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ORIGINAL PAPER

Four rice QTL controlling number of spikelets per panicle expressed the characteristics of single Mendelian gene in near isogenic backgrounds

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Abstract Development of quantitative trait loci (QTL) near isogenic lines is a crucial step to QTL isolation using the strategy of map-based cloning. In this study, a recombinant inbred line (RIL) population derived from two *indica* rice varieties, Zhenshan 97 and HR5, was employed to map QTL for spikelets per panicle (SPP). One major QTL (*qSPP7*) and three minor QTL (*qSPP1, qSPP2* and *qSPP3*) were identified on chromosomes 7, 1, 2 and 3, respectively. Four sets of near isogenic lines (NILs) BC_4F_2 targeted for the four QTL were developed by following a standard procedure of consecutive backcross, respectively. These QTL were not only validated in corresponding NILs, but also explained amounts of phenotypic variation with much larger LOD scores compared with those identified in RILs. SPP in the four QTL-NILs expressed bimodal or discontinuous distributions and followed the expected segregation ratio of single Mendelian factor by progeny test. Finally, *qSPP1, qSPP2*, *qSPP3* and *qSPP7* were respectively mapped to a locus, 0.5 cM from MRG2746, 0.6 cM from MRG2762, 0.8 cM from RM49 and 0.7 cM from MRG4436, as co-dominant markers on the basis of progeny tests. These results indicate no matter how small effect minor QTL is, QTL may still express the characteristics of single Mendelian factor in NILs and isolation of minor QTL will be possible using high quality NILs. Pyramiding these QTL into a variety will largely enhance rice grain yield.

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Introduction

Rice (*Oryza sativa* L.) is a staple food and more than 50% of the human population in the world lives on rice. Increasing yield of rice is a permanent topic for over-increasing population. Complex traits such as rice yield components are quantitative inheritance, which are contributed by multiple genes each with small effect, namely quantitative trait loci (QTL). Number of spikelets per panicle (SPP) is highly associated with number of grains per panicle (GPP), which is a very important component of yield. These traits have been frequently studied because of their importance in rice genetic improvement with the advent of the molecular markers (Xiao et al. 1996; Zhuang et al. 1997; Xiong et al. 1999; Xing et al. 2002, 2008).

In previous reports of identification of QTL for SPP and GPP, mapping populations were mainly primary populations such as F_2, doubled haploid lines (DH) and RILs (Xiao et al. 1996; Lu et al. 1996; Zhuang et al. 1997; Yu et al. 1997; Xing et al. 2002). While conventional experiments (primary materials and insufficient population size) limit minor QTL identification and give low resolutions of QTL, which is frequently located in a large confidential interval not in one site (Darvasi et al. 1993). Near isogenic lines (NILs), which only a little target region of genome is segregating, can eliminate the genetic background noise and have been widely used in plants to validate and fine map QTL (Alpert and Tanksley 1996; Wang et al. 2005; Chu et al. 2005, Zhang et al. 2006; Fan et al. 2006). NILs have been received more attention recently because major

QTL can express the characteristic of qualitative gene in near isogenetic background. Last decade, great progresses were made in QTL isolation for some traits such as plant height and flowering time, which are regarded to be controlled by qualitative and quantitative genes (Ishimaru et al. 2004; Yamamoto et al. 1998, 2000). For example, several QTL for plant height and heading date in rice were cloned using NILs (Monna et al. 2002; Spielmeyer et al. 2002; Takahashi et al. 2001). However, a few studies on QTL fine mapping for the typical quantitative trait SPP and GPP were conducted based on near isogenic lines (Zhang et al. 2006; Xing et al. 2008). Only two QTL for SPP have been cloned recently (Ashikari et al. 2005; Xue et al. 2008).

Advanced backcross QTL analysis strategy (AB-QTL) was widely used for NILs development (Alpert and Tanksley 1996; Wang et al. 2005; Yi et al. 2006; Li et al. 2007). However, NILs in previous report were mainly aimed to major QTL. Very few studies aimed to minor QTL when developing NILs. Most quantitative traits were simultaneously controlled by several minor QTL and few major QTL. After all, the number of major QTL for a given trait is very limited. More attention should be put to minor QTL. In addition, it is very difficult to repeatedly detect minor QTL using primary mapping population because of background noise and QTL by environment interaction. Consequently, NIL population blocking the genetic background noise can validate the minor QTL and provide chance to precisely map them as single Mendelian factor.

In terms of the successful examples of QTL isolation,high density map of QTL region and ideal mapping populations are two prerequisites for QTL map-based cloning (Ashikari et al. 2005; Fan et al. 2006). So far, construction of high density map is no longer a bottle-neck with the completion of whole genome sequencing in rice. That is to say, one gene/QTL can be easily fine-mapped to a small region if it exhibited the characteristics of single segregation of Mendelian factor. And then, the sequence of targeted region can be easily touched in the public database, candidate gene will be quickly determined by gene prediction of that region after fine mapping. Thus, combination of candidate gene strategy and map-based cloning will be necessary and efficient for gene isolation now. In this case, lots of boring chromosome-walking work will be greatly reduced or even avoided. Currently, development of advanced mapping population such as chromosome segment substitution lines (CSSLs), single segment introgression lines (SSILs) and near isogenic lines (NILs) is getting more important. NILs have been proven to be very ideal materials for QTL fine mapping and cloning due to its pure genetic background (Yamamoto et al. 1998; Takahashi et al. 2001).

