

Molecular Dissection of the Relationships among Tiller Number, Plant Height and Heading Date in Rice

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Abstract : Appropriate plant height, tiller number and heading date are important traits for maximizing rice production. In order to understand the genetic basis of the relationships among these three plant traits, we mapped quantitative trait loci (QTLs) using a recombinant inbred population and detected two-locus interactions for plant height and tiller number at two growth stages and for heading date in two years. There were significant negative correlations between tiller number and plant height, and between tiller number at maturity and heading date. A significant positive correlation was observed between heading date and plant height at maturity. A total of 29 QTLs for the three traits were identified over the two years. Results show that QTLs and majority of two-locus interactions for plant height and tiller numbers at 35 days after transplanting were different from those at maturity, indicating that different genes and interactions control the traits at different developmental stages. A large proportion of QTLs and interactions could only be detected in one year, suggesting that QTLs and two-locus interactions for the traits were dependent on the environment. Results suggest that pleiotropy and/or close linkage of genomic regions and pleiotropy of common two-locus combinations may be the genetic basis for the close correlations among the three traits. A QTL with a large effect for heading date, which was located in RG424-RZ667 on chromosome 6, also showed large effects on tiller number and plant height at maturity.

Key words : Heading date, Molecular marker, Plant height, Quantitative trait locus (QTL), Rice, Tiller number.

Plant height, tiller number, and heading date are closely associated with biomass production and grain yield in rice (Tanaka et al., 1966; Yoshida, 1972; Akita, 1989). Since the end of the 1950s, rice varieties with short stature were released, resulting in a high harvest index and high yield (Chandler, 1969). However, recent researches indicated that short plant height of semi-dwarf rice and wheat might limit canopy photosynthesis and biomass production (Kuroda et al., 1989; Gent, 1995). Tillering plays an important role in determining final grain yield in rice since it has a significant effect on panicle number. Too few tillers limit grain yield, while excess tillers lead to high tiller abortion, small panicle size, poor grain filling, and further reduced grain yield (Peng et al., 1994). Heading date is a major determining trait for adaptation of rice varieties. Long growth duration, which often results in large biomass production, is also associated with lower harvest index (Vergara et al., 1966; Akita, 1989). Varieties with too short growth duration may not produce a high yield due to limited vegetative growth and biomass production, while those with too long growth duration may not give a high yield because of excessive vegetative growth and lodging (Yoshida, 1981). Therefore, optimizing

the relation among the three traits is necessary for improving yield potential in rice breeding programs.

Tiller number is usually negatively correlated with plant height in rice. In comparison with normally-tillered cereals, plants with a single culm are taller and flower earlier (Richards, 1988). In Poaceae, flowering terminates apical growth, and increased plant height is often associated with late flowering (Lin et al., 1995). Genetically, genes at several chromosomal locations have pleiotropic effects on both heading date and plant height (Li et al., 1995; Yu et al., 2002), and there was a reduction in height of about one centimeter for every day earlier heading (Li et al., 1995). However, relationships among tiller number, plant height and heading date have not been reported, and the genetic basis for common observations still remains unclear.

Molecular marker techniques greatly facilitate genetic dissection of the relationships among traits and make it possible to dissect the polygenes for quantitative traits into Mendelian factors. Many researchers have attempted to dissect the genetic basis of heading date (Mackill et al., 1993; Li et al., 1995; Yano et al., 1997; Lin et al., 1998; Yamamoto et al., 1998; Xing et al., 2001; Yu et al., 2002), tiller number (Ray et al., 1996; Yan et al., 1998b; Li et al., 1999; Wu et al., 1999), and

Table 1. Plant height, tiller number and heading date of parents and recombinant inbred (RI) lines grown at IRRI farm in 1999 and 2000.

Traits [†]	Minghui 63	Zhenshan 97	F ₁	LSD	Recombinant inbred lines			
					Mean ± SD	Range	Skew	Kurt
1999								
PH1	65.1	68.3	67.5	2.19 [‡]	66.7±2.7	43.3 - 95.7	0.38	3.42
PH2	115.6	86.0	106.5	5.14 [§]	100.5±2.1	70.4 - 163.2	0.33	2.59
TN1	27.8	17.8	23.2	4.12 [§]	21.8±3.3	14.2 - 33.5	0.43	1.05
TN2	15.3	15.3	15.4	1.51 [‡]	14.7±1.9	10.8 - 21.3	0.81	0.72
HD	87.3	60.3	70.0	2.51 [§]	73.8±0.9	54.0 - 96.7	-0.05	-0.89
2000								
PH1	71.2	73.1	73.2	2.80 [‡]	75.5±5.2	54.0 - 91.0	-0.23	1.20
PH2	110.2	93.8	100.2	8.32 [§]	97.1±3.1	74.0 - 161.1	1.33	1.80
TN1	25.5	21.6	25.4	3.04 [‡]	21.1±2.7	13.2 - 32.7	0.29	0.41
TN2	14.8	13.5	14.4	4.78 [§]	14.3±1.2	9.7 - 20.8	0.49	0.46
HD	87.3	65.0	71.0	4.65 [§]	73.7±1.0	54.7 - 95.5	-0.07	-0.61

[†] PH1 and TN1 refer to the plant height and tiller number per hill at 35 days after transplanting. PH2 and TN2 refer to the plant height and tiller number per hill at maturity. HD refers to the heading date.

[‡] LSD_{0.05}, [§] LSD_{0.01} for the trait.

plant height (Beavis et al., 1991; Li et al., 1995; Lin et al., 1995; Huang et al., 1996; Yan et al., 1998a; Xing et al., 2001; Yu et al., 2002). However, most of them put their interests on identification of quantitative trait loci (QTLs) for each individual trait. This paper reports the identification of QTLs and two-locus interactions for tiller number, plant height and heading date of rice carried out using a recombinant inbred population with special emphasis on the genetic basis underlying the relationship among the three traits.

