

Detection of QTLs Influencing Panicle Length, Panicle Grain Number and Panicle Grain Sterility in Rice (*Oryza sativa* L.)

Jafar Ahamadi¹, M. H. Fotokian², S. Fabriki-Orang³

¹ Biotechnology Department, Imam Khomeini International University, Gazvin, Iran (Corresponding author)

² Shahed University, Tehran, Iran

³ Tarbiat Modarres University, Tehran, Iran

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Abstract

The detection, characterization and use of quantitative traits loci, QTL, have significant potential to improve the efficiency of selective breeding of species. Therefore, a population with 59 advanced backcross lines (BC₂F₃), derived from a cross between IR64 and Tarome molaei, were studied in Tonekabon Rice Research Station of Iran in order to map QTLs for panicle length, number of grain per panicle, and panicle grain sterility in rice. The parental screening with 235 SSR markers in agarose and polyacrylamide gels revealed 114 markers with clear polymorphic bands. To search for QTLs associated with panicle length, number of grain per panicle, and panicle grain sterility, we constructed a genetic linkage map using 114 microsatellite markers. Positive and negative transgressive segregations were observed in BC₂F₃ lines for all traits. Using multiple interval mapping (MIM), a total of 20 putative QTLs were detected, of which eight were for panicle length, three for number of grains, and nine for panicle grain sterility. The maximum number of QTLs were mapped on chromosomes 1 and 2 with eight QTLs. These QTL markers could possibly be utilized for marker-assisted selection.

Key words: QTL, mapping, rice, *Oryza sativa* L., SSR.

Introduction

Quantitative trait locus, QTL, mapping has been in wide use for nearly two decades, during which molecular markers have become available in conjunction with interval mapping methods (Lander and Botstein 1986). The goal of QTL mapping is to determine the loci that are responsible for variation in quantitative traits. In some situations, determination of the number, location, and the interaction of these loci is the ultimate goal. However, the identification of the actual genes and their functions are also of interest. For example, breeding studies attempt to identify the loci that improve crop yield or quality, and then bring the favorable alleles together into elite lines. The identification of genomic regions that carry growth QTL allows breeders to use marker-assisted selection to precisely move beneficial

QTL alleles into elite agricultural strains for crop improvement. QTL mapping also helps quantitative and population geneticists to define the genetic architecture of growth traits (Mauricio 2001). In rice (*Oryza sativa* L.), panicle length, panicle grain number, and panicle grain sterility are crucial determinants of grain yield together with plant panicle number (Matsushima 1995). Genetic improvement of panicle length, grain number, and grain sterility are therefore a major concern for rice breeders to attain high yields (Yonezawa 1997). The recent development of molecular marker techniques has enabled researchers to identify gene loci affecting agronomic traits of interest, QTLs, on the linkage map of rice (Yano and Sasaki 1997). Yan et al. 1999, Kobayashi et al. 2003a and Kobayashi et al. 2003b, conducted QTL analysis for plant type traits, such as culm length, panicle number, and flag leaf length, across temperate and tropical climates. DNA markers are used to map important traits in the rice genome and can be used in marker-aided selection of these traits. Simple sequence repeat (SSR) markers are particularly useful for gene mapping and marker-based selection, since these

* To whom correspondence should be addressed

Jafar Ahamadi

E-mail: njahmadi910@yahoo.com

Tel: 098-02818371139

markers are amenable to high-throughput analysis and are informative in many types of genetic crosses. Previous studies in rice have contributed to the development of several hundred microsatellite markers and a genetic map consisting of 320 SSRs (Akagi et al. 1996; Chen et al. 1997; Panaud et al. 1996; Temnykh et al. 2000; Wu and Tanksley 1993). These markers have been used to analyze diversity (Cho et al. 2000; Harrington 2000; Olufowote et al. 1997; Yang et al. 1994) and to locate genes and QTLs on rice chromosomes using both intra- and interspecific crosses (Bao et al. 2000; Bres-Patry et al. 2001; Moncada et al. 2001; Xiao et al. 1998; Zou et al. 2000). SSRs are increasingly useful for integrating the genetic, physical, and sequence-based maps of rice, and they simultaneously provide breeders and geneticists with an efficient tool to link phenotypic and genotypic variation (Temnykh et al. 2001). A total of 2,414 new di-, tri-, and tetra-nucleotide non-redundant SSR primer pairs, representing 2,240 unique marker loci have been developed and experimentally validated for rice, *Oryza sativa* L. (McCouch et al. 2002).

To detect QTLs controlling traits of agronomic importance in rice, 194 recombinant inbred lines (F_8), with crossing of two elite homozygous lines 9024 and LH422, which represent the indica and japonica subspecies of rice, were genotyped with 141 RFLP markers and evaluated in a field trial for 13 quantitative traits including grain yield. Transgressive segregants were observed for all traits examined. The number of significant QTLs ($LOD \geq 2.0$) detected affecting each trait ranged from one to six. The percentage of phenotypic variance explained by each QTL ranged from 5.1 to 73.7%. For those traits for which two or more QTLs were detected, increases in the traits were conditioned by indica alleles at some QTLs and japonica alleles at others. No significant evidence was found for epistasis between markers associated with QTLs and all the other markers. Pleiotropic effects of single QTLs on different traits are suggested by the observation of clustering of QTLs. No QTL for traits was found to map to the vicinity of major gene loci governing the same traits qualitatively. Evidence for putative orthologous QTLs across rice, maize, oat, and barley is discussed (Xiao et al. 1995).