In present study, a recombinant inbred line population derived from two *indica* rice varieties, Zhenshan 97 and HR5 with striking differences in SPP and GPP, was used for QTL mapping. The standard AB-QTL strategy was used to develop isogenic lines for a series of QTL detected in the mapping population. Our objectives are (1) to dissect the genetic bases of SPP and GPP; (2) to validate the QTL in the near isogenic background; (3) to map target QTL as single Mendelian factor.

Materials and methods

Field experiment of RILs for QTL analysis

A recombinant inbred line population (RILs, F_6 and F_7) was employed to identify QTL controlling number of spikelets per panicle (SPP) and number of grains per panicle (GPP). This population consisted of 190 RILs derived by single-seed descendent from a cross between two parents, Zhenshan 97 and HR5, which have striking differences in SPP and GPP. For the field test, the seeds were sown in a seedling bed in May, 2001 and 2002. The RILs (F_6 and F_7) and two parents were transplanted to a bird-net-equipped field in the experimental farm in Huazhong Agricultural University in the 2001 and 2002 rice-growing season in Wuhan, China. Field experiments were carried out following the randomized complete block design with two replicates. Fourteen seedlings of approximate 22-day-old for 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. The 10 plants in the middle of the two rows in each plot were harvested individually to score SPP and GPP.

DNA marker and trait measurements

DNA was extracted from leaf samples using a modified CTAB protocol (Murray and Thompson 1980). PCR was done using a hotstart Taq polymerase (TaKaRa) with the following conditions: 95°C for 2 min, 35 cycles of 95°C for 30 s, 55–58°C for 45 s, and 72°C for 1.5 min, followed by 72°C for 10 min. SSR markers were used for developing genetic linkage map. The SSR primers of the RM series were designed according to the reports (Temnykh et al. 2000, 2001) and those of the MRG series were according to the rice genome sequences of Monsanto Company (McCouch et al. 2002). SPP was measured as the total number of spikelets of the whole plant divided by its total number of panicles. GPP was scored as the total number of filled grains of the whole plant divided by its total number of panicles.

QTL-NIL development

HR5 segments containing four QTL, *qSPP-1, qSPP-2, qSPP-3* and *qSPP-7* identified in the RILs, were separately

introgressed into Zhenshan 97 by successive backcrossing with molecular marker aided selection. In this process, two flanking markers tightly linked to each of the four QTL were used to select the positive backcrossing progeny for continuous backcross. In detail, four pairs of markers, MRG2746 and RM490, MRG2762 and RM3515, RM135 and RM49, MRG4436 and RM2 were used for selecting *qSPP-1, qSPP-2, qSPP-3* and *qSPP-7*, respectively.

A total of 126 SSR polymorphic markers between HR5 and Zhenshan 97 and evenly distributed on 12 chromosomes, were used for genetic background screening in the BC_4F_1 generation. The positive individuals of the four target QTL, whose genetic constitution was highly similar to the recurrent parent, Zhenshan 97, were selected for selfing. Finally, four BC_4F_1 plants with the least background noise, BC_4F_1-1, BC_4F_1-2, BC_4F_1-3 and BC_4F_1-7, were selected to produce their corresponding BC_4F_2, hereafter named as NIL-1, NIL-2, NIL-3 and NIL-7, respectively.

Progeny test of four NILs

4 BC_4F_2 populations (NIL-1, NIL-2, NIL-3 and NIL-7), each segregating at *qSPP-1, qSPP-2, qSPP-3* and *qSPP-7* respectively, were cultivated in a bird-net experimental paddy field at Huazhong Agricultural University in the 2005 rice-growing season in Wuhan, China. The sizes of populations NIL-1, NIL-2, NIL-3 and NIL-7 were 162, 187, 191 and 140, respectively. All BC_4F_2 plants were harvested for SPP and GPP measurement. In the 2006 rice-growing season, the progenies of the four populations (BC_4F_3) and the two parents were cultivated in the same field. Twenty-four plants of each BC_4F_2 progeny were grown in two rows, 20 plants in the middle were investigated for SPP and GPP measurement and data analysis. Due to germination problem, 134, 158, 174 and 140 BC_4F_3 families of NIL-1, NIL-2, NIL-3 and NIL-7 were used for progeny test, respectively. The genotype of the target QTL in each BC_4F_2 plant was also inferred from the segregation of SPP and GPP. Plant height and heading date was also scored in BC_4F_2 of NIL-7 population and its progeny BC_4F_3.

Genetic linkage map and QTL analysis

The molecular linkage map for whole genome and target region were constructed using Mapmaker 3.0 (Lincoln et al. 1992). Kosambi function was used to calculate genetic distance. Forward stepwise regression and backward elimination regression methods were used to choose significant markers for each trait. The composite interval mapping based on the mixed linear model was carried out for QTL analysis with RILs by using WinQTL Cartographer v2.0 (Wang and Zeng 2003). All of the significant markers were selected to control the genetic background, and window size (10 cM) was used to control the genetic background. Thresholds for logarithm of odds (LOD) score were estimated by permutation tests (Churchill and Doerge 1994) with 1,000 replicates. The threshold value of LOD at genome-wide significance level of 0.05 was 2.1. Interval mapping was used for QTL detection with NILs using MAPMAKER/QTL (Lander and Botsein 1989; Lincoln et al. 1993).