Materials and Methods

1. Plant materials and field experiment

A population consisting of 241 recombinant inbred (RI, F₁₀ and F₁₁) lines constructed by single seed descent from a cross between Zhenshan 97 and Minghui 63 (*Oryza sativa* L. ssp. indica) was employed. Field experiments were conducted at International Rice Research Institute (IRRI) in the Philippines in the dry seasons (January to May) of 1999 and 2000. A randomized complete block design was used with three replications. The two parents, the F₁ (Shanyou 63) and 241 RI lines were transplanted in five rows with 12 hills per row in 1999 and 15 hills per row in 2000. Transplanting was done on 6 January in 1999 and on 12 January in 2000 using 14-day-old seedlings. Other crop management practices were identical in both years. Plant spacing was 20 × 20 cm with three seedlings per hill. A total of 220 kg N ha⁻¹ in the form of urea was applied in four divided doses [60 kg ha⁻¹ at one day before transplanting, 60 kg ha⁻¹ at about 20 day after transplanting (DAT), 60 kg ha⁻¹ at about 40 DAT, and 40 kg ha⁻¹ at about 70 DAT] to ensure N sufficiency

for all entries. Phosphorus (30 kg P ha⁻¹ as single superphosphate), potassium (40 kg K ha⁻¹ as KCl), and zinc (5 kg Zn ha⁻¹ as zinc sulfate heptahydrate) were incorporated in all plots one day before transplanting. Pests and diseases were intensively controlled.

2. Measurement of plant height, tiller number and heading date

The first measurement of plant height and tiller number per hill was carried out on nine hills in the middle of each plot at 35 DAT. Plant height was the distance between the soil surface and the tip of the highest leaf within a hill. A shoot with at least one visible leaf was counted as a tiller. In this study, the main stem or mother tiller was counted as one tiller and included in the total tiller number. The same measurement was carried out on 12 harvested hills at maturity. Plant height at maturity was the distance between the plant base and the tip of the highest leaf or panicle, whichever was taller within a hill. In this study, heading date was defined as when 50% of hills in a plot had at least one panicle completely emerged and was quantified as days from seeding to heading.

3. Data analysis

The trait measurements averaged over the three replications were used for analysis. Heritability (H_b^2) based on RI lines were estimated according to the following equation: $H_b^2 = \sigma_g^2 / (\sigma_g^2 + \sigma_e^2 / re + \sigma_{ge}^2 / e)$, where r is the number of replications per year, e is number of environments (years), σ_g^2 is genetic variance, σ_{ge}^2 is variance of genotype × environment interaction, and σ_e^2 is residual variance.

The molecular-marker linkage map and markers used were as described by Xing et al. (2002). The map consisted of 221 marker loci and covered a total of 1796 cM. The chromosomal locations and the number of putative QTLs were determined using QTLMAPPER (Wang et al., 1999) in a mixed linear model. A LOD threshold of 2.4 and a probability of 0.005 were used for declaring the existence of a putative QTL. The possible digenic interaction was investigated over the entire genome using EPISTAT (Chase et al., 1997), and then the significant digenic interaction at $p \leq 0.01$ was determined by two-way analysis of variance using STATISTICA (Statsoft Inc., 1991).

Results

1. The performance of the traits

At 35 DAT, the two parents showed small differences in plant height in both 1999 and 2000 (Table 1). However, at maturity Minghui 63 was significantly taller than Zhenshan 97. The two parents showed large differences in tiller number at 35 DAT and Minghui 63 was the high value parent in both 1999 and 2000, while no apparent difference was observed at maturity. There was a greater decline in tiller number at maturity in Minghui 63 than Zhenshan 97. Significant differences in heading date between the two parents were observed in the two years. Zhenshan 97 flowered

earlier than Minghui 63 by more than 22 days.

For all traits, continuous distribution among RI lines was observed in the two years. Transgressive segregation was found for each trait studied. Generally, skewness values were less than 1.0 except for plant height at maturity in 2000, suggesting normal distributions for most of the traits in this study.

2. Correlation analysis

Overall, significant and negative correlations between tiller number and plant height were observed at each growth stage in the two years (Table 2). However, correlations between tiller number and plant height measured at different growth stages were rather weak and inconsistent. Heading date was significantly negatively correlated with tiller number at maturity in both 1999 and 2000, while tiller number at 35 DAT had relatively weak correlation with heading date. Although there was no apparent and consistent correlation between heading date and plant height at 35 DAT, positive correlations between heading date and plant height at maturity were observed in the two years. The correlation between tiller number at 35 DAT and at maturity was relatively weak and this was also true for plant height. A close correlation for the same trait between the two years was observed, and correlation coefficients ranged from 0.67 for tiller number at 35 DAT to 0.93 for heading date (Table 3), which suggested similar performances of the three traits across seasons, as shown by the high heritability (Table 3).

Table 2. Simple correlations among plant height, tiller number and heading date in 1999 (upper) and 2000 (lower).

Traits [†]	PH2	TN1	TN2	HD
PH1	0.17*	-0.53**	-0.22**	-0.04
	0.39**	-0.51**	-0.37**	0.18*
PH2		0.14*	-0.57**	0.79**
		0.04	-0.60**	0.64**
TN1			0.20**	0.25**
			0.26**	0.14*
TN2				-0.59**
				-0.63**

[†] PH1 and TN1 refer to the plant height and tiller number per hill at 35 days after transplanting. PH2 and TN2 refer to the plant height and tiller number per hill at maturity. HD refers to the heading date.

*, **Indicate the significance at 0.05 and 0.01 probability levels, respectively.

