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ORIGINAL PAPER

Fine mapping of a major quantitative trait loci, *qSSP7*, controlling the number of spikelets per panicle as a single Mendelian factor in rice

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Abstract In our previous studies, one putative QTL affecting number of spikelets per panicle (SPP) was identified in the pericentromeric region of rice chromosome 7 using a recombinant inbred population. In order to define the QTL (*qSPP7*), RI50, a recombinant inbred line with 70% of genetic background same as the female parent of Zhenshan 97, was selected to produce near-isogenic lines for the target region in the present study. In a BC_2F_2 population consisting of 190 plants, the frequency distribution of SPP was shown to be discontinuous and followed the expected Mendelian ratios (1:2:1 by progeny test) for single locus segregation. *qSPP7* was mapped to a 0.4 cM region between SSR marker RM3859 and RFLP marker C39 based on tests of the BC_2F_2 population and its progeny. Its additive and dominant effects on SPP were 51.1 and 24.9 spikelets, respectively. Of great interest, the QTL region also had effects on grain yield per plant (YD), 1,000 grain weight (GW), tillers per plant (TPP) and seed setting ratio (SR). Significant correlations were observed between SPP and YD ($r = 0.66$) and between SPP and SR ($r = -0.29$) in the progeny test. 1082 extremely small panicle plants of a BC_3F_2 population containing 8,400 individuals were further used to fine map the QTL. It turns out that *qSPP7* co-segregated with two markers, RM5436 and RM5499 spanning a physical distance of 912.4 kb. Overall results suggested that recombination suppression occurred in the region and positional cloning strategy is infeasible for *qSPP7* isolation. The higher grain yield of Minghui 63 homozygote as compared to the heterozygote suggested that Minghui 63 homozygote at *qSPP7* in hybrid rice could further improve its yield.

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Introduction

With the advent of DNA molecular markers, QTL mapping has become a routine strategy for the discovery of genes involved in complex quantitative traits. Thousands of QTL have been mapped for important agronomical traits in rice (http://www.gramene.org/db/cmap/map_set_info?species_acc=rice&map_type_acc=qtl). Although primary mapping populations including F_2, recombinant inbred lines (RILs) and doubled haploid lines (DHs) have been widely used for QTL mapping in rice (Li et al. 1995; Yu et al. 1997; Xu et al 2004; Fan et al. 2005; Marri et al. 2005), QTL can only be localized to a genomic region (confidential region) rather than a locus in those populations. Following the primary mapping, advanced populations such as near isogenic lines (NIL) and chromosome segment substitution lines (CSSL) can be used to map QTL to a locus as a Mendalian factor by blocking the genetic background noise (Lin et al. 2002; Zhang et al. 2006; Wang et al. 2006). Based on this strategy, several QTLs have been isolated in tomato (Frary et al. 2000) and rice (Yano et al. 2000; Takahashi et al. 2001; Li et al. 2003) in recent years. The common aspect in all the QTL cloning work is to exploit high quality NILs as advanced mapping populations.

Food shortage is one of the most serious global problems. The United Nations Food and Agricultural Organization (FAO) estimates that 852 million people worldwide

were undernourished between 2000 and 2002 (http://www.fao.org/documents/show_cdr.asp?url_file0/docrep/007/y5650e/y5650e00.htm). Rice is a staple food for many countries. As the world population increases, rice production has to be raised by at least 70% over the next three decades to meet growing demands. In the long run, development of high yield varieties is one of the most important goals in rice cultivation. Unfortunately, yield improvement efficiency is deemed to be very low due to its complex property affected by number of spikelets per panicle (SPP), 1,000-grain weight (GW) and tillers per plant (TPP). Of these factors, SPP was shown to be highly correlated with yield and acts as a crucial component in determining rice yield (Hua et al. 2002). Therefore, dissection of its genetic basis would be of great value in breeding high yield variety. So far, QTLs for SPP have been mapped in lots of populations, which were derived from inter-subspecific and intraspecific crosses (Li et al. 1997; Yu et al. 1997; Xing et al. 2002). Although many QTL controlling SPP were mapped in rice, only *Gn1a*, has been cloned. *Gn1a*, controlling grain productivity in rice, was elucidated to be a gene encoding cytokinin oxidase/dehydrogenase (*OsCKX2*), an enzyme that degrades the phytohormone cytokinin. Reduced expression of *OsCKX2* causes cytokinin accumulation in inflorescence meristems and increases the number of reproductive organs, resulting in enhanced grain yield (Ashikari et al. 2005).