Results

Phenotypic variation of SPP and GPP

The male parent, HR5, had highly significant more SPP and GPP than the female parent, Zhenshan 97, in both years (Table 1). Large variations in RILs were observed for SPP and GPP in 2001 and 2002, and their phenotypic variations each showed a normal frequency distribution. No line had more SPP than the parent HR5 in both years. While, a few recombinant inbred lines had small panicle with average SPP less than Zhenshan 97 in both years (Table 1). The occurrence of some extreme recombinant inbred lines is expected due to environmental rather than genetic reasons since the RIL population size was much larger than the parents. GPP of 3 and 2 RILs in both years was less than the means of Zhenshan 97 − 2 standard deviations (SD) observed in Zhenshan 97 distributions. Chi square tests

Table 1 Performance of spikelets per panicle (SPP) and grains per panicle (GPP) in the parents and recombinant inbred lines (RILs) in 2001 and 2002

Traits	Heritability (%)	Zhenshan 97 (40 plants)		HR5 (40 plants)		RILs (190 lines)		No of lines in RILs	
		Mean ± SD	<ZS97[a] (M − 2SD)	M ± SD	>HR5[b] (M + 2SD)	Range	M ± SD	>HR5[a] (M + 2SD)	<ZS97[b] (M − 2SD)
SPP (2001)	84.0	97.2 ± 9.7	1	368.2 ± 44.7	1	99.8–275.8	191.5 ± 40.0	0	0
SPP (2002)	84.7	91.8 ± 18.3	3	327.4 ± 28.0	0	80.5–272.0	157.6 ± 40.8	0	0
GPP (2001)	71.1	85.3 ± 8.2	1	322.9 ± 39.3	1	63.1–247.3	154.0 ± 38.8	0	2
GPP (2002)	69.2	82.1 ± 14.9	2	264.7 ± 28.9	2	58.3–243.0	129.4 ± 37.1	0	3

[a] *ZS97* <ZS97 − 2SD

[b] *HR5* >HR5 + 2SD

were used to compare the number of lines expected in the RILs to exceed the means of Zhenshan 97 − 2 SD (assuming that the size of Zhenshan 97 populations were equal to the RILs) with the actual number of RILs that exceeded those thresholds. The number of observed extreme individuals did not significantly exceed the expected ($P < 0.05$), suggesting no transgressive segregation in Zhenshan 97 direction. SPP expressed higher heritability than GPP, whose broad heritability is about 70%. This indicated that two traits were mainly controlled by genetic factors rather than environment factor in these field experiments.

Whole genome linkage map

A total of 263 polymorphic SSR markers evenly distributed on 12 chromosomes were used for construction of genetic linkage map with the RILs population. The 263 SSR markers were assigned into 14 linkage groups. It spanned 1552.7 cM in length with an average distance of 5.9 cM between adjacent markers.

QTL identified in RILs

Three QTL *qSPP-1, qSPP-3* and *qSPP-7* controlling SPP were detected in 2001; and two QTL *qSPP-2* and *qSPP-7* were detected in 2002, respectively (Table 2). Only *qSPP-7* was commonly detected in both years. In total, four QTL were identified. They were located between markers MRG2746 and RM490, MRG2762 and MRG3515, RM135 and RM49, RM4436 and RM2, respectively (Fig. 1). Among them, *qSPP-7* could explain 19.1 and 23.6% of trait variation in 2001 and 2002, respectively. *qSPP-2* showed a minor effect with contribution to 3.8% of trait variation in 2002. HR5 alleles increased SPP values at all the four QTL. All the detected QTL in 2001 and 2002 totally explained 38.3 and 25.6% of SPP variation, respectively.

As for GPP, a total of four QTL, *qGPP-1, qSPP-2, qSPP-3* and *qGPP-7* were detected. Among them, only *qGPP-1* and *qGPP-7* were detected simultaneously in both years. They shared the same intervals as QTL for SPP. They individually explained a range of 3.8–22.9% of the total phenotypic variation. *qGPP-2* had the lowest LOD (2.2) score. Similarly, HR5 alleles increased GPP values at all the four QTL. The QTL totally explained 30.4 and 29.7% of GPP variation in 2001 and 2002, respectively.