3. QTLs for plant height

For plant height at 35 DAT (PH1), a total of five and four QTLs were detected, together explaining 46.03% of total variation in 1999 and 27.26 % of total variation in 2000, respectively (Table 4 and Fig. 1). Three QTLs (qPH1-1-1, qPH1-3-1 and qPH1-4-1) for PH1 were identified in the two years. Both qPH1-5-1 and qPH1-6-2 showed their effects only in 1999, while qPH1-6-1 only in 2000.

Four QTLs for plant height at maturity (PH2) were detected in the two years. All alleles came from Minghui 63 except for qPH2-3-1 that was detected only in 2000. The qPH2-6-1 was localized in the interval of RG424-RZ667 on chromosome 6, which

Table 3. Simple correlations for an identical trait between the two years and trait heritability.

Traits [†]	PH1	PH2	TN1	TN2	HD
Correlation between two years	0.71**	0.91**	0.67**	0.73**	0.93**
Heritability (%)	82.6	93.5	80.4	84.2	95.6

[†] PH1 and TN1 refer to the plant height and tiller number per hill at 35 days after transplanting. PH2 and TN2 refer to the plant height and tiller number per hill at maturity. HD refers to the heading date.

**Indicate the significance at 0.01 probability levels.

Table 4. Putative QTLs for plant height, tiller number and heading date in 1999 and 2000.

Tri [†]	QTL [‡]	Chrom	1999				2000				
			Interval	LOD	Add [§]	Var% [¶]	Interval	LOD	Add	Var %	
PH1	qPH1-1-1	1	RM81A-RG173	6.79	1.58	11.48	RM81A-RG173	3.22	1.02	6.71	
	qPH1-3-1	3	RM148-R1925	4.85	1.10	5.62	RM148-R1925	5.62	1.16	8.64	
	qPH1-4-1	4	RM241-C2807	9.05	-1.74	13.94	RM241-C2807	4.65	-1.05	7.12	
	qPH1-5-1	5	RM31-C1447	8.88	-1.65	12.64					
	qPH1-6-1	6					R3139-C474	3.28	0.86	4.79	
	qPH1-6-2	6	R2749-R1952a	2.90	0.71	2.35					
					Total				Total		
										46.03	27.26
PH2	qPH2-3-1	3					RZ403-C1087	4.37	-1.98	6.06	
	qPH2-4-1	4	C107-C1016	2.99	1.67	2.26					
	qPH2-4-2	4					G235-G102	5.59	2.16	7.20	
	qPH2-6-1	6	RG424-RZ667	22.51	6.90	38.40	RG424-RZ667	18.41	4.45	26.50	
					Total				Total	40.66	39.76
TN1	qTN1-1-1	1	RM81A-RG173	4.13	-0.84	7.45	RM81A-RG173	7.97	-1.03	12.28	
	qTN1-5-1	5	C624-RZ649	2.75	0.63	4.19					
	qTN1-6-1	6	R2147-P	3.17	0.77	6.21					
	qTN1-6-2						RG424-RZ667	2.51	0.55	2.94	
	qTN1-6-3	6	R2749-R1952a	5.47	-0.95	9.39					
	qTN1-6-4						C688-C1496	5.63	-0.83	7.97	
	qTN1-7-1	7	R1789-RM234	4.82	-0.93	9.00	R1789-RM234	2.67	-0.63	4.59	
	qTN1-8-1	8					RG333-C1121	4.59	0.71	5.97	
	qTN1-9-1	9	RG667-RG570	3.41	0.70	4.55	RG667-RG570	3.04	0.59	4.06	
					Total				Total	32.56	37.02
TN2	qTN2-1-1	1					C2340-C567	6.29	0.44	8.32	
	qTN2-3-1	3	R1925-RM227	5.37	-0.43	6.23					
	qTN2-6-1	6	RG424-RZ667	21.93	-0.96	32.08	RG424-RZ667	16.60	-0.74	23.72	
	qTN2-10-1	10	RM258-C677	6.39	0.48	8.01	RM258-C677	5.58	0.40	7.01	
					Total				Total	46.32	39.05
HD	qHD-6-1	6	RG424-RZ667	31.66	5.84	43.30	RG424-RZ667	34.03	5.36	44.10	
	qHD-7-1	7	R1440-C1023	5.36	2.12	5.69					
	qHD-10-1	10					RG561-RM258	3.83	1.52	3.54	
	qHD-10-2	10					C1633-RM239	7.12	-2.29	8.06	
	qHD-11-1	11					Clone2-C405b	6.20	1.93	5.73	
	qHD-11-2	11	C794-R2918	6.25	2.26	6.50					
					Total				Total	55.49	59.69

[†] PH1 and TN1 refer to the plant height (cm) and tiller number per hill at 35 days after transplanting. PH2 and TN2 refer to the plant height and tiller number per hill at maturity. HD refers to the heading date (day).

[‡] QTL nomenclature follows that of McCouch et al. (1997).

[§] The negative value of additive effect indicates that the allele from Zhenshan 97 is in the direction of increasing the trait score.

[¶] Var% represents variation explained by each QTL.

alone explained 38.40% of total variation in 1999 and 26.50% of total variation in 2000. The allele at this QTL from Minghui 63 increased plant height by 6.90 cm in 1999 and 4.45 cm in 2000, respectively. The qPH2-4-1 or qPH2-4-2 was detected only in one year.

4. QTLs for tiller number

For tiller number at 35 DAT (TN1), nine QTLs were identified in the two years (Table 4 and Fig. 1). Among the nine identified QTLs, qTN1-1-1, qTN1-7-1, and qTN1-9-1 were detected on the same chromosomal regions in both years. However, they explained a different percentage of total variation across the two years. For example, qTN1-7-1 explained 9.00% of

total variation in 1999 but only 4.59% in 2000. The qTN1-1-1 accounted for 7.45% of total variation in 1999 and 12.28% of total variation in 2000. This allele was from Zhenshan 97 and increased tiller number by approximately one in the two years. The QTL qTN1-6-1 detected in 1999 was near qTN1-6-2 detected in 2000, and qTN1-6-3 detected in 1999 was near qTN1-6-4 detected in 2000.