In our previous studies, a QTL affecting the number of spikelets per panicle was repeatedly mapped to one pericentromeric region flanked by RFLP markers C1023 and R1440 on rice chromosome 7 in several primary mapping populations (Yu et al. 1997, 2002; Xing et al. 2001, 2002). In the present study, we aimed to precisely estimate its genetic effect under near isogenic background and fine map the QTL as a single Mendelian factor based on a large number of NILs.

Materials and methods

Development of NIL

In our previous study, one QTL, *qSPP7*, was mapped to the interval between R1440 and C1023 on chromosome 7 using a recombinant inbred population derived from the cross between Zhenshan 97 and Minghui 63 (Xing et al. 2002). The recombinant inbred line 50 (RI50), was selected to backcross with the female parent Zhenshan 97 and a BC_1F_3 population was obtained with two selfings. RI50 was homozygous for Minghui 63 alleles in the targeted QTL region on chromosome 7. Moreover, approximately 70 percent of RI50 genetic background was the same as that of Zhenshan 97 based on 221 polymorphic genome-wide markers (Xing et al. 2001). One line of BC_1F_3 population (BC_1F_3-120) with homozygous Minghui 63 alleles, showing uniform number of SPP, was selected to continue backcrossing with Zhenshan 97 to produce BC_2F_2 and BC_2F_3 populations. According to the genotypes of the flanking markers, one BC_2F_2 plant (BC_2F_2-SPP7) with homozygous Minghui 63 regions surrounding *qSPP7* was chosen to backcross with Zhenshan 97 to produce the BC_3F_1. In order to screen the genetic background of BC_2F_1, 150 SSR markers evenly distributed on 12 chromosomes were selected from all polymorphic markers between Zhenshan 97 and Minghui 63 (McCouch et al. 2002). Eight chromosome fragments covering about 150 cM on chromosomes 1, 3, 5, 6, 7, 9 and 12 (two segments), were heterozygote in BC_2F_1. The markers situated in these 8 fragments were further used to screen several BC_3F_1 plants. Finally, the one with the least background markers, that is, only three small regions on chromosomes 5, 9 and 12 were heterozygote besides about 40 cM region containing the targeted QTL on chromosome 7 (Fig. 1), was selected for BC_3F_2 production. The populations of 190 BC_2F_2 and BC_3F_2 individuals were used to evaluate the gene effects, respectively. The BC_3F_1 plant was divided into several clones at seedling stage in order to harvest amounts of selfed seeds. 8,400 BC_3F_2 plants were used for fine mapping the genes.

Field trial and trait measurement

The 190 BC_2F_2 plants with RI50, Zhenshan 97 and Minghui 63 were sown on 17 May 2002 and transplanted to the field

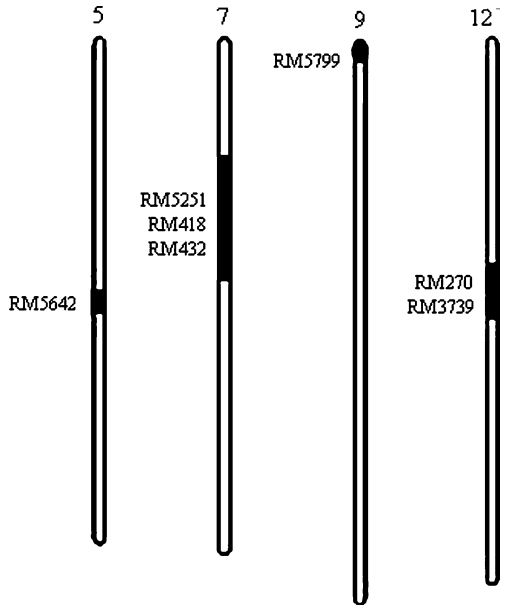

Fig. 1 The graphic genotype of the BC3F1 plant. The *black* and *white* chromosome segments were heterozygote and Zhenshan 97 homozygote, respectively. Only four chromosomes contained segments of donor parent, Minghui 63. The other eight chromosomes were fixed with Zhenshan 97

with distance of 16.5 cm between plants within a row, and of 25 cm between rows on 11 June 2002 in Wuhan (East longitude 114°, North latitude 30°), where the average day length during the period between June and August is 13.5 h. BC_3F_1 ratoons from Hainan were divided into seven individuals and transplanted in the field on 1 May 2003. Zhenshan 97, Minghui63, BC_2F_3-SPP7, the 190 BC_2F_3 families and the large BC_3F_2 population with 8,400 plants were sown on 19 May 2003. Plants were then transplanted to the field on 10 June 2003 in Wuhan. From each BC_2F_3 family, 16 healthy plants were selected for the progeny test. Field management essentially followed the normal agricultural practices, with fertilizer applied (per hectare) as follows: 48.75 kg N, 58.5 kg P and 93.75 kg K as the basal fertilizer; 86.25 kg N at the tilling stage and 27.6 kg N at the booting stage. Each plant was harvested individually at maturity.