QTL validation

In the process of developing advanced backcross line for each QTL,besides the foreground selection for the target QTL, background selection was also conducted. In BC_4F_1 generation, eight heterozygous plants at each target QTL region were screened using 126 markers. And then the best individuals with the least genetic background noise of 5.5, 4.3, 4.9 and 5.0% were used to produce BC_4F_2 populations NIL1, NIL2, NIL3 and NIL7, respectively. In NIL1, besides *qSPP1* region, marker intervals between RM274 and RM26 on chromosome 5 and between MRG6240 and MRG4766 on chromosome 11 were segregated. In NIL2, besides *qSPP2* region, marker interval between RM133 and RM190 on chromosome 6 was segregated. In NIL3, besides *qSPP3* region, marker intervals between RM228 and RM590 on chromosome 10 and between RM117 and RM101 on chromosome 12 were segregated. In NIL7, besides *qSPP7* region, marker interval between RM274 and RM26 on chromosome 5 was segregated. That is to say, besides target QTL region, only one or two small chromosomal regions not associated with SPP were segregated in each NIL. In addition, ZZ genotypes in NIL1, NIL2 and NIL7 had very

Table 2 Putative QTL for SPP and GPP identified in recombinant inbred lines in 2001 and 2002

Traits	QTL	Marker interval	2001			2002		
			LOD	*A*	R^2 (%)	LOD	*A*	R^2 (%)
SPP	*qSPP1*	MRG2746-RM490	4.3	12.2	8.1			
	qSPP2	MRG2762-RM3515				2.4	8.4	3.8
	qSPP3	RM135-RM49	5.8	15.2	13.4			
	qSPP7	MRG4436-RM2	10.5	18.7	19.1	11.1	21.7	23.6
	Total contribution				38.3			25.6
GPP	*qGPP1*	MRG2746-RM490	3.7	12.1	8.8	2.4	7.2	3.8
	qGPP2	MRG2762-RM3515	2.2	6.8	5.8			
	qGPP3	RM135-RM49				2.7	10.3	6.9
	qGPP7	MRG4436-RM2	8.2	17.6	18.1	10.4	19.4	22.9
	Total contribution				30.4			29.7

A additive effect, positive additive effect means HR5 allele increasing trait values, R^2 QTL contribution to trait variation

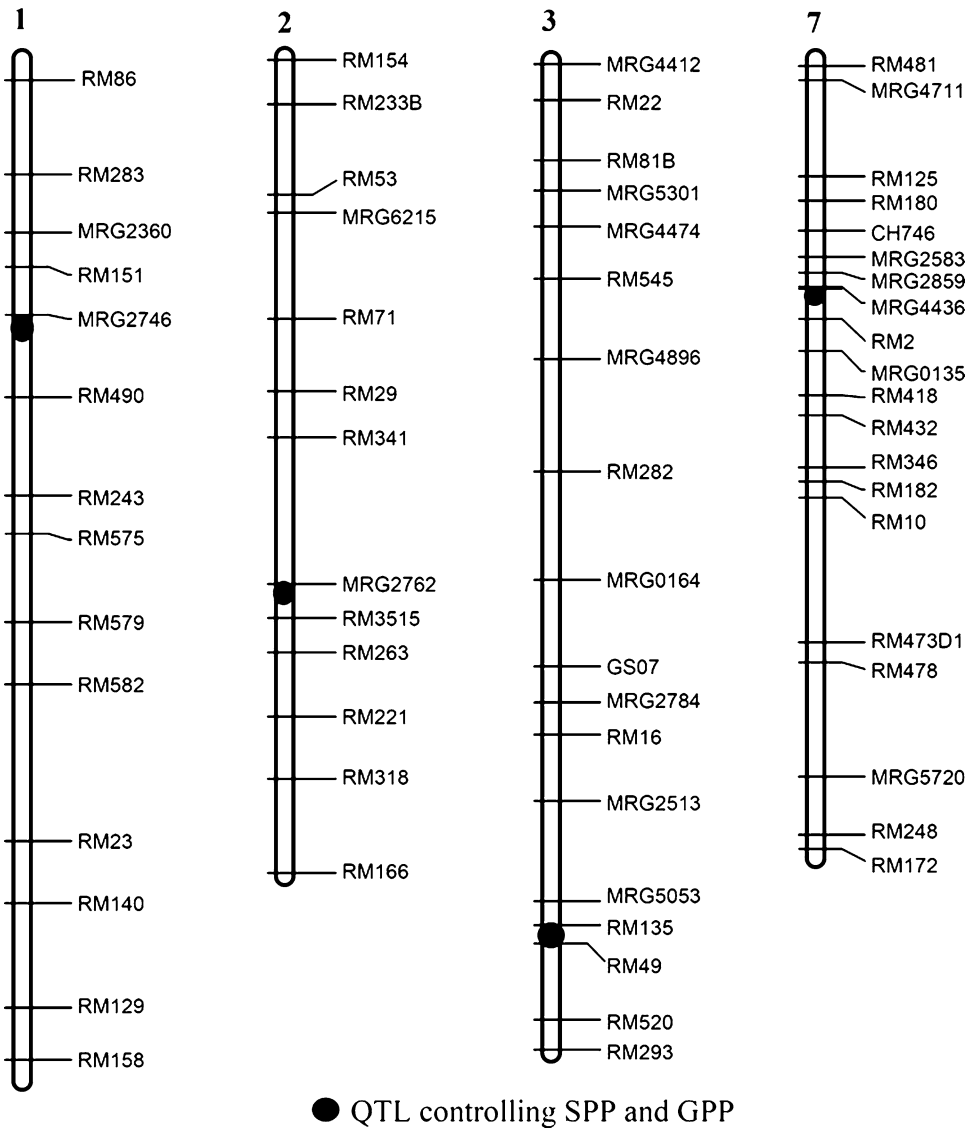

Fig. 1 The genetic linkage map of 4 chromosomes showing the positions of QTL detected in RIL population. *Black circle* indicates the LOD peaks of putative QTL identified in RILs

similar SPP to that of Zhenshan 97 in both years, but ZZ genotype in NIL3 had a slightly larger SPP than Zhenshan 97 at the level of $P = 0.05$ (Table 3). This also confirmed that NIL quality was good. Each local linkage maps of target region was constructed using corresponding NILs.