For tiller number at maturity (TN2), four QTLs were detected over the two years. The qTN2-1-1 was identified only in 2000 while qTN2-3-1 was detected only in 1999. The QTLs, qTN2-6-1 and qTN2-10-1, showed their effects on tiller number at maturity in both years. The qTN2-6-1 explained 32.08% of total

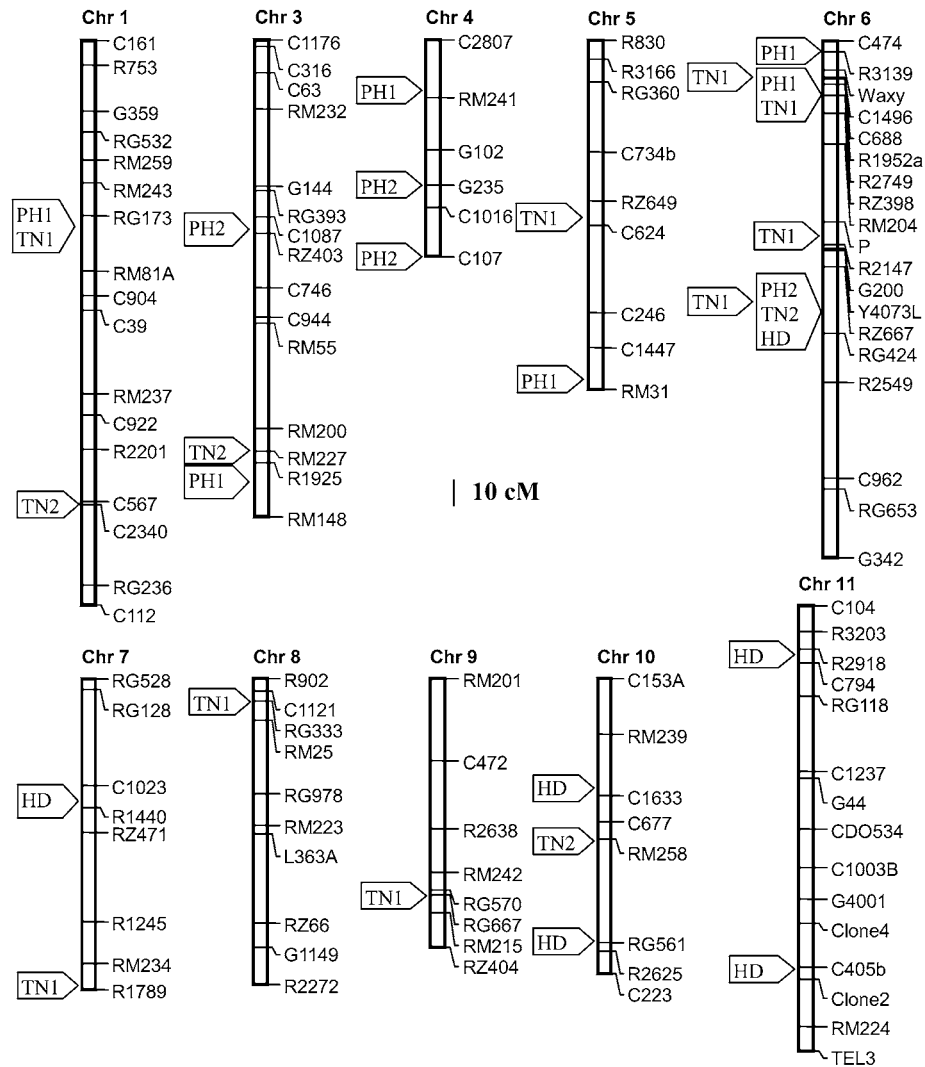


Fig. 1. Locations of QTLs for tiller number, plant height and heading date in recombinant inbred lines derived from a cross between Zhenshan 97 and Minghui 63. PH1 and TN1 refer to plant height and tiller number per hill at 35 days after transplanting. PH2 and TN2 refer to plant height and tiller number per hill at maturity. HD refers to heading date.

variation in 1999 and 23.72% of total variation in 2000. This allele was from Zhenshan 97 and increased tiller number by approximately one in the two years.

5. QTL for heading date

Three QTLs for heading date (HD) were detected in 1999, which together explained 55.49 % of total variation, while four QTLs were detected in 2000, which explained 59.69% of total variation (Table 4). The QTL with a large effect, qHD-6-1 on chromosome 6 was detected in both years, and it accounted for 43.30% of total variation in 1999 and 44.10% of total variation in 2000. The other QTLs with small effects were identified only in one year.

6. Analyses of two-locus interactions

Table 5 presents the numbers of two-locus

combinations showing significant interactions ($p \leq 0.01$) involving markers distributed in the entire genome and of two-locus combinations with effects on at least two traits. Several general comments can be made about these interactions: (1) More interactions for plant height and tiller number were detected at an early stage of growth in comparison with those at maturity in both years. For example, 597 interactions were identified for plant height at 35 DAT, while only 243 were recorded at maturity in 1999. (2) The proportions of common interactions detected in both years ranged from 8.99% for tiller number at maturity to 34.75% for heading date. (3) The percentages of common interactions for plant height or tiller number between the two stages (35 DAT and maturity) ranged from 0.72% to 3.56%. (4) A total of 220 and 139 two-locus combinations were detected to have pleiotropic

effects on at least two traits in 1999 and 2000, respectively. In 1999, 80 and 33 combinations had simultaneous effects on tiller number and plant height at 35 DAT and at maturity, respectively, 65 on heading date and plant height at maturity, 10 on heading date, plant height and tiller number at maturity. Similar results were also detected in 2000, as presented in Table 5. A total of 55 common two-locus combinations had pleiotropic effects by affecting at least two traits over the two years. Twelve combinations simultaneously affected tiller number and plant height at 35 DAT, while 21 combinations affected both heading date and plant height at maturity. (5) Interactions between QTL and loci in the rest of the genome were assessed, using markers flanking QTL. A total of 31 two-locus interactions were identified between markers flanking the identified QTLs for different traits (data not shown). One interaction between QTL-linked markers was found (qPH1-1-1 and qPH1-6-2). The majority of the interactions occurred between markers not linked to QTL affecting the identical trait (data not shown).