Yield per plant (YD) was scored as the total weight (g) of the grains from the entire plant. The number of tillers per plant (TPP) scored as the number of reproductive tillers for each plant. The number of spikelets per panicle (SPP) was measured as the number of spikelets divided by TPP. Seed setting rate (SR) was scored as the number of grains per panicle divided by SPP, 1000-grain weight (GW) as YD divided by the number of grains multiplied by 1,000. Five plants of Zhenshan 97, Minghui 63, BC_2F_3-SPP7 and BC_3F_1 were used to score the phenotype. The arithmetic mean of each homozygous BC_2F_3 family in the targeted region was regarded as the trait value of the genotype. For the heterozygote family (whose segregation ratio within each family—frequently distorted away from 1:2:1), the weighted mean of the 16 plants was regarded as the trait values of corresponding heterozygous BC_2F_2 plants. The weighted mean of trait value (y) was calculated by the formula: $y = a/4 + b/4 + c/2$, in which, a, b and c represent the trait mean value of Zhenshan 97 homozygote, Minghui 63 homozygote and heterozygote genotypes within a family, respectively.

Genotyping of populations

Genomic DNA was extracted from 190 BC_2F_2 plants, 190 BC_3F_2 plants and 1,082 plants with small panicle (<96 spikelets) out of 8,400 BC_3F_2 plants by using simple fast CTAB method (Murray and Thompson 1980). Co-dominant SSR markers located in the region were used to screen the polymorphism between parents. The SSR genotyping was executed as described by Wu and Tanksley (1993).

Linkage analysis and QTL analysis

Genetic linkage map was constructed by using Mapmaker/EXP 3.0 (Lincoln et al. 1992). The genetic distance was determined by Kosambi function. Due to very few regions segregated in the populations of the 190 BC_2F_2 plants and 190 BC_3F_2 plants, simple interval mapping were employed for QTL analyses using Mapmaker/QTL (Lander and Botstein 1989). The LOD thresholds ranged from 2.3 to 2.8 for the 5 traits, determined by 1,000 random permutations at a false positive rate of 0.05 for each trait. Performance of the self-pollinated progeny (BC_2F_3) was used to determine the individual BC_2F_2 genotypes at the QTL. Then he QTL was treated as a marker and were directly located into the linkage group by using Mapmaker/EXP 3.0.

Fine mapping the gene using the extremely small panicle plants

Based upon the assumption that all 1082 small panicle (<96 spikelets) plants were homozygous for the recessive allele (Zhenshan 97) at the targeted locus, the recombination frequency between a marker and the gene locus was calculated by using of a maximum likelihood estimator: $c = (N_1 + N_2/2)/N$ (Allard 1956; Zhang et al. 1994), in which N is the total number of the extremely small panicle plants investigated, N_1 is the number of plants homozygous for Minghui 63, and N_2 is the number of plants heterozygous for the parents. 20 progeny plants of each recombinant were grown in the next normal rice growing season to re-evaluate its phenotype.

Results

The trait performance of Zhenshan 97, Minghui 63, BC_2F_3-SPP7, BC_3F_1

Minghui 63 showed significant higher trait values than Zhenshan 97 in SPP, GW, SR and YD; however, Zhenshan 97 had more TPP (Table 1). Except SPP, no significant difference was identified in the other four traits between BC_3F_1 (ratoon) and BC_2F_3-SPP7 with homozygous Minghui 63 alleles in the targeted region. BC_2F_3-SPP7 and BC_3F_1 had much higher values in SPP and YD as compared to Minghui 63, the original parent of the population with higher trait values.