In BC_4F_2 of NIL1, NIL2, NIL3, SPP showed bimodal distribution with 127, 112 and 136 as trait value boundaries, respectively (Fig. 2). In NIL7, SPP expressed a discontinuous distribution with a gap between 127 and 134 SPP (Fig. 2). The segregation of SPP in each population agreed with the expected ratio (3:1) of single Mendelian factor. These results indicated a likely single-locus controlled SPP in each NIL. However, for GPP, no clear bimodal distribution was observed in NIL1, NIL2, NIL3 and NIL7 (figure not shown). For SPP, the progenies of each F_2 individuals in each NIL population could be classified into three types: uniform small number of SPP, uniform large number of SPP and variant number of SPP. These three types were matched to the three genotypes of Zhenshan 97 homozygote, HR5 homozygote and heterozygote. Average SPP values of ZZ genotypes in the four NIL populations were significantly smaller than those of other two corresponding genotypes. The same result was also observed for GPP but in NIL2. In most cases, average SPP and GPP values of HH genotypes were not significantly different from those of corresponding ZH genotypes (Table 3). Number of plants with ZZ, ZH and HH genotypes in each NIL population was listed in Table 3. Chi square test showed that the ratio of three genotypes in each NIL population fitted the Mendelian segregation ratio (1:2:1) of single gene. Thus, *qSPP1*, *qSPP2*, *qSPP3* and *qSPP7* like co-dominant markers were mapped to a locus 0.5 cM from MRG2746, 0.6 cM from MRG2762, 0.8 cM from RM49, 0.7 cM from MRG4436, respectively (Fig. 3).

Table 3 Performance of the three genotypes at the targeted QTL in the 4 NIL-F2 populations and their progenies in 2005 and 2006

Pop	Gen	χ^2	BC_4F_2 (2005)		BC_4F_3 (2006)	
			SPP	GPP	SPP	GPP
Zhenshan 97			102.4 ± 8.5	84.1 ± 6.9	117.3 ± 7.7	83.9 ± 5.7
NIL1-F_2	ZZ (26)	2.28	98.5.7 ± 9.7A[a]	82.5 ± 10.6A	116.3 ± 6.7A	78.4 ± 6.4A
	ZH (71)		136.7 ± 13.4B	109.9 ± 15.3B	137.8 ± 6.4B	92.4 ± 8.9B
	HH (37)		144.1 ± 13.9B	117.7 ± 14.9B	149.7 ± 10.2C	98.7 ± 9.8B
NIL2-F_2	ZZ (39)	2.44	100.7 ± 8.3A	86.6 ± 9.4A	120.2 ± 4.9A	82.1 ± 6.0A
	ZH (77)		126.8 ± 8.3B	103.8 ± 11.4B	136.2 ± 5.3B	88.9 ± 6.6AB
	HH (42)		131.6 ± 8.9B	106.6 ± 10.0B	144.9 ± 7.0B	95.4 ± 8.8B
NIL3-F_2	ZZ (36)	3.06	114.1 ± 8.1A	86.6 ± 10.2A	129.1 ± 6.6A	87.5 ± 10.5A
	ZH (91)		153.0 ± 14.9B	119.9 ± 16.2B	158.4 ± 10.8B	106.8 ± 11.4B
	HH (37)		166.6 ± 22.1C	132.1 ± 23.6C	172.7 ± 9.3C	118.6 ± 9.3C
NIL7-F_2	ZZ (37)	0.14	108.9 ± 12.2A	76.5 ± 11.9A	110.6 ± 6.6A	88.3 ± 8.4A
	ZH (71)		160.9 ± 19.1B	108.3 ± 15.6B	159.6 ± 7.2B	112.0 ± 7.3B
	HH (32)		172.1 ± 18.2B	112.4 ± 16.7B	176.9 ± 9.8C	128.8 ± 15.4C

NIL1, NIL2, NIL3 and NIL7 represent the NILs for QTL on chromosomes 1, 2, 3 and 7, respectively. ZZ, HH and ZH means the genotypes at QTL with homozygous Zhenshan 97 and HR5 alleles, and heterozygous, respectively. Number in the bracket means the number of plants in each genotype. $\chi^2 = 5.99$ with 2 *df* at $P = 0.05$

