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ORIGINAL PAPER

Quantitative trait loci for panicle size, heading date and plant height co-segregating in trait-performance derived near-isogenic lines of rice (*Oryza sativa*)

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Abstract Near-isogenic lines (NILs) are ideal materials for precise estimation of quantitative trait loci (QTL) effects and map-based gene isolation. With the completion of the rice genome sequence, QTL isolation based on NILs is becoming a routine. In this study, a trait-performance derived NIL strategy was adopted to develop NILs. Two plants were identified within one inbred line of recombinant inbred lines (RILs, F_7 generation), exhibiting a significant difference in panicle size. By marker screening of the whole genome the genetic background of the two plants was estimated to be 98.7% identical. These two plants were selected as parents to produce a near-isogenic F_2 (NIL-F_2) population, consisting of 125 individuals, in which spikelets per panicle (SPP), grains per panicle (GPP), heading date (HD) and plant height (PH) were recorded. These four traits expressed discontinuous or bimodal distribution in the NIL-F_2 population and followed the expected segregation ratios for a single Mendelian factor by progeny tests. A partial dominant QTL for the four traits was mapped to the same interval flanked by RM310 and RM126 on chromosome 8. The QTL region explained 83.0, 80.2, 94.9 and 93.8% of trait variation of SPP, GPP, HD and PH in the progenies, respectively. Progeny tests also confirmed co-segregation of QTL for the four traits, tall plants consistently flowering late and carrying large panicles. Different NILs development strategies are discussed.

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Introduction

Complex traits, such as yield components, are inherited in a quantitative manner and typically controlled by a number of major and minor quantitative trait loci (QTL). Spikelets per panicle (SPP), grains per panicle (GPP), heading date (HD) and plant height (PH) are four traits frequently studied because of their importance in rice genetic improvement. Panicle size, normally measured by SPP and GPP, is an important component of yield. HD is a critical trait for rice adaptation to different cultivation areas and cropping seasons, and PH is one of the most important traits related to plant status and yield potential. Especially in the last decade, a number of studies were conducted to dissect the genetic basis of these traits with the advent of the molecular markers (Yu et al. 1997, 2002; Yano et al. 1997; Zhuang et al. 1997; Yamamoto et al. 2000; Xing et al. 2002).

Beneficial alleles for crop variety improvement could be identified both in superior and inferior varieties or wild relatives (Paterson et al. 1991; Xiao et al. 1998; Xiong et al. 1999; Tian et al. 2005). Once beneficial genes are identified, they can be directly introgressed into varieties by marker assisted selection (MAS). Primary mapping populations and insufficient population size limit identification of minor QTL and results in low resolution of QTL mapping. Thus, QTL are

frequently located in extended confidence intervals not in one site (Darvasi et al. 1993), resulting in linkage redundancy (i.e., too many genes in the QTL region) in turn complicating MAS. Blocking noise from the genetic background is necessary to precisely map QTL as a single Mendelian factor. Near-isogenic lines (NILs), segregating in small, defined regions of the genome, could eliminate the genetic background noise and make a given QTL express the characteristic of a qualitative gene. Over the past 5 years, several studies employed NILs to isolate QTL in rice and tomato (Frary et al. 2000; Spielmeyer et al. 2002; Li et al. 2004; Ashikari et al. 2005; Ren et al. 2005; Fan et al. 2006).

With the complete genome sequence of rice, chromosome-walking will be greatly reduced or even avoided in the process of map-based gene isolation. Hence, NIL development will play more important roles in QTL isolation. In this study, a straightforward and efficient strategy for characterizing a QTL was reported based on trait-performance derived NILs (TP-NILs).

Materials and methods

Discovery of TP-NILs in a F_7 recombinant inbred lines

In order to dissect the genetic basis of yield components in rice, a population consisting of 190 recombinant inbred lines (RILs) was developed by single-seed descent from a cross between two elite *indica* cultivar parents, Zhenshan 97 (ZS97) and HR5. ZS97 exhibits small panicles, short plants and early heading. HR5 exhibits large panicles, tall plants and late heading. F_7 generation plants of the RILs was used in a randomized complete block design with two replications in 2002 in Wuhan. Fourteen seedlings (approximately 22 days old) of each entry were transplanted into a two-row plot, with a distance of 17 cm between plants within a row, and 27 cm between rows. At the stage of flowering, one inbred line (RIL39) was found segregating in several traits in both replications. Within this inbred line, plant N15, with small panicle size, short PH and early heading, and plant N16, with large panicle size, tall PH and late heading, were selected as parents of a near-isogenic F_2 (NIL-F_2) population and crossed in the autumn of 2003 in Wuhan.