Phenotypic variations in BC_2F_2 and BC_2F_3

The frequency distribution of SPP showed discontinuous variation with a clear gap between 110 and 120 spikelets based on the progeny test in the BC_2F_2 population (Fig. 2). According to the progeny test, the BC_2F_2 individuals could be classified into three subgroups for SPP expressing uniform large panicle, uniform small panicle and varied SPP among 16 plants within each family. The three subgroups

Table 1 Trait performance of Minghui 63, Zhenshan 97, BC_2F_3-SPP7 and BC_3F_1 grown in the natural field in 2003

Plant	SPP	GW (g)	SR (%)	TPP	YD (g)
Zhenshan 97	109.2a	23.5a	72.7a	10.5b	19.6a
Minghui 63	148.6b	28.0b	77.1b	9.4a	29.9c
BC_2F_3-SPP7	185.4d	22.9a	74.2a	8.8a	27.7b
BC_3F_1*	165.2c	23.8a	75.3a	9.1a	26.9b

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

* The trait value of BC_3F_1 was gotten from BC_3F_1 ratoon, which was divided into seven individuals and transplanted in the field

corresponded to the three genotypes, respectively: the homozygotes of Minghui 63 (MM) and Zhenshan 97 (ZZ), and heterozygote (MZ) at the targeted QTL. On average, the number of spikelets per panicle of genotype MM was 102 more than that of ZZ. Genotype ZM was intermediate to those of the homozygotes. As expected, the frequency distribution of SPP in the BC_2F_2 population followed the Mendelian ratios (1:2:1 by progeny test) for single locus segregation ($\chi^2 = 1.07$, $P = 0.586$). SPP displayed bimodal distribution in the 190 BC_3F_2 (Fig. 2). The 190 plants were separated into two distinct groups: plants with small panicle (50 plants) which could be homozygous for Zhenshan 97 alleles at the targeted gene locus and plants with large panicle (140 plants) which could be the mixture of homozygotes for Minghui 63 alleles and heterozygotes. Also, frequency distribution was also in agreement with the expected Mendelian ratio (1:3) for single locus segregation ($\chi^2 = 0.11$, $P = 0.740$). In contrast, the other 4 traits showed continuous but not normal distribution (Fig. 2). Therefore, it was impractical to clearly classify them into subgroups according to trait performance by the progeny test.

The BC_2F_2 population was classified into three subgroups of ZZ, MM and MZ according to SPP performance in the progeny test, the average values of the five traits were calculated for the three subgroups. Paired t test was used to compare trait value differences between subgroups. The averaged values of GW and SR among three subgroups

