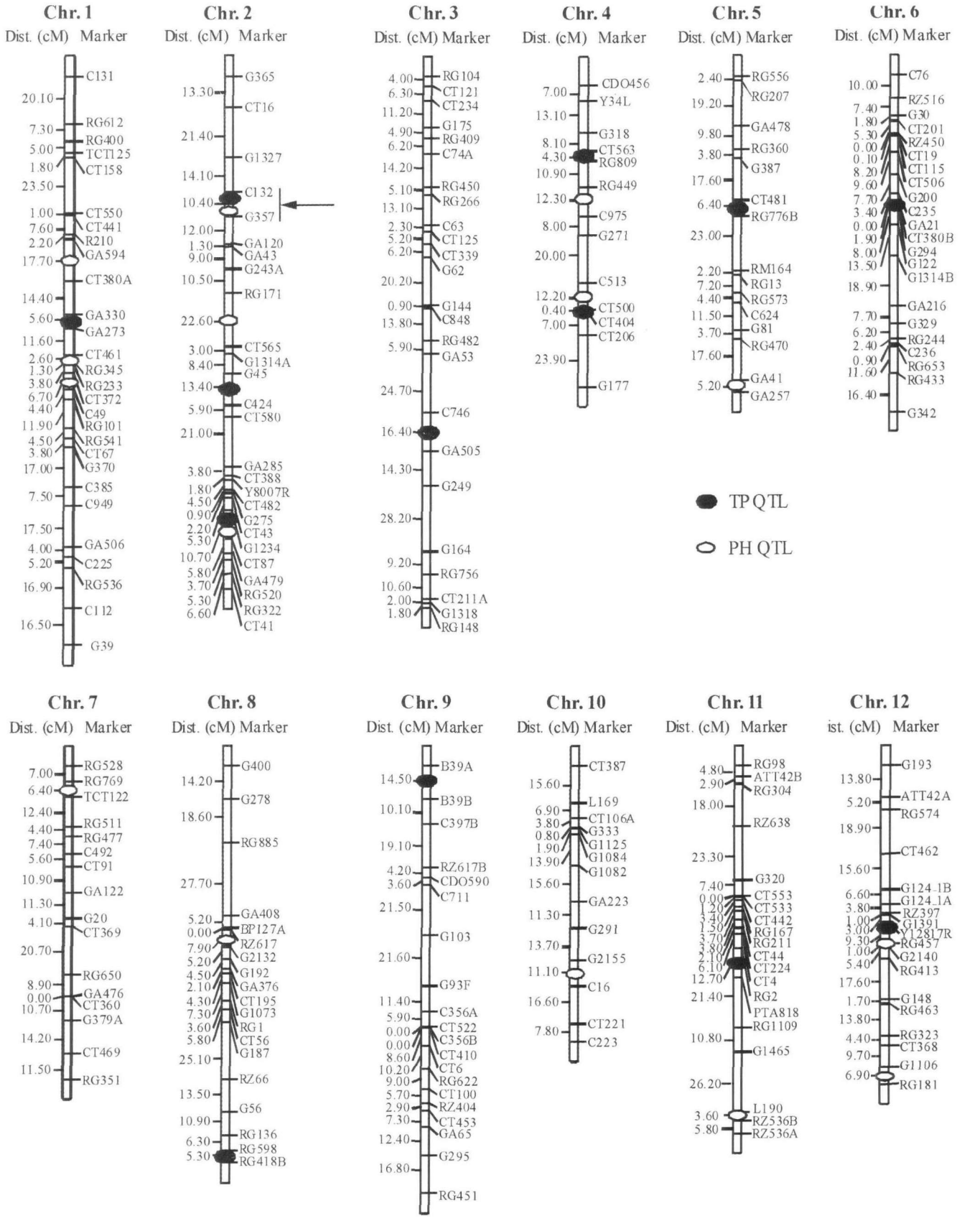

Fig. 2. Main-effect QTLs mapped in the DH population at five measured stages at two nitrogen levels based on unconditional and conditional composite interval mapping. Arrow shows *dft1* allele region.

In this study, we reported the delimitation of a 91 kb region of *dft1*, a first primary mapped to the same region of the small-LOD peak QTL, *ph-2* and *tp-2*, which was identified in rice growing at low-nitrogen levels and detected by QTL mapping. It may be that *dft1* is identical to the QTL *ph2-3* or *tp2-2*; however, we do not yet have enough evidence conclude this with