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

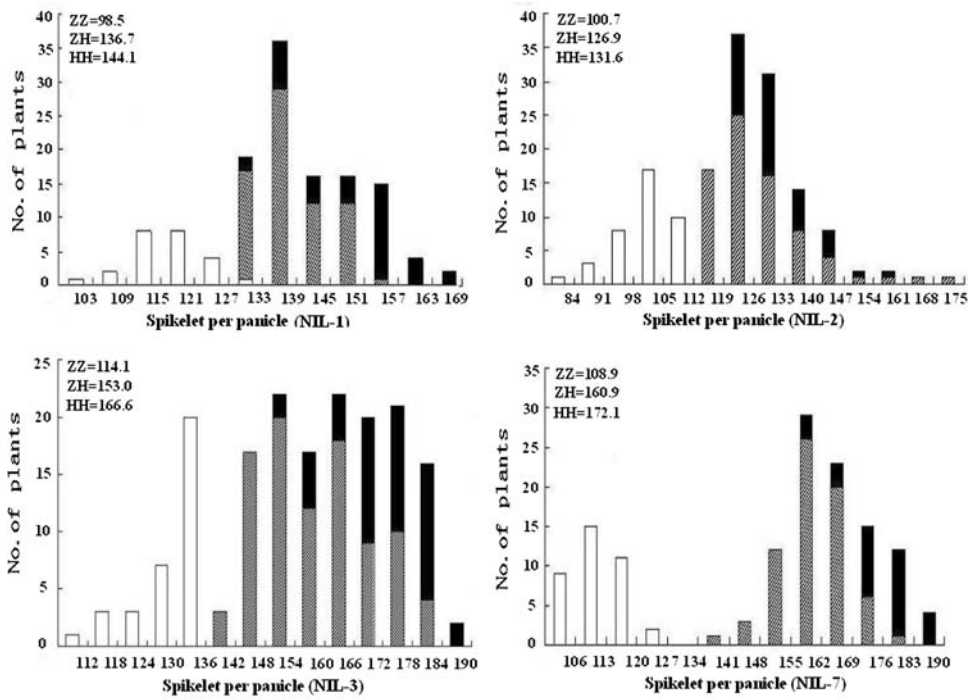

Fig. 2 Frequency distributions of SPP in populations of NIL-1, NIL-2, NIL-3 and NIL-7. *White bar, black bar* and *stripe bar* represented Zhenshan 97 homozygote (ZZ), HR5 homozygote (HH) and heterozygote (ZH), respectively. The values of ZZ, ZH and HH indicate the average values of three genotypes in corresponding BC_4F_2 population, respectively

Accordingly, four QTL with large LOD scores were individually identified in the four NIL-F_2 populations. They located in the same intervals as those identified in RIL population (Table 4). However, the genetic effects were much larger than those estimated in RILs. In each NILs, QTL for SPP and GPP also shared the same intervals. More than 50% of SPP variation in each NILs could be explained by the corresponding single QTL, indicating these QTL had major effects on SPP. *qSPP7* had the largest effect on SPP with LOD score of 74.2. And all QTL for GPP had less effect than their corresponding *qSPP*. All alleles from HR5 increased SPP and GPP values.

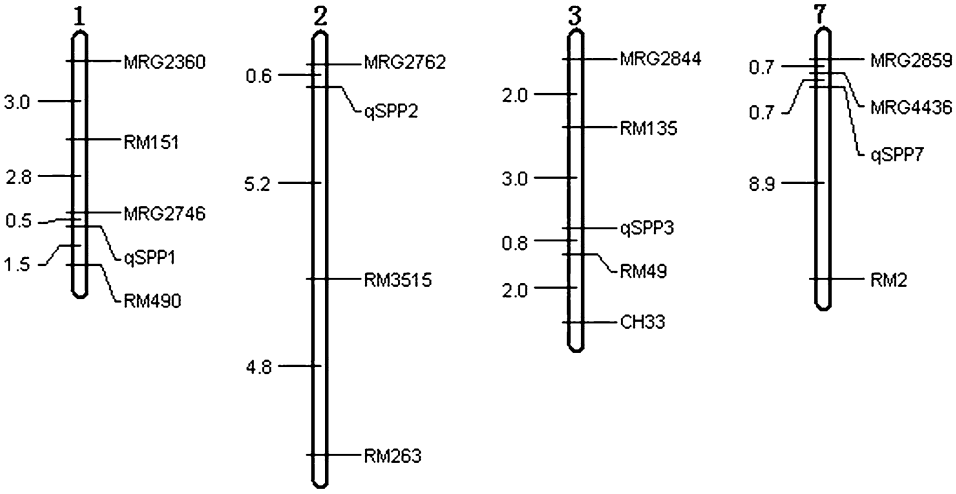

Fig. 3 Four local linkage maps of target regions constructed with the corresponding BC_4F_2 populations. NIL1, NIL2, NIL3 and NIL7 segregated in the chromosomal fragments between MRG2360 and RM490, MRG2760 and RM263, MRG2844 and CH33, and RM2859 and RM2, respectively. *qSPP1*, *qSPP2*, *qSPP3* and *qSPP7* were mapped as a co-dominant marker on the basis of progeny test, respectively

Table 4 QTL effects on SPP and GPP estimated in the progeny test of BC_4F_2 populations