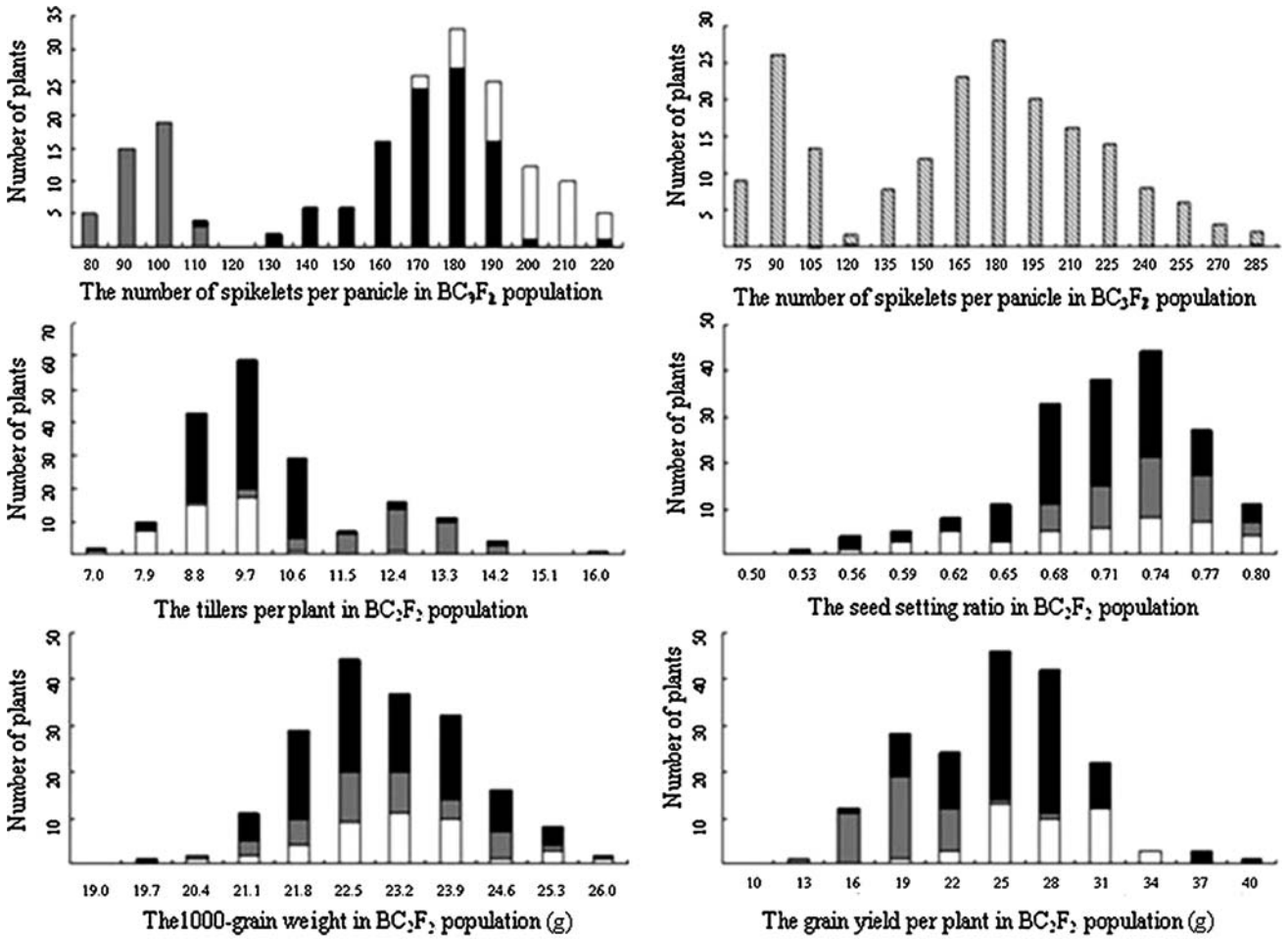

Fig. 2 Frequency distributions of the traits in BC_2F_2 population and BC_3F_2 population. Black, white and gray bars indicated the genotypes of heterozygous, homozygous for Minghui 63 alleles and homozygous for Zhenshan 97 alleles in BC_2F_2 population at *qSPP7*, respectively. The three genotypes at *qSPP7* were inferred by BC_2F_3 progeny test

Table 2 Correlation coefficient for yield traits calculated with BC_2F_3 population

Trait	SPP	SR	YD	GW
SR	−0.29**			
YD	0.66**	0.22**		
GW	0.03	−0.05	0.20*	
TPP	−0.54**	0.35**	−0.12	−0.03

*,** Significant at the level of $\alpha = 0.05, 0.01$ respectively

indicated no significant difference. On the contrary, significant difference existed between ZZ and the other two subgroups ($P = 0.001$) with regard to YD and TPP. MM genotype had the YD of 26.5 g significant higher than that of MZ, 24.2 g ($P = 0.05$).

The correlation coefficients between yield and its four components were presented in Table 2. The correlation coefficient between SPP and YD was the highest ($r = 0.66$) among all the investigated pair-tests, significant negative correlations were observed between SPP and SR ($r = -0.29$), between SPP and TPP ($r = -0.54$). No significant correlation was detected between SPP and GW ($r = 0.03$).

Validation of QTL for SPP

In our previous studies, one putative QTL affecting SPP and YD was identified in the pericentromeric region of rice chromosome 7, between RFLP markers C1023 and R1440 (Fig. 3a). In this study, QTL analysis was further performed in the same target region using BC_2F_2 and BC_3F_2 populations.

The genotypes of the corresponding BC_2F_2 plants at *qSPP7* locus were easily determined on the performance of their progenies. Thus, *qSPP7* treated as a marker was located in the locus between SSR marker RM3859 and RFLP marker C39, 0.2 cM away from each marker (Fig. 3b). *qSPP7* showed partial dominance for SPP.

In the BC_3F_2 population, one QTL with a LOD value of 56.1 for SPP was located in the interval between RM3859 and C39 on chromosome 7. For simplicity, and hereafter, the QTL was referred as *qSPP7*, which was 0.2 cM away from RM3859 and C39, and co-segregated with RM5436. Its additive and dominant effects were 59.8 and 28.3 spikelets per panicle with a contribution of 74.2% to the phenotypic variance.

Accordingly, based on the BC_2F_2 population and its progenies, two major QTLs controlling YD and TPP, were respectively mapped to the same interval where *qSPP7* was located (Table 3). Also, a minor QTL for SR was also detected in the same interval. Minghui 63 allele increased SPP and YD, but decreased TPP and SR. No QTL for GW was mapped. The QTL for SR expressed over-dominance, but the others showed partial dominance.