Discussion

1. Detection of QTL

Taken together, more QTLs for tiller number and plant height were identified at an early growth stage rather than at maturity in the two years. Most QTLs for tiller number and plant height detected at an early growth stage could not be detected at maturity. Generally, the parent with a lower trait score also provided chromosomal regions for increasing phenotypic score.

Several QTLs shared the same chromosomal regions. The QTL in the vicinity of the genomic region around RZ667 on chromosome 6 was simultaneously detected for tiller number at 35 DAT, tiller number and plant height at maturity, and heading date in the two years (Fig. 1). The qPH1-1-1 on chromosome 1 was detected to share the same chromosomal region (RM81A-RG173) with qTN1-1-1 in 1999 and 2000. Furthermore, qPH1-6-2 for plant height shared the same interval (R2749-R1952a) with qTN1-6-3 for tiller number in 1999, while qPH1-6-1 was near qTN1-6-4 in 2000 (Fig. 1).

Different genes are expressed at various developmental stages according to views of developmental genetics (Yan et al., 1998a). Therefore, research on quantitative traits at different development stages is necessary. In this study, QTL mapping generally revealed that QTLs for plant height or tiller number at the two stages were different, suggesting that QTLs for both plant height and tiller number were developmental-stage-dependent. Similar results have been reported by Yan et al. (1998a, b), Wu et al. (1999) and Cao et al. (2001). Therefore, the present results suggest that both plant height and tiller number may be controlled by different genes

expressed selectively at different developmental stages.

Interactions with other genes and environmental factors such as temperature, nutrient supply, water availability and photoperiod, may modify a given gene expression. Numbers of QTLs detected in different environments are often used to determine genotype \times environment interaction (Stuber et al., 1992; Lu et al., 1996). In the present study, approximately 65% (19/29) of QTLs were detected only in one year, suggesting environmental effects on QTL detection. The genotype \times environment interaction was trait-dependent. For example, 50% of QTLs for plant height at 35 DAT were detected in both years, while only one of six QTLs for heading date were identified in the two years. Moreover, environmental factors also had influences on the percentage of total variation explained by the identical QTL in the two years. For example, qPH1-4-1 for plant height at 35 DAT explained 13.94% of total variation in 1999, while it accounted for 7.12% in 2000. In the same mapping population growing in Wuhan, China, Xing et al. (2001) identified a QTL with a relatively large effect for heading date with explanations of 19.6% and 15.4% of total variation in two years, respectively. This QTL was located in R1440-C1023 on chromosome 7. A similar QTL with relatively small effects (5.69%) on heading date was also mapped in the same chromosomal region in 1999 in the present study. Similarly, the region (RG424-RZ667) were detected to have a large effect on heading date in the two years in this study, while it had a small effect on heading date (6.7%) only in one year when the same mapping population was grown in Wuhan, China (Xing et al., 2001). These results strongly suggest that the traits are affected by genotype \times environment interaction, although performances of traits were similar in the two years in this study.

Overall, Xing et al. (2001) detected 4 QTLs for plant height at maturity on chromosomes 1, 6, 7, and 8 and 4 QTLs for heading date on chromosomes 6 and 7. Yu et al. (2002) located 6 QTLs for plant height on chromosome 1, 3, 5, 7, and 11 and 6 QTLs for heading date on chromosomes 1, 3, 5, 7, and 11. The QTLs for plant height at maturity and heading date that were detected in this study were largely different from those reported by Xing et al. (2001) and Yu et al. (2002), although the same mapping population was used. The discrepancy is probably attributed to the different growing conditions. Another possibility is that some minor QTLs were undetected at the threshold set for QTL declaration.

Correspondence between known major genes and detected putative QTL has been reported. For example, Beavis et al. (1991) found that the chromosomal location of several major genes for dwarfing in maize were in agreement with those of QTLs for plant height. In the study presented here,

a QTL for plant height at maturity was located in the region of RG424-RZ667 on chromosome 6, in which a major gene for plant height (*d9*) was also located (Huang et al. 1996). A large effect QTL, qHD-6-1 for heading date, was localized at the interval of RG424-RZ667 on chromosome 6 in the present study. Several papers have shown that a region around RZ667 on chromosome 6 has a large effect on heading date (Yano et al., 1997; Lin et al., 1998; Yamamoto et al., 1998). According to published papers and genetic maps, a major photoperiod-sensitivity gene (*Se-1*) may localize in the same region near RZ667 on chromosome 6 (Yokoo et al., 1980; Poonyarit et al., 1989; Mackill et al., 1993; Yan et al., 1998b; Maheswaran et al., 2000). Yano et al. (2000) identified a genomic region of 12 kb as a candidate for *Hd-1*, which is allelic to *Se-1* and has high homology with CONSTANTS, a gene for flowering time in *Arabidopsis*. Therefore, our results suggest that qHD-6-1 in the studied population might be the locus with the main effect for heading date in the environment involved in the study, or may well correspond with the locus *Se-1*. The positional correspondence between the QTLs and major genes may be the evidence for supporting the hypothesis that genes at QTL were alleles of known major genes for the identical trait (Huang et al., 1996; Yano and Sasaki, 1997). Fine mapping of QTL should be necessary for determining the exact relationships between them.