Populations	QTL	Interval	LOD	*A*	*D*	R^2
NIL1	*qSPP1*	MRG2746-RM490	32.6	16.7	4.8	68.1
	qGPP1	MRG2746-RM490	13.7	10.1	3.8	38.0
NIL2	*qSPP2*	MRG2762-MRG3515	46.5	12.3	3.7	73.0
	qGPP2	MRG2762-MRG3515	14.1	6.6	0.2	32.7
NIL3	*qSPP3*	RM135-RM49	43.2	21.8	8.0	68.8
	qGPP3	RM135-RM49	25.7	15.6	3.8	49.7
NIL7	*qSPP7*	MRG4436-RM2	74.2	33.7	15.4	92.0
	qGPP7	MRG4436-RM2	36.0	21.0	2.8	69.7

A additive effect, positive additive effect means HR5 allele increasing trait values, *D* dominance effect, R^2 QTL contribution to trait variation

Table 5 Variation of plant height and heading date in population of NIL7

			BC_4F_2		BC_4F_3	
Traits	Genotypes	χ^2	Range	Mean	Range	Mean
Heading date (d)	ZZ (37)	0.14	57.0–72.0	66.2	58.1–62.1	60.2
	ZH (71)		75.0–90.0	83.8	66.3–79.8	76.1
	HH (32)		83.0–95.0	90.6	82.9–88.2	84.8
Plant height (cm)	ZZ (37)	0.14	70.0–93.0	81.9	78.5–88.4	83.9
	ZH (71)		90.0–109.0	101.8	88.1–108.7	103.6
	HH (32)		96.0–110.0	103.5	103.9–112.4	108.4

ZZ, HH and ZH means the genotypes at QTL with homozygous Zhenshan 97 and HR5 alleles, and heterozygous, respectively. Number in the bracket means the number of plants in each genotype. $\chi^2 = 5.99$ with 2 *df* at $P = 0.05$

Pleiotropic effects of *SPP7*

Except large variation was observed for SPP and GPP in NIL7, both heading date and plant height also expressed wide variation (Table 5). One QTL detected in the region between MRG4436 and RM2 had effects on plant height and heading date (Table 6). It could explain more than 80% of trait variation of plant height and heading date, respectively. Progeny test showed 140 BC_4F_2 plants could be also classified into the same three subgroups as did by SPP. This confirmed the QTL for plant height and heading date was co-segregated with *SPP7*. That is to say, SPP7 had pleiotropic effects on SPP, GPP, plant height and heading date.

Table 6 Pleiotropic effects of *qSPP7* on plant height and heading date based on the progeny test of BC_4F_2 of NIL7

Traits	LOD	*A*	*D*	R^2 (%)
Heading date (d)	66.3	12.8	6.1	85.4
Plant height (cm)	64.7	11.1	9.5	84.3

A additive effect, positive additive effect means HR5 allele increasing trait values, *D* dominance effect, ns non significant at the level of $P = 0.05$, R^2 trait variation explained by QTL

Discussion

Direct evidence for detected QTL in RIL

The most important finding in present study is that the four QTL detected in RIL population were individually confirmed in a Mendelian fashion and mapped to a locus using NILs. Due to genetic background noise in primary QTL mapping population, QTL location were only defined to a large confidential interval of about 10 cM (Darvasi et al. 1993; Takahashi et al. 2001) and its effect was not easily and precisely estimated. Especially, the identity of some minor QTL with a low LOD score would be suspected. It is expected that a real QTL should be able to be repeatedly identified across multiple populations in some extent. In fact, there were some examples showing such expectation, e.g. major semi-dwarfing allele, *sd-1* and two newly cloned QTL, *GS3* and *GW2*, were frequently mapped by several populations (Sasaki et al. 2002; Spielmeyer et al. 2002; Monna et al. 2002**;** Fan et al. 2006; Song et al. 2007). The QTL identified in this study also located in the similar intervals reported by other previous studies. For examples, *qSPP1* and *qGPP1* was mapped into the similar interval as *sp1* and *Gn1* (Xiong et al. 1999; Ashikari et al. 2005). *qSPP2* and *qGPP2* shared the similar interval with *tns2, nfg2* and *gpp2.1* (Zhuang et al. 1997; Septiningsih et al. 2003). *qSPP3* shared the similar interval with *qSNP-3-2* (Zhang et al. 2004). *qSPP7* and *qGPP7* also shared the similar interval with *sp7* and *gn7* (Xiong et al. 1999; Xing et al. 2002).

Although the four QTL was repeatedly detected among different populations, it only increased the possibility of a real QTL lying, but could not provide direct evidence for its reliability. However, in the present study, four QTL-NILs obtained by introgressed HR5 target region into Zhenshan 97 intuitively proved their identities. *qSPP7* was a major QTL with a LOD score of more than 9.0 in RILs. In its QTL-NIL population, SPP frequency distribution showed a clear discontinuous segregation. For the other three minor QTL, SPP frequency distribution also exhibited a clear bimodal segregation in their QTL-NIL populations, respectively. Moreover, SPP segregation was in agreement with ratio of 3:1, which is the expectation of single Mendelian factor. All these segregation patterns definitely indicated there was a gene/QTL controlling SPP in each NIL population. In addition, the genotype of each BC_4F_2 plant at the targeted QTL could be inferred by progeny tests (BC_4F_3), thus the target QTL like a molecular marker was mapped to a locus not a confidential region. Hence, the location of QTL was definite (Fig. 3).