Development of a NIL-F_2 population

F_2 seeds were produced from one hybrid plant in the spring of 2004 in Hainan. In the summer of 2004, 125 F_2 plants were planted into bird-net-equipped field in the experimental farm in Huazhong Agricultural University. All F_2 plants were subsequently genotyped and phenotyped for SPP, GPP, HD and PH.

Progeny test

In May 2005, F_3 families were sowed into seedling beds. Twenty seedling plants, approximately 20 days old, for each family and each parent were transplanted into a two-row plot in the field (the experimental farm, Huazhong Agricultural University) with a distance of 17 cm between plants within a row, and 27 cm between rows. One hundred and nine families were used to conduct the progeny test due to bad germination of 16 families.

DNA marker and data analysis

DNA was extracted from fresh leafs at seedling stage employing the CTAB method (Murray and Thompson 1980). The parents of the F_2 population, N15 and N16, were genotyped with 126 SSR markers, polymorphic between ZS97 and HR5 and evenly distributed on the 12 chromosomes, to estimate the genetic background. The SSR assay was conducted as described by Wu and Tanksley (1993). The molecular linkage map was constructed using Mapmaker 3.0 (Lincoln et al. 1992), employing the Kosambi function calculate genetic distance. Interval QTL mapping was performed with both F_2 and F_3 data employing Mapmaker/QTL 1.1 (Lander and Botstein 1989; Lincoln et al. 1993). In the progeny test, on one hand, the genotype of each F_2 plant at the target gene was directly inferred for gene mapping based on its progeny test. Namely, if the trait variation among 20 plants in a progeny was small, which the average within-progeny standard deviation of homozygotes as ZS97 (ZZ) and HR5 (HH) is 2.8 and 1.5 for HD, 2.6 and 1.2 for PH, 11.9 and 6.8 for SPP, 10.4 and 5.6 for GPP, the corresponding F_2 plant genotype should be regarded as one of the two parental genotypes dependent on the trait expression. If the trait variation among 20 plants in a progeny was large, which the average within-progeny standard deviation of heterozygote (ZH) is 6.7 for HD, 8.2 for PH, 32.4 for SPP and 27.6 for GPP, the corresponding F_2 plant genotype at the target gene is treated as heterozygous genotype. On the other hand, the mean data of each progeny was used for QTL analysis. For the progeny with uniform performance, the arithmetic mean was regarded as its phenotype value of the progeny. For the segregated progeny, the weighted mean was regarded as its phenotype value. Because of impossible to distinguish the genotypes ZH and HH by phenotype and frequent

skewed segregation in a progeny, the weighted mean was calculated as $(3H + 1S)/4$, which H and S means the average values of tall plants (large panicle) and short plants (small panicle), respectively.

Results

Genetic background differences between N15 and N16

Based on SSR genotyping, three genomic intervals were found to be polymorphic between N15 and N16. One interval, between SSR markers MRG5720 and MRG0357, was located on chromosome 7, while two intervals, between SSR markers MRG4432 and RM547 and SSR markers RM433 and RM447, respectively, were located on chromosome 8. For N16, the three intervals were from HR5, and for N15, the three intervals were from ZS97. The remaining genomic regions were identical between N15 and N16. The three intervals polymorphic between N15 and N16 span a total of 19.8 cM. This equals approximately 1.3% of the total genome length (1,549.5 cM), based on the linkage map of our RILs population, agreeing with the expected heterozygote ratio of the F_7 generation (1.6%).

Trait performance of N15 and N16

Significant phenotypic differences were observed for all traits between N15 and N16 as well as between ZS97 and HR5 (Table 1). HR5 consistently showed the highest trait values and performed as a typical large panicle variety with approximately 380 SPP. While lower than for HR5, N16 consistently showed higher trait values than N15 in all the four traits. The performance of N15 was very similar to ZS97, a significant difference only detected for HD in 2004 (Table 1).

Trait distributions in the F_2 population

In the F_2 population and its progeny, transgressive segregation was observed in both directions for PH, in one direction to the parent N15 for SPP and GPP, and one direction to the parent N16 for HD (Fig. 1). SPP expressed a discontinuous variation with a gap between 109 and 120 SPP. PH also expressed a discontinuous variation with a gap between 84 and 88 cm. GPP and HD showed bimodal distributions with 94 GPP and 67 days, respectively, as trait value boundaries. In the progeny test, three classes of progenies were observed, the first class showing uniform expression with late flowering, large size panicle and tall plants, the second class showing large trait variations in the four traits and the third class showing uniform expression with early flowering, small size panicle and short plants (Table 2). The number of progenies in the above three classes are 23, 61 and 25, respectively, which is in agreement with the expected ratio (1:2:1) of one single segregating gene.