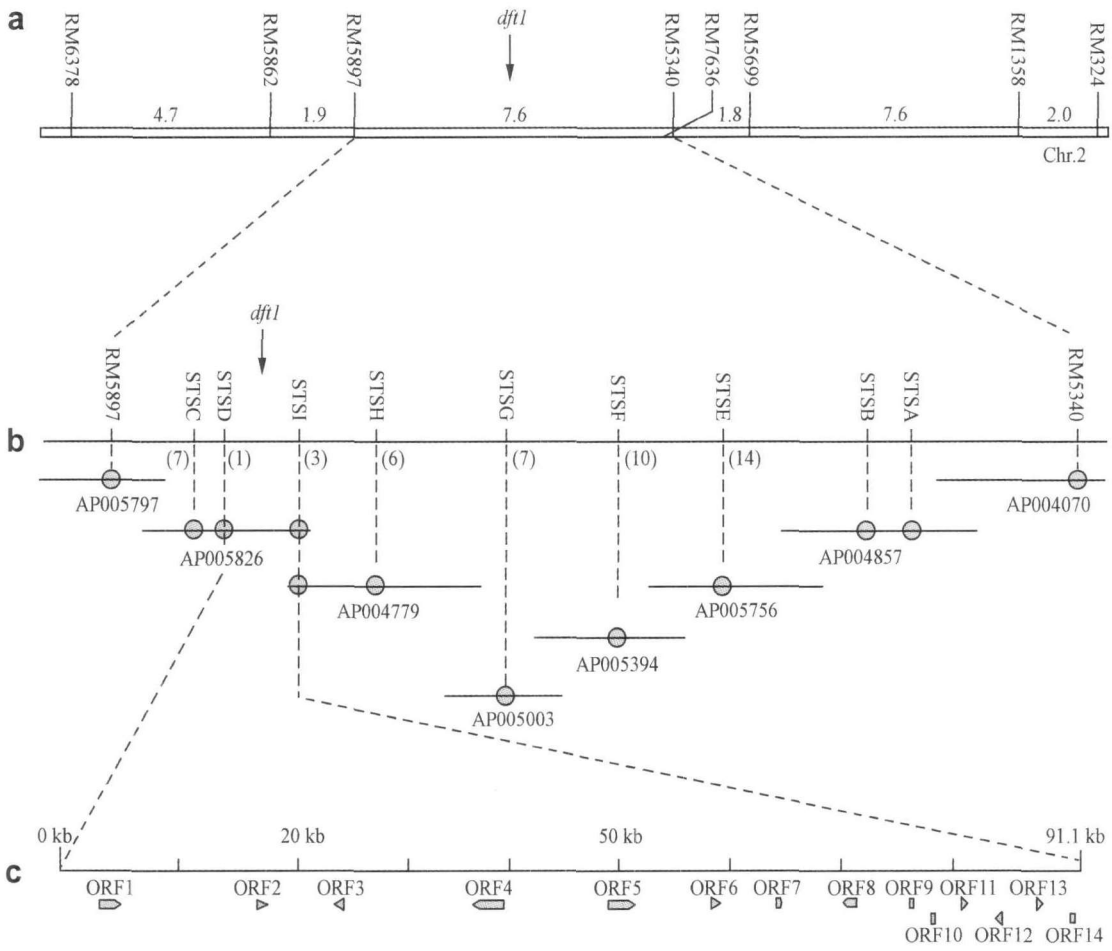

Fig. 3. A rough-scale, high-resolution genetic and physical map of the *dft1* allele region on chromosome 2. (a) Primary map of *dft1* gene. Numbers between the SSR markers indicate genetic distance (cM). (b) High-resolution genetic map of *dft1* allele region based on recombination events (the numbers under the horizontal line) between markers and *dft1* locus. (c) Candidate region of *dft1* gene and presumed ORFs in this interval predicated by TIGR.

Table 4
Sequences of the STS markers designed for fine-mapping of the *dft1* gene.

Marker	Forward sequence (5′–3′)	Reverse sequence (5′–3′)	Product size in Nipponbare (bp)	Annealing. temperature (°C)	Anchored BAC
STSA	TACTTGTTTTTGAGAGGCGG	GTTTTCTTCAAGTTTGAGTTA	118	55	AP004857
STSB	AGAAATCGGGTGAGATGGTGT	CTCCACCCTGACAGCATTAG	119	55	AP004857
STSE	CTATTGGTGGTTAGGGCTGAT	AGTTGATTTAGATGTGTTTTA	146	56	AP005756
STSF	ATGCTCCCAAACCTACTTCC	CACCGTCTACCTTGGATTGC	94	56	AP005394
STSG	TCAGGGAGGACACTGTTCGG	GGAGACAACGAGACGGGAGATA	140	60	AP005003
STSH	CAGGGAGGACACTGTTCGG	TTGAGGAGCGGAGGAGACA	151	58	AP004779
STSC	CTTTCTGCTCCTCGTCCAT	CCGCTCGTCAAGCTATTTA	181	54	AP005826
STSD	GCTCTGCTTCCTTCCTGCTA	CGATCGATAATGTGACTCATCC	246	59	AP005826
STSI	CTCAACCGAATCTGACAAG	CACCGTTAGTTAAGGAGAAAGT	184	50	AP005826

certainty. Even so, it is difficult to describe the relationship between the micro effect of the QTL and the extreme phenotype. Is the phenotype caused by the direct function of the gene itself, or by interactions between the gene and the complex background? If the phenotype of the *dft1* gene can be rescued using a complementation test we can show that even the small-LOD peak QTL affects the phenotype to a certain extent. If we can identify the fragment that, when absent, demonstrates the same phenotype as its parental line, DH78, coactions between the gene and the complex background will be clarified. In any case, we can claim that at least one gene affects TP and PH in the putative QTL region.

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