In the present study, a QTL for tiller number at an early growth stage was located near marker Waxy on chromosome 6. Ray et al. (1996) also found a QTL for tiller number near Waxy. Both qTN1-1-1 and qTN1-9-1 for tiller number at an early stage were found in two environments. Two QTLs affecting tiller angle in rice were found in the interval of RG532-RG173 on chromosome 1 and near RG667 on chromosome 9 by Li et al. (1999). These results suggested that the regions near RG173 and near RG667 have pleiotropic effects on tiller number and tiller angles. Though these associations are quite preliminary, they suggest that the plant type with a large angle tends to have more tillers.

2. Detection of epistasis

Epistatic interactions play an important role in the genetic basis of quantitative traits (Lark et al., 1995; Yu et al., 1997; Xing et al., 2002). In this study, a large number of two-locus interactions were detected at $p \leq 0.01$ in both years by analysis of variance. Firstly, pairwise epistatic interaction analyses showed that the proportion of common two-locus combinations detected in both years for studied traits ranged from 8.99% to 34.75% (Table 5), suggesting that epistasis is also dependant on the environment. Liao et al. (2001) reported that epistatic loci could be interchangeable depending probably on the environment. Secondly, the proportions of common two-locus combinations

Table 5. Summary of two-locus interaction analysis ($p \leq 0.01$).

	Significant interaction			
	1999	2000	Common	
PH1 [†]	597	434	156	(17.83%) [‡]
PH2	243	237	121	(33.70%)
Common	6 (0.72%)	22 (3.39%)	4	(1.47%)
TN1	431	315	99	(15.30%)
TN2	398	208	50	(8.99%)
Common	22 (2.73%)	18 (3.56%)	3	(2.05%)
HD	240	237	123	(34.75%)
Two-locus combinations with pleiotropic effects				
	222	139	55	
TN1 PH1	80	29	12	(12.37%)
TN2 PH2	33	14	6	(14.63%)
HD TN1	12	16	2	(7.69%)
HD TN2	23	28	4	(8.51%)
HD PH1	6	2	2	(33.33%)
HD PH2	65	37	21	(25.92%)
HD PH1 TN1	1	0	0	(0.00%)
HD PH2 TN2	10	6	1	(6.67%)

[†] PH1 and TN1 refer to the plant height and tiller number per hill at 35 days after transplanting. PH2 and TN2 refer to the plant height and tiller number per hill at maturity. HD refers to the heading date.

[‡] Percentage in brackets is the proportion of common two-locus combinations.

for either plant height or tiller number detected at two stages in this study were less than 4%. These results indicate that different interactions affected the trait performance at different developmental stages. Cao et al. (2001) also suggested that most epistasis was only revealed at some specific stages by conditional mapping. Generally, two-locus interactions for tiller number, plant height and heading date are considered to be dependent on the environment and developmental stages. In addition, the proportions of common interactions seem to suggest that epistasis is more sensitive to the developmental stage than to the environment.

3. Relations among tillers, plant height and heading date

Plants with single culms are taller compared with normally-tillered cereals (Richards, 1988), implying that lowering tillering ability may increase plant height. In our study, significant negative correlations were observed between plant height and tiller number at each of the two growth stages in the two years. The negative relationship may be attributed to competitions for limited nutrient supply and available assimilates between tillering and stem elongation. On the other hand, mutual shading due to taller stature may cause limitation of carbohydrates for smaller tillers, and further result in their death. According to Aastveit and Aastveit (1993), pleiotropy, linkage and environmental effects are the main causes of correlations among traits. In this paper, several intervals (such as RM81A-RG173, R2749-R1952a,

and RG424-RZ667) had pleiotropic effects on plant height and tiller number, providing the genetic basis for the strong correlations between them. Similar results have been reported by Veldboom et al. (1994). Quantitatively, for example, allele at RM81A-RG173 from Minghui 63 may increase plant height by 1.58 cm, and allele from Zhenshan 97 may increase tiller number by approximately one at an early stage in 1999. Moreover, 113 and 43 two-locus combinations were identified to simultaneously affect plant height and tiller number over the two growth stages in 1999 and 2000, respectively. So pleiotropic effects of digenic interactions may play a role in causing the relation between plant height and tiller number.

A significant positive correlation was observed between plant height at maturity and heading date. This suggests that delay of heading date in rice might result in an increase in plant height. Similar results were also observed in sorghum (Lin et al., 1995) and in rice (Li et al., 1995; Yu et al., 2002). A QTL with a relatively large effect for heading date shared the interval of RG424-RZ667 with qPH2-6-1 for plant height at maturity. Co-location of QTLs for plant height and heading date is consistent with the result of Lin et al. (1995), Li et al. (1995) and Yu et al. (2002). As shown by Yu et al. (2002), our results also demonstrated that epistasis plays an important role as the genetic basis of relationship between plant height and heading date. Furthermore, 65 and 37 two-locus combinations had effects on both plant height at maturity and heading date simultaneously in 1999 and 2000, respectively. Therefore, both co-location of QTLs and common interactions were involved as the genetic basis of the high correlation existed between plant height at maturity and heading date.

Plants with a single culm flower earlier than normal-tillered cereals (Richards, 1988). The result of Richards (1988) from wheat with a tiller inhibitor gene, however, did not clarify the association between heading date and tiller number. In this study, a QTL qHD-6-1 was located near qTN1-6-1 for tiller number at 35 DAT with additive effects in the same direction. This is in agreement with the slight positive correlation between heading date and tiller number at an early stage. However, a QTL with a large effect for heading date (qHD-6-1) shared the same chromosomal region with a QTL with a large effect for tiller number at maturity (qTN2-6-1) with additive effects in an opposite direction. This is consistent with the significant negative correlation between tiller number at maturity and heading date. Longer vegetative growth duration seems to extend tillering duration, while excess tillering results in more tiller abortion at a late stage due to limitation of horizontal space, available carbohydrates, and nutrients (Peng et al., 1994). On the other hand, 35 and 44 two-locus combinations exerted their pleiotropic effects on both

heading date and tiller number in 1999 and 2000, respectively, suggesting the role of epistasis in causing the relation between the two traits.