Correlation among traits

Highly significant correlation was detected among the four traits either based on the F_2 population or the F_3 progeny (Table 3). While the correlation coefficients ranged from 0.42 to 0.94 in the F_2 population, they consistently exceeded 0.90 in the F_3 progeny. For the F_3 progeny phenotypic data were collected from 20 plants, as compared to single plants in the F_2 population, and could thus more reliably reveal correlations among the four traits.

QTL analysis for the traits

Ten SSR markers polymorphic between N15 and N16 were used to genotype the F_2 population. Four markers, MRG3520 and MRG0357 (located in the long arm of chromosome 7) and RM433 and RM447 (located in the long arm of chromosome 8) did not show association with the traits. The remaining six markers were mapped into one linkage group covering a 6.6 cM region in the short arm of chromosome 8 (Fig. 2). QTL analysis identified one QTL controlling all four traits mapping in a 1.4 cM interval between markers RM310

Table 1 Trait performance of genotypes N15, N16 and their original parents in 2004 and 2005

Parent	SPP		GPP		PH		HD	
	2004	2005	2004	2005	2004	2005	2004	2005
HR5	381.5 a	395.3 a	269.8 a	271.8 a	104.8 a	112.3 a	91.3 a	84.4 a
N16	178.4 b	180.4 b	129.2 b	156.9 b	103.8 b	102.8 b	69.8 b	71.7 b
N15	119.9 c	122.7 c	96.3 c	107.5 c	84.0 b	79.5 c	61.5 c	58.7 c
ZS97	124.7 c	119.5 c	92.0 c	104.7 c	84.7 b	82.1 c	63.5 d	60.0 c

Values within columns followed by the same letter are not statistically significant ($P = 0.01$) according to Duncan's test