High-resolution mapping of *qSPP7*

The 190 BC_3F_2 plants could be divided into two subgroups with 112 spikelets per panicle as the boundary (Fig. 2). Zhenshan 97 alleles at *qSPP7* exhibited small SPP in contrast to Minghui 63 alleles and heterozygous alleles. In order to avoid using heterozygous plants, 1,082 plants with extremely small panicle (<96 spiketes), which accounted for about 1/8 of 8,400 BC_3F_2 plants, were selected for map based gene cloning. Two SSR markers of RM5451 and RM445 flanking the QTL (Fig. 3) were used to detect the recombinant events between markers and the targeted gene. Analysis of RM5451 identified 66 recombination events between the marker and *qSPP7* on one side, and analysis of RM445 detected 146 recombination events between the

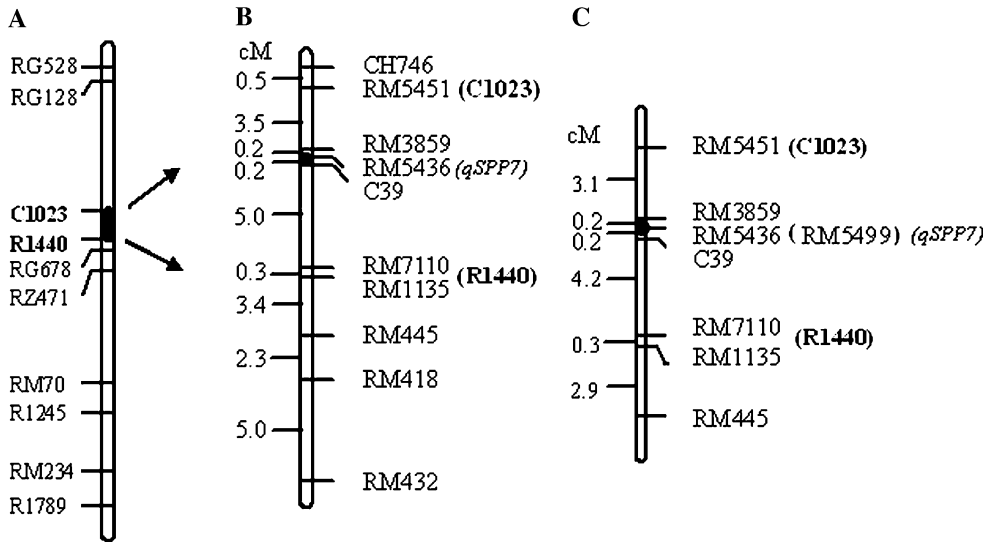

Fig. 3 Linkage maps showing the location of *qSPP7* based on three populations. **a** Linkage map of chromosome 7 showing the QTL region based on the recombinant inbred lines from the cross between Zhenshan 97 and Minghui 63, the *black bar* indicated 1-LOD confidence interval (Xing et al. 2001). **b** The QTL (*qSPP7*) location was mapped based on the 190 BC_2F_3 progeny test. The *black dot* indicates the centromere position. **c** The QTL (*qSPP7*) location was determined based on 1082 extreme small panicle BC_3F_2 plants. The *black dot* indicates the centromere position

Table 3 Genetic effects of the QTL region between RM3859 and C39 on the four traits by Mapmaker/QTL

Traits	Population	LOD	*A*	*D*	*D*/*A*	R^2 (%)
SPP	BC_2F_3*		51.1	24.7	0.48	
	BC_3F_2	56.1	59.8	28.3	0.47	74.2
YD (g)	BC_2F_3	22.3	4.4	2.7	0.61	43.2
TPP	BC_2F_3	32.4	−1.6	−1.0	0.63	61.9
SR (%)	BC_2F_3	3.0	−1.6	−2.1	1.31	7.4

A additive effect on the Minghui 63 allele, *D* dominant effect on the Minghui 63 allele, R^2 percentage of total phenotypic variance explained by the QTL