The reliability of QTL identified in RILs can be not only confirmed, but also their relative effects can be directly compared each other in NILs due to their similar genetic backgrounds**.** *qSPP7* showed the largest additive of 33.7 SPP; *qSPP2* showed the smallest one of 12.3 SPP. It is also agreed with the ranking in RILs. In the near Zhenshan 97 background, HR5 homozyogtes at *qSPP7*, *qSPP3*, *qSPP2* and *qSPP1* have SPP of 172.1, 166.6, 131.6 and 144.1, respectively, which is significantly more than Zhenshan 97 of 97 SPP.

SPP suitable for gene isolation

In the present study, a general coincidence of the locations of QTL for SPP and GPP was observed, and all intervals harboring QTL for SPP also harbored QTL for GPP, meanwhile, the effects of QTL controlling SPP and GPP had the same direction of HR5 allele increasing trait values. The 1-LOD interval for SPP and GPP is fully overlapped each other in the NILs. In addition, there was no QTL for seed setting ratio in this study. Thus, the position difference between QTL for SPP and for GPP might be caused by the environment. These suggested that these four intervals each might have a pleiotropic QTL rather than the close linkage of different QTL. Hence, QTL for SPP and GPP in the same region would be the same one. Thus, only one trait needs to be focused on when isolating the QTL.

SPP stays stable after panicle differentiation. It is not affected by the environment during flowering. However, GPP is greatly affected by the external environment factors at flowering time and after flowering. For example, the extreme high or low temperature during flowering and long continuous raining can influence grain filling and result in variable GPP. Hence, SPP frequently showed higher heritability than GPP. In fact, rice plant was always suffered more environment stress from flowering to grain filling stage, and caused a large variation of GPP even within the same genotype. In the NILs, SPP distribution showed bimodal or discontinuous, indicating single gene controlling this trait. While for the trait of GPP, their frequency distribution seemed more complex, probably indicating gene and gene by environment interaction controlling this trait. In addition, it is difficult to exactly score the trait of GPP due to difficult distinguishing partially filled grains from unfilled grains (null). However, measurement of SPP will be easier

and more precise than GPP. Accordingly, SPP would be firstly focused on for QTL isolation.

NILs strategy feasible for minor QTL cloning

SPP and GPP are two important components of yield, which expressed typical characteristics of quantitative trait such as continuous distribution in primary population. It seems to be impossible to isolate individual QTL in primary population. However, using NILs, two QTL, *Gn1* and *Ghd7*, controlling GPP on chromosomes 1 and 7, have been cloned (Ashikari et al. 2005; Xue et al. 2008). Hence, to develop NILs might be the way which must be taken to isolate QTL using map-based cloning strategy.

In the present study, *qSPP1*, *qSPP2* and *qSPP3* explained 8.1, 3.8, 13.4% of the total phenotypic variation in RILs population, respectively. Moreover, *qSPP1*, *qSPP2* and *qSPP3* were only identified in one year. However, these QTL can enlarge genetic effects and explain amounts of trait variation in corresponding NILs and their progenies in 2005 and 2006, respectively. This illustrated the same conclusion as that Takahashi et al. (2001) reported: advanced backcross progeny enabled us to identify a quantitative trait locus even though it exhibited a relatively small effect on the phenotype. Thus, minor QTL identified in RILs would be cloned using NILs with pure enough genetic background.

qSPP7 had pleiotropic effects on SPP, GPP, plant height and heading date in this study, it was closely linked to marker MRG4436 (namely RM5436). The newly cloned gene, *Ghd7*, had pleiotropic effects on SPP, GPP, plant height and heading date and was tightly linked to RM5436 (Xue et al. 2008). They were located in the similar region and exhibited pleiotropic effects on same traits. Therefore, *qSPP7* might be the gene of *Ghd7*. *qSPP1* is located in the similar region of cloned QTL, *Gn1a* (Ashikari et al. 2005). However, *qSPP1* is not the same as *Gn1a* because *qSPP1* flanked by markers MRG2746 and RM490, which corresponds to the physical distance of 6.15 Mb to 6.60 Mb, is obviously different from *Gn1a* location of 5.26 Mb. No QTL around the *qSPP2* and *qSPP3* regions has been cloned at the moment. Therefore, it is worth isolating *qSPP1, qSPP2* and *qSPP3.* No doubt, NIL1, NIL2 and NIL3 would be the good materials for fine mapping and cloning of *qSPP1, qSPP2* and *qSPP3*, respectively.

Application for rice breeding program

In present study, we confirmed the identities of four QTL using NILs, *qSPP1*, *qSPP2, qSPP3* and *qSPP7*, which controlled the component of yield. Three QTL, *qSPP1*, *qSPP2* and *qSPP3*, could increase SPP without affecting heading date and plant height compared with the recurrent parent, Zhenshan 97. They are valuable genes for rice yield improvement. *qSPP7,* had a larger effect on grain yield than the other three QTL, but it simultaneously delayed heading date and increased plant height. Considering that a proper increase in plant height and delay of heading date can benefit photosynthesis and accumulation of carbohydrate, *qSPP7* is still a good candidate QTL in rice breeding program. HR5 has positive alleles at all the four detected QTL, which was in agreement with no transgressive segregation in RILs. HR5 alleles can be introgressed to recipient with marker aided selection for rice yield improvement.

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