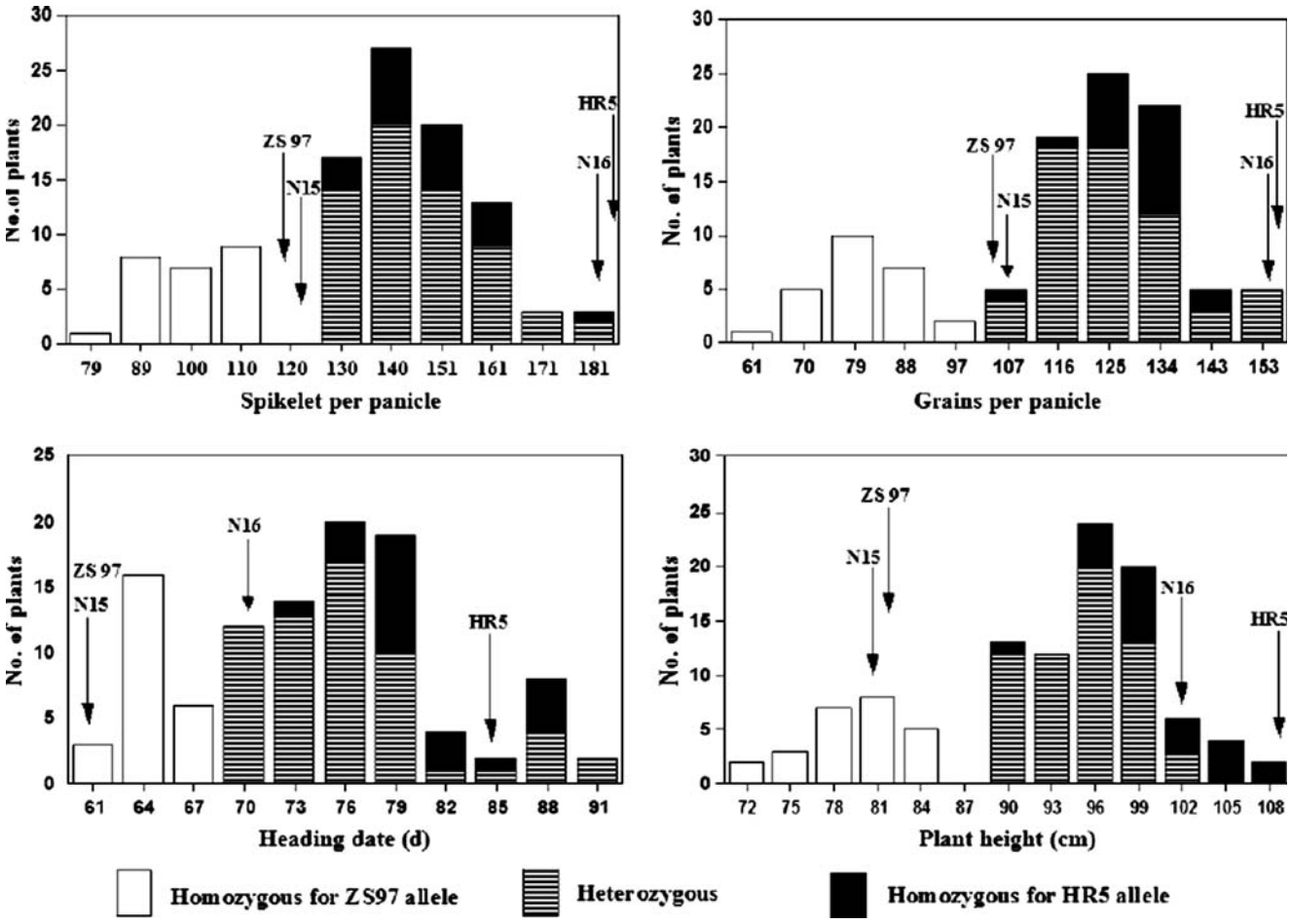

Fig. 1 Frequency distribution of trait performance in the F_2 population

Table 2 Range of trait variation and means of the three QTL-genotypes (ZZ, ZH, HH) in the F_2 population and F_3 progeny

Trait	Generation	Year	ZZ	ZH	HH
			$N = 25$	$N = 61$	$N = 23$
SPP	F_2	2004	93.9	142.3	143.0
	F_3	2005	94.0	136.9	150.6
GPP	F_2	2004	75.8	121.5	125.5
	F_3	2005	78.9	117.8	129.0
HD (d)	F_2	2004	64.3	75.3	80.0
	F_3	2005	64.2	73.5	76.7
PH (cm)	F_2	2004	78.5	94.1	99.0
	F_3	2005	81.1	96.4	101.6

ZZ homozygous for Zhenshan97 allele, *ZH* heterozygous, *HH* homozygous for HR5 allele, *SPP* spikelets per panicle, *GPP* grains per panicle, *HD* heading date, *PH* plant height

Table 3 Correlation coefficients among the four traits

Trait	SPP	GPP	HD	PH
SPP		0.98*	0.93*	0.91*
GPP	0.94*		0.90*	0.91*
HD	0.42*	0.52*		0.95*
PH	0.76*	0.77*	0.42*	

The correlation coefficients of the F_2 and F_3 are below and above the diagonal, respectively

*Significant at the level of $\alpha = 0.01$

and RM126 (Table 4). This QTL explained 83.0, 80.2, 94.9 and 93.8% of the trait variation in SPP, GPP, HD and PH of the F_3 progenies, respectively, consistently explaining more of the trait variation of the F_3 progeny than of the F_2 population (Table 4). Similarly, the LOD values of the QTL detected in the F_3 progeny were higher than that detected in the F_2 population. The additive effects of the QTL on SPP, GPP, HD and PH were larger than that of the corresponding dominance effects estimated both in individual F_2 data and progeny data, indicative of partial dominance of the QTL. In general, plants carrying the HR5 alleles showed increased phenotypic values for all four traits.

Table 4 QTL effects on the four traits in the QTL-NIL population

Trait	Generation	QTL	QTL position[a]	LOD	A[b]	D[c]	D/A	Var[d] %
SPP	F_2	*QSpp8*	RM126 + 0.0 cM	31.2	24.9	22.6	0.9	73.5
	F_3	*QSpp8*	RM126 + 0.0 cM	40.0	28.0	14.4	0.5	83.0
		gene[e]	RM310 + 0.4 cM		28.3	14.6	0.5	
GPP	F_2	*QGpp8*	RM126 + 0.0 cM	35.6	25.0	20.0	0.8	78.1
	F_3	*QGpp8*	RM126 + 0.0 cM	36.6	25.0	13.5	0.5	80.2
		gene[e]	RM310 + 0.4 cM		25.1	13.9	0.6	
HD	F_2	*QHd8*	RM126 + 0.0 cM	24.8	8.3	2.0	0.2	66.0
	F_3	*QHd8*	RM126 + 0.0 cM	67.3	6.3	3.1	0.5	94.9
		gene[e]	RM310 + 0.4 cM		6.3	3.1	0.5	
PH	F_2	*QPh8a*	RM126 + 0.0 cM	39.1	10.1	5.2	0.5	81.1
	F_3	*QPh8b*	RM126 + 0.0 cM	63.5	10.5	4.5	0.4	93.8
		gene[e]	RM310 + 0.4 cM		10.3	5.1	0.5	