The number of two-locus combinations shared by two traits seem be associated with a correlation coefficient between the two identical traits. A total of 65 combinations shared by heading date and plant height at maturity in 1999, between which the correlation was 0.79. On the other hand, only six combinations shared by heading date and plant height at 35 DAT, between which the correlation was -0.04. Similar cases were observed for other traits (Table 2 and Table 5). In this study, the chromosomal region of RG424-RZ667 had pleiotropic large effects on heading date, plant height, and tiller number at maturity. Additionally, a total of ten and six two-locus combinations had effects on plant height and tiller number at maturity, and heading date simultaneously. These results also suggested that pleiotropy of QTLs and two-locus interactions may be involved in the relationship among the three traits.

In summary, the results clearly illustrate that the close linkage of QTLs for different traits, pleiotropic effects of chromosomal regions and two-locus combinations, which had effects on multiple traits, may be the genetic basis of the relationships among heading date, tiller number and plant height.

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References

- Aastveit, A.H. and Aastveit, K. 1993. Effects of genotype-environment interactions on genetic correlation. *Theor. Appl. Genet.* 86 : 1007-1013.
- Akita, S. 1989. Improving yield potential in tropical rice. In *Progress in Irrigated Rice Research. Proc. Intl. Rice Res. Conf., Hangzhou, China.* 41-73.
- Beavis, W.D., Grant, D., Albertsen, M. and Fincher, R. 1991. Quantitative trait loci for plant height in four maize populations and their associations with qualitative genetic loci. *Theor. Appl. Genet.* 83 : 141-145.
- Cao, G., Zhu, J., He, C., Gao, Y., Yan, J. and Wu, P. 2001. Impact of epistasis and QTL×environment interaction on the developmental behavior of plant height in rice (*Oryza sativa* L.). *Theor. Appl. Genet.* 103 : 153-160.
- Chandler, R.F. Jr. 1969. Plant morphology and stand geometry in relation to nitrogen. In J.D. Eastin, F.A. Haskins, C.Y. Sullivan and C.H.M. van Bavel eds., *Physiological Aspects of Crop Yield.* ASA, Madison, Wisconsin, USA. 265-285.
- Chase, K., Adler, F.R. and Lark, K.G. 1997. Epistat: a computer program for identifying and testing interactions between pairs of quantitative trait loci. *Theor. Appl. Genet.* 94 : 724-730.
- Gent, M.P.N. 1995. Canopy light interception, gas exchange, and biomass in reduced height isolines of winter wheat. *Crop*