* Genotypes of the corresponding BC_2F_2 plants at the gene locus were identified by BC_2F_3 progeny test, and then the QTL treated as a single Mendelian factor was mapped to the linkage group. Its genetic effect estimated on the progeny data. Additive effects equal to the half of the trait value difference between two homozygotes, dominance effects equal to the trait value difference between heterozygote and the middle value of two homozygotes

marker and *qSPP7* on the other side. Assay with four additional markers of RM3859, RM1135, RM7110 and C39, which are more internal to *qSPP7*, identified 4 recombination events on the RM5451 side and 94, 88 and 4 recombination events on the RM445 side, respectively (Fig. 4). Using the formula given by Zhang et al. (1994), the recombination frequencies between RM3859 and *qSPP7*, and between C39 and *qSPP7* were both calculated to be 0.18%, which equaled to 0.2 cM from *qSPP7* to both RM3859 and C39 (Fig. 3c). Consequently, two SSR markers RM5436 and RM5499 were chosen to screen the eight recombinants from each sides of the gene. However, no recombination event was left for the two markers. RM5436 and RM5499 were located in the BAC clones of AP003756 and AP003836, respectively. The physical distance between the two markers is of 912.4 kb (http://www.gramene.org/Oryza_sativa_japonica/mapview?chr=7). Thus, the *qSPP7* was defined by RM3859 and C39, and co-segregated with a large region.

Discussion

In this study, a major QTL for SPP was fine mapped to a 0.4 cM region, which had effects on TPP, YD and SR as well. This mapping result benefited mainly from the use of near isogenic lines and should be of great value for transferring the favorable alleles to rice varieties.

The target region exhibited similar effects on SPP in both the F_2 population (Yu et al. 1997; 2002) and the RIL population (Xing et al. 2001; 2002) derived from the same cross between Zhenshan 97 and Minghui 63. The QTL-NIL populations in the present study revealed even richer information on this QTL. Besides the same direction of the genetic effect with Minghui 63 alleles increasing trait values across different generations, the NIL population provided more accurate estimation of genetic effects on SPP due to the minimization of its genetic background noise. Obviously, these genetic effects in F_2 and RILs were underestimated. As expected, SPP frequency showed normal distribution in F_2 and RIL populations. In contrast, it expressed a bimodal or discontinuous SPP frequency distribution in the NILs, which could be used to determine the genotype of individual plants at *qSPP7* according to their progeny test. In fact, several examples of successful QTL cloning were benefited greatly from development of high quality NILs (Yamamoto et al. 2000; Lin et al. 2003) in the last decade. There were few heterozygous plants falling into the category of small number of SPP (Fig. 2) in this study could cause false recombinants in the fine mapping process. This problem was solved in two ways: first, only 1/8 of the plants with the smallest number of SPP were chosen from the 8,400 plants to screen the recombinants, which can greatly minimize the risk of false recombinants; second, the reliability of all screened recombinants were further confirmed by progeny test, which showed uniform small number of SPP among 20 progeny plants. Hence, it is highly confident that all recombinants are authentic in this study.

Although centromeres are often associated with a depression of meiotic recombination adjacent to the

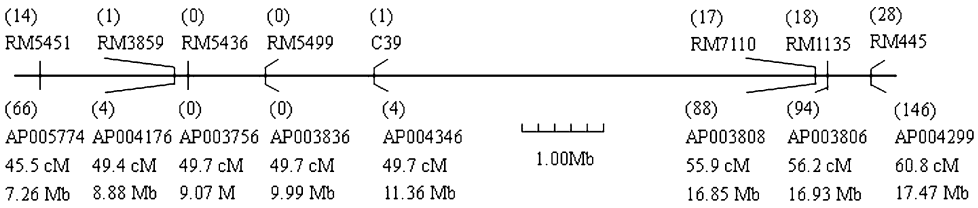

Fig. 4 Genetic and physical map in the local region of *qSPP7*. Numbers of recombinants detected in 190 BC_2F_2 plants and 1082 BC_3F_2 plants between the markers and *qSPP7* were indicated in the parentheses in the *top* and *bottom* of markers, respectively. SSR marker physical position was given at two forms of physical distance and the accession number of BAC clone contained the SSR marker (http://www.gramene.org/Oryza_sativa_japonica/). The genetic positions of SSR markers were mined from the website http://rgp.dna.affrc.go.jp/cgi-bin/statusdb/stattable.pl?chr=7&lab=RGP