[a] Position of QTL means the LOD peak position of QTL

[b] Additive effect on the HR5 allele

[c] Dominant effect on the HR5 allele

[d] Var percentage of total phenotypic variance explained by the QTL

[e] QTL treated as a single Mendelian factor on the basis of progeny test, its genetic effect estimated on the progeny data. Additive effects equal to the middle value of two homozyogtes, dominance effects equal to the trait value difference between heterozygote and the middle value of two homozyogtes

Gene mapping

The progeny test confirmed the co-segregation among the four traits, with short plants consistently accompanying small size panicle and early flowering, and vice versa. Plants homozygous for the HR5 allele (HH) showed high trait values, plants homozygous for the ZS97 allele (ZZ) showed low trait values, and heterozygous plants (ZH) showed segregating trait values (Table 2). The QTL was treated as a marker and mapped to the interval between RM544 and RM310, 1.8 cM away from the LOD peak position (RM126) of the QTL, but in the 1-LOD confidence interval (Fig. 2). The average trait values of each family are in accordance with its F_2 data (Table 2). Each ZZ progeny showed low trait values, discriminating it from ZH and HH progeny. The average differences between homozygotes were 56.6 SPP, 50.1 GPP, 12.5 days in HD and 20.5 cm in PH.

Discussion

Strategies for NIL development

Near-isogenic lines have received considerable attention recently and have been employed in several QTL cloning studies (Spielmeyer et al. 2002; Ashikari et al. 2005; Ren et al. 2005; Nishimura et al. 2005).

Near-isogenic line development by consecutive backcrossing (CB-NIL) and MAS have been widely used (Yamamoto et al. 1998, 2000; Li et al. 2004). Its progress is very similar to the advanced backcross QTL analysis, initially described by Tanksley and Nelson (1996). The CB-NIL strategy generally includes: (1) hybrid production between two varieties, (2) four continuous backcrosses to one (recurrent) parent with MAS for targeted QTL in each backcross generation, (3) NILs production by selfing. Thus, CB-NIL development generally takes 3 years. The CB-NIL strategy has the advantage that a new variety, similar to the recurrent parent but with a superior given QTL, could be developed when producing QTL-NIL, if the recurrent parent is an elite variety (Bernacchi et al. 1998; Yamamoto et al. 1998). However, QTL information is needed before starting MAS in the backcross generations. Thus, QTL-NIL could be obtained approximately 3 years after QTL analysis.

Compared to CB-NIL development, TP-NIL development, as used in this study, allow for the direct identification of NILs within advanced RILs such as F_5 or F_6. The genetic principle of TP-NIL is that continuous selfing results in a homozygous genetic background. In theory, more than 93 or 96% of the genome in the RIL F_5 or F_6 generations, respectively, is homozygous. However, unlike the recurrent parent of CB-NILs, the genetic background of TP-NIL is a random combination of two parental genotypes. If the heterozygous region(s) of a given RIL contain a major QTL, selfing should produce progenies with significant variation in associated trait values. Once a common, segregating QTL is detected between two individual plants of the same RIL, and differing in trait values, the QTL could be associated with the trait. Subsequently, individual

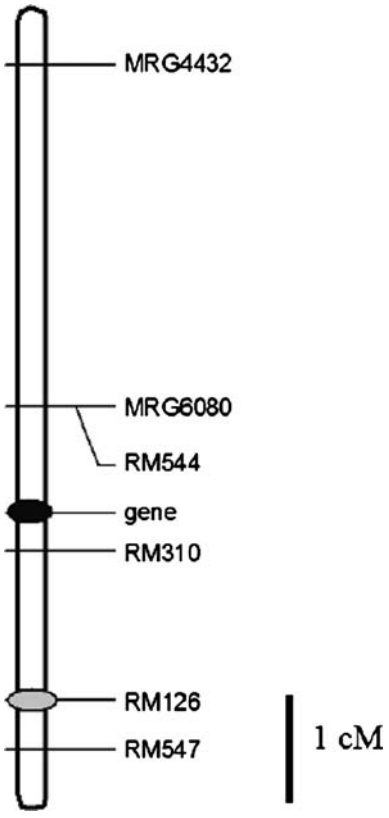

Fig. 2 The target linkage map showing QTL position. The *black spot* indicates the gene position mapped as a co-dominant marker on the basis of progeny test. The *gray spot* indicates the LOD peak position in the QTL analysis