- Sci. 35 : 1636-1642.
- Huang, N., Courtois, B., Khush, G.S., Lin, H.X. and Wang, G.L. 1996. Association of quantitative trait loci for plant height with major dwarfing genes in rice. *Heredity* 77 : 130-137.
- Kuroda, E., Ookawa, T. and Ishihara, K. 1989. Analysis on difference of dry matter production between rice cultivars with different plant height in relation to gas diffusion inside stands. *Jpn. J. Crop Sci.* 58 : 374-382.
- Lark, K.G., Chase, K., Adler, F., Mansur, L.M. and Orf, J.H. 1995. Interactions between quantitative loci in soybean in which trait variation at one locus is conditional upon a specific allele at another. *Proc. Natl. Acad. Sci. USA* 92 : 4656-4660.
- Li, Z., Paterson, A.H., Pinson, S.R.M. and Stansel, J.W. 1999. RFLP facilitated analysis of tiller and leaf angles in rice (*Oryza sativa* L.). *Euphytica* 109 : 79-84.
- Li, Z., Pinson, S.R.M., Stansel, J.W. and Park, W.D. 1995. Identification of quantitative trait loci (QTLs) for heading date and plant height in cultivated rice (*Oryza sativa* L.). *Theor. Appl. Genet.* 91 : 374-381.
- Liao, C.Y., Wu, P., Hu, B. and Yi, K.K. 2001. Effects of genetic background and environment on QTLs and epistasis for rice (*Oryza sativa* L.) panicle number. *Theor. Appl. Genet.* 103 : 104-111.
- Lin, S.Y., Sasaki, T. and Yano, M. 1998. Mapping quantitative trait loci controlling seed dormancy and heading date in rice, *Oryza sativa* L., using a backcross inbred lines. *Theor. Appl. Genet.* 96 : 997-1003.
- Lin, Y.R., Schertz, K.F. and Paterson, A.H. 1995. Comparative analysis of QTLs affecting plant height and maturity across the Poaceae, in reference to an interspecific sorghum population. *Genetics* 141 : 391-411.
- Lu, C., Shen, L., Tan, Z., Xu, L., He, P., Chen, Y. and Zhu, L. 1996. Comparative mapping of QTLs for agronomy traits of rice across environments using a doubled haploid population. *Theor. Appl. Genet.* 93 : 1211-1217.
- Mackill, D.J., Salam, M.A., Wang, Z.Y. and Tanksley, S.D. 1993. A major photoperiod-sensitivity gene tagged with RFLP and isozyme markers in rice. *Theor. Appl. Genet.* 85 : 536-540.
- Maheswaran, M., Huang, N., Sreeragasamy, S.R. and McCouch, S.R. 2000. Mapping quantitative trait loci associated with days to flowering and photoperiod sensitivity in rice (*Oryza sativa* L.). *Mol. Breed.* 6 : 145-155.
- McCouch, S.R., Cho, Y.G., Yano, M., Paul, E. and Blinstruub, M. 1997. Report on QTL nomenclature. *Rice Genet. Newsl.* 14 : 11-13.
- Peng, S., Khush, G.S. and Cassman, K.G. 1994. Evolution of the new plant ideotype for increased yield potential. In K.G. Cassman ed., *Breaking the Yield Barrier. Proceedings of a Workshop on Rice Yield Potential in Favorable Environments.* International Rice Research Institute, Los Baños, Philippines. 5-20.
- Poonyarit, M., Mackill, D.J. and Vergara, B.S. 1989. Genetics of photoperiod sensitivity and critical daylength in rice. *Crop Sci.* 29 : 647-652.
- Ray, J.D., Yu, L., McCouch, S.R., Champoux, M.C., Wang, G. and Nguyen, H.T. 1996. Mapping quantitative trait associated with root penetration ability in rice (*Oryza sativa* L.). *Theor. Appl. Genet.* 92 : 627-636.
- Richards, R.A. 1988. A tiller inhibitor gene in wheat and its effect on plant growth. *Aust. J. Agric. Res.* 39 : 749-757.
- Stuber, C.W., Lincoln, S.E., Wolff, D.W., Helentjaris, T. and Lander, E.S. 1992. Identification of genetic factors contributing to heterosis in a hybrid from two elite maize inbred lines using molecular markers. *Genetics* 132 : 823-839.
- Tanaka, A., Kawano, K. and Yamaguchi, J. 1966. Photosynthesis, respiration, and plant type of the tropical rice plant. *Intl. Rice Res. Inst. Tech. Bull.* No. 7.
- Veldboom, L.R., Lee, M. and Woodman, W.L. 1994. Molecular marker-facilitated studies in an elite maize population: I. Linkage analysis and determination of QTL for morphological traits. *Theor. Appl. Genet.* 88 : 7-16.
- Vergara, B.S., Tanaka, A., Lilis, R. and Puranabhavung, S. 1966. Relationship between growth duration and grain yield of rice plants. *Soil Sci. Plant Nutr.* 12 : 31-39.
- Wang, G.L., Zhu, J., Li, Z.K. and Paterson, A.H. 1999. Mapping QTLs with epistatic effects and QTL×environment interactions by mixed linear model approaches. *Theor. Appl. Genet.* 94 : 1255-1264.
- Wu, G.W., Li, W.M., Tang, D.Z., Lu, H.R. and Worland, A.J. 1999. Time-related mapping of quantitative trait loci underlying tiller number in rice. *Genetics* 151 : 297-303.
- Xing, Y.Z., Tan, Y.F., Hua, J.P., Sun, X.L., Xu, C.G. and Zhang, Q. 2002. Characterization of the main effects, epistatic effects and their environmental interactions of QTLs in the genetic basis of yield traits in rice. *Theor. Appl. Genet.* 105 : 248-257.
- Xing, Y.Z., Xu, C.G., Hua, J.P., Tan, Y.F. and Sun, X.L. 2001. Mapping and isolation of quantitative trait loci controlling plant height and heading date in rice. *Acta Botanica Sinica* 43 : 721-726.
- Yamamoto, T., Kuboki, Y., Lin, S.Y., Sasaki, T. and Yano, M. 1998. Fine mapping of quantitative trait loci Hd-1, Hd-2 and Hd-3, controlling heading date of rice, as single Mendelian factors. *Theor. Appl. Genet.* 97 : 37-44.
- Yan, J.Q., Zhu, J., He, C.X., Benmoussa, M. and Wu, P. 1998a. Molecular dissection of the developmental behavior of plant height in rice (*Oryza sativa* L.). *Genetics* 150 : 1257-1265.
- Yan, J.Q., Zhu, J., He, C.X., Benmoussa, M. and Wu, P. 1998b. Quantitative trait loci analysis for the developmental behavior of tiller number in rice (*Oryza sativa* L.). *Theor. Appl. Genet.* 97 : 267-274.
- Yano, M., Harushima, Y., Nagamura, Y., Kurata, N., Minobe, Y. and Sasaki, T. 1997. Identification of quantitative trait loci controlling heading date in rice using a high-density linkage map. *Theor. Appl. Genet.* 95 : 1025-1032.
- Yano, M. and Sasaki, T. 1997. Genetic and molecular dissection of quantitative traits in rice. *Plant Mol. Bio.* 35 : 145-153.
- Yano, M., Katayose, Y., Ashikarib, M., Yamanouchic, U., Monac, L., Fuseb, T., Babac, T., Yamamoto, K., Umehara, Y., Nagamura, Y., Sasaki, T. 2000. Hd1, a major photoperiod sensitivity quantitative trait locus in rice, is closely related to the Arabidopsis flowering time gene *CONSTANTS*. *Plant Cell* 12 : 2473-2484.
- Yokoo, M., Kikuchi, F., Nakane, A. and Fujimaki, H. 1980. Genetic analysis of heading date by aid of close linkage with blast resistance in rice. *Bull. Natl. Inst. Agric. Sci. Ser. D.* 31 : 95-126.
- Yoshida, S. 1972. Physiological aspects of grain yield. *Annu. Rev. Plant Physiol.* 23 : 437-464.
- Yoshida, S. 1981. *Fundamentals of Rice Crop Science.* International Rice Research Institute, Los Baños, Philippines.

Yu, S.B., Li, J.X., Xu, C.G., Tan, Y.F., Gao, Y.J., Li, X.H., Zhang, Q. and Saghai Maroof, M.A. 1997. Importance of epistasis as the genetic basis of heterosis in an elite rice hybrid. Proc. Natl. Acad. Sci. USA 94 : 9226-9231.

Yu, S.B., Li, J.X., Xu, C.G., Tan, Y.F., Li, X.H. and Zhang, Q. 2002. Identification of quantitative trait loci and epistatic interactions for plant height and heading date in rice. Theor. Appl. Genet. 104 : 619-625.