pericentromeric regions (Haupt et al. 2001), no recombination suppression was reported in the pericentromeric region on rice chromosome 3 (Fan et al. 2006). Thus, it is possible to narrow down the QTL located in the pericentromeric regions to a candidate gene by map-based cloning in this study. Unfortunately, severe recombination suppression was observed in the targeted interval within pericentromeric region (Fig. 4). The recombinants between markers RM5451, RM445 and RM7110, and *qSPP7* in the 1082 BC_3F_2 plants were about five-fold as those in the 190 BC_2F_2 plants, respectively. While, the recombinants in the 1082 BC_3F_2 plants decreased to four-fold as those in the 190 BC_2F_2 plants for markers RM3859 and C39, indicating the presence of light recombination suppression at the two marker loci. There was no recombinant in both two populations for markers RM5436 and RM5499 covered a distance of more than 900 kb. This indicated the presence of severe recombination suppression in the region between RM5436 and RM5499. No clear recombination suppression was observed in the regions between RM5451 and RM3859, RM1135 and RM7110. But clear recombination suppression was occurred in the region between RM7110 and C39. Therefore, recombination suppression was occurred at least in the region between RM3859 and C39 although the right boarder of recombination suppression region could not be precisely defined due to lack of polymorphic markers in the large region between RM7110 and C39. The genetic distance between RM3859 and C39 was 0.4 cM based on mapping result in either 190 BC_3F_2 plants or 1082 extreme BC_3F_2 plants. RM3859 and C39 are located in the BAC clones AP004176 and AP004346, respectively. The physical distance between these two clones was estimated to be as large as 2,400 kb or so (http://www.gramene.org/Oryza_sativa_japonica/mapview?chr=7). The ratio of physical-to-genetic distance in this targeted region was about 6,000 kb/cM, which is 20 times higher than the estimated average ratio (250–300 kb/cM) (Arumuganathan and Earle 1991). This is consistent with the ratio of 2740 kb/cM, or 10 times higher than that of the rest of the genome reported by Wu et al. (2002). In tomato, it can be as high as 10- to 40-fold (Tanksley et al. 1992). In the present study, no recombinant was found between *qSPP7* and markers in the large region of 912.4 kb. Therefore, we failed to further narrow down the gene using a large population consisting of 1082 plants after *qSPP7* was mapped to a 0.4-cM region using the small BC_2F_2 population consisted of 190 individuals. Taken together, there was no resolution difference between the large and small populations for QTL fine mapping. That is to say, resolution of gene mapping could not be improved for a region with clear recombination suppression by enlarging a population even though there are lots of polymorphic markers in the region. This comes up with the conclusion that positional cloning strategy would not be feasible for isolation of the targeted gene, *qSPP7*.

Besides GW, one QTL for YD and TPP and SR was simultaneously detected in the *qSPP7* surrounding region. The QTL for SR expressed over-dominance, the QTL for other traits acted in partially dominant (Table 3). These indicated that the homozygote with Minghui 63 alleles is promising to produce higher yield than does the heterozygote. Given the traits of yield components are highly correlated, ridge regression analysis was used for the phenotype data set collected from BC_2F_3 to build a regression equation of the form: $YD = -70.454 + 0.16 \times SPP + 2.08 \times TPP + 0.92 \times GW + 39.23 \times SR$. From the equation, the theoretical YD can be easily predicted. For example, the additive and dominance effects of *qSPP7* were estimated in BC_2F_3, the theoretical YD difference between genotypes MM and MZ at *qSPP7* is 3.2 g. This is well illustrated by the fact that the Minghui 63 homozygotes at *qSSP7* showed larger average values in the traits than the heterozygotes in the BC_2F_3 progenies. Moreover, the averaged grain yield per plant of Minghui 63 homozygotes was 26.5 g, which was significantly higher ($P = 0.05$) than heterozygotes of 24.2 g in the BC_2F_3 progenies. This result was also consistent with the conclusion that heterozygotes were not always advantageous for F_1 performance on the basis of an "immortalized F_2" population produced from the same cross between Zhenshan 97 and Minghui 63 (Hua et al. 2002). Thus, the ideal genotype in the *qSPP7* surrounding region in the hybrid should be Minghui 63 homozygotes for hybrid production. Markers flanking the QTL could be directly used for improvement of parent Zhenshan 97. Theoretically, the new hybrid Shanyou 63 with homozygous Minghui 63 alleles at *qSPP7* should exhibit higher YD than does its original hybrid Shanyou 63, an elite rice hybrid between Zhenshan 97 and Minghui 63 in last two decades in China. The new version Shanyou 63 is in the process of development.

In summary, the QTL of *qSPP7* will play a crucial role in developing high yielding rice varieties. Efforts in its isolation are worth being made in combination with bioinformatic strategy.

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