plants, differing at a given "candidate-QTL," can be crossed for NIL development, requiring only one additional round of selfing. If plants, heterozygous at the "candidate QTL" region, is identified in the segregating RIL, progeny produced by selfing could be regarded as high quality NILs. Therefore, TP-NIL development can be started in the process of RIL population development or in parallel with field experiments for QTL mapping. Normally, 20 plants of each line in the F_6 generation are recommended to investigate phenotypic differences for the purpose of NILs development. This strategy can save 2.5 years for QTL-NIL development as compared to CB-QTL strategy. Moreover, noise from the genetic background in the NILs can be minimized. In the lines reported this study, only 0.9% of the genetic background, apart from the QTL region, segregating. Although for most genome segments, heterozygous plants can be expected in the process of developing a population of 200 RILs, only major QTL-NILs, resulting in significant phenotypic differences would be identified. According to our experience, one or two RILs with monofactorial segregation at the phenotypic level can be identified among 200 F_6 or F_7 rice RILs derived from parents selected based on contrasting (high–low) trait values (unpublished data). For RILs derived from parents not displaying significant differences in trait values, the TP-NIL strategy should probably not be applied as minor QTL-NILs might not be identified without prior knowledge on QTL position(s).

Thus, while CB-NIL is a directional and efficient strategy for minor QTL-NIL development, TP-NIL is a straightforward strategy for major QTL-NIL development. In fact, both strategies are tightly associated with the QTL mapping process of population development, field experiments and marker genotyping. A combination of both strategies could prove a flexible and efficient strategy for developing a series of QTL-NILs.

The QTL is not identified in RIL population

The RIL population, from which N15 and N16 were selected, was developed for QTL analysis. However, in this population, no QTL was detected for the four traits at LOD threshold of 2.0 in the targeted region in both years, except for a QTL for PH identified in 2002 (unpublished data). Major QTLs can be reliably detected in similar environments using the same mapping population, only the minor QTLs have a high risk of not being repeatedly detected in several environments (Lu et al. 1996; Xing et al. 2002; Wan et al. 2005). Hence, probably the major QTL on the four traits reported here not identified in RILs is mainly caused by genetic interactions but environment interaction. This case was happened in *Hd6* mapping. Yano et al. (1997) did not identify the QTL of *Hd6* in a population of 186 F_2 plants. However, Yamamoto et al. (2000) identified this QTL using an advanced backcross population from the same cross. Due to its interaction with *Hd2*, *Hd6* could not be detected in the primary population, even though its genetic effect of *Hd6* is large (Yamamoto et al. 2000). It can be speculated, that the QTL reported here interacts with one or more loci of the genetic background in the RILs population, thus preventing its detection.

Cluster QTLs or a pleiotropic QTL in the target region

Previous studies have reported co-localization of QTL controlling SPP, GPP, HD and PH in rice. Lin et al. (1996) and Xiao et al. (1996) detected QTL controlling SPP in the short arm of chromosome 8 using RILs derived from *indica* and *japonica* subspecies. Zhuang et al. (1997) reported a QTL in the interval between markers RZ562 and RG978 on chromosome 8 simultaneously controlling SPP and PH by using an F_2 popula-

tion. Xiong et al. (1999) reported a QTL in the interval between markers RG333 and C1121 on chromosome 8, controlling both HD and PH in a population derived from a cross between a wild rice species and a cultivar. Moreover, a QTL controlling SPP was identified in a closely linked region delimited by markers R2285 and RG333 on chromosome 8. Lin et al. (2003) reported fine mapping of *Hd5* between markers C166 and R902 on chromosome 8. The above-mentioned regions, flanked by different markers, share physical genome region with the QTL detected in this study. Thus, our results and previous studies support the location of a QTL simultaneously controlling SPP, GPP, HD and PH on the short arm of chromosome 8. The progeny test in this study confirmed that the QTL controlling the four traits are co-segregating, indicating a pleiotropic QTL at this position. However, due to limited population size, further studies, e.g., in a larger NIL-F_2 population, are needed in order to firmly establish the presence of one pleiotropic- or several closely linked QTL.

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