The identification of QTLs for yield components and plant height with an F_2 and two equivalent F_3 populations of an indica-indica cross of rice were conducted for three trials. A total of 44 QTLs were detected in 18 intervals of nine chromosomes, including three for the number of panicles, six for the number of filled grains, six for total number of paniclelets, three for paniclelet sterility, seven for 1000-grain weight, five for grain weight per plant, eight for plant height, and seven for panicle length. In all three trials, QTLs were frequently detected for related traits in the same intervals. The directions of additive effect of QTLs for related traits in a given interval were in agreement with few exceptions, no matter whether they were detected in the same trial or not. When gene pleiotropism was considered, 23 of the 29 QTLs for yield and its components and nine of the 15 QTLs for plant stature were detected in more than one trial. This indicated that the detection of chromosomal segments harboring QTLs was hardly affected by environmental factors (Zhuang et al. 1997). The genetic basis for three grain

yield components of rice, 1000-kernel weight, grain number per panicle, and grain weight per panicle, was investigated using restriction fragment length polymorphism markers and F_4 progeny testing from a cross between rice subspecies japonica (cv. Lemont from the USA) and indica (cv. Teqing from China). Following identification of 19 QTLs affecting these traits, we investigated the role of epistasis in the genetic control of these phenotypes. Among 63 markers distributed throughout the genome that appeared to be involved in 79 highly significant ($P < 0.001$) interactions, most (46 or 73%) did not appear to have main effects on the relevant traits, but influenced the trait(s) predominantly through interactions. These results indicate that epistasis is an important genetic basis for complex traits such as yield components, especially traits of low heritability such as grain number per panicle and grain weight per panicle. The identification of epistatic loci is an important step toward resolution of discrepancies between QTL mapping and classical genetic dogma, contributes to better understanding of the persistence of quantitative genetic variation in populations, and impels reconsideration of optimal mapping methodology and marker-assisted breeding strategies for improvement of complex traits (Li et al. 1997).

The objective of this study was to identify and characterize QTLs for panicle length, number of grain per panicle and panicle grain sterility in rice. For this objective, BIL lines derived from a cross between two genetically divergent high-yielding varieties, IR64 and Tarome molaei, were studied. Therefore, QTL analyses using the BIL lines could provide information useful for rice breeders to improve panicle length, number of grain per panicle, and panicle grain sterility, and ultimately, rice yields.

Materials and Methods

Plant materials

IR64, as recurrent parent, was crossed to Tarome molaei, as donor parent, and then F_1 plants were backcrossed with IR64 to produce BC_2F_1 progeny. Fifty-nine advanced backcross-inbred lines, BC_2F_5 , developed by self-fertilization from BC_2F_1 plants (IR64/ Taromee molaei // IR64/// IR64) by the single-seed descent method. Fifty-nine advanced BILs, BC_2F_5 , and their parental lines were sown in May 2006, and twenty 30-day-old seedling of each line were transplanted in June and grown as spaced plants under natural conditions in chaparsar-tonekabon, the rice Experimental Station of Iran, in a randomized complete block design with two replications to reduce the effects of environmental factors. For each line, ten plants were harvested 40 days after heading, when mature, and evaluated individually for panicle length (PL), panicle grain number (PGN), and panicle grain sterility (PGS) to calculate the mean value for QTL analysis.

DNA preparation

DNA was extracted from young leaves following the method of CTAB (Murray and Thompson 1980; Pal et al. 2001), and dissolved in TE buffer. DNA quality and quantity were estimated

spectrometrically, and the concentration adjusted to 20 ng/μl. PCR amplification was carried out in 10 μl solutions containing 10 mM Tris-HCl, pH 8.3, 50 mM KCl, 1.5 mM MgCl₂, 50 μM each of dNTP, 0.01% Gelatin, 0.5 μM of forward and reverse primers, 0.5 U Taq polymerase, and 10 ng of DNA template. The PCR regime was: 94°C for 5 min, followed by 35 cycles of 60 s denaturing at 94°C, 60 s annealing (at between 55 and 67 °C), and 120 s extension at 72°C, plus a final extension of 7 min at 72°C completed the cycles performed on a research thermocycler. The PCR products were separated by electrophoresis on a 2% agarose gel and 4% polyacrylamide gels stained with ethidium bromide and visualized by UV fluorimetry.

SSR assay and linkage analysis

For the SSR assay, 114 SSR primer pairs of 235 microsatellite markers (SSRs) derived from Cornell SSR linkage map (McCouch et al. 2002) tested on IR64 and Tarome molaei, showed polymorphism between the parental DNAs. A total of 114 SSR primer pairs were analyzed for the population. Linkage groups and the order of markers were determined using MAP-MAKER/EXP 3.0 (Lander et al. 1987) based on 114 SSRs to span 1825.0 cM. To avoid detecting pseudo-linkage of markers, relatively higher threshold levels (LOD. 3.0) were employed to establish linkage groups. Linkage groups were reconfirmed using the "GROUP" command with a LOD score 3.0 and recombination fraction 0.4. The RIPPLE command was used to verify the order of markers on each chromosome. The Kosambi mapping function was used to transform the recombination frequency to genetic distances, centi-Morgan, (Kosambi 1944).

QTL mapping

Multiple interval mapping was used to identify QTLs using the software package QTL Cartographer ver. 2.5 (Wang et al. 2004). The level of significance for QTLs in this study was determined as LOD ≥ 3 at P < 0.05. The proportion of observed phenotypic variance explained by each QTL was estimated by the coefficient of determination, R² (McCouch et al. 1997).

Results and Discussion

Phenotypic variation for yield-related traits

Analysis of variance revealed significant differences (P < 0.01) between the two parental lines in all yield related traits assessed in the current study (Table 1). Therefore it could be expected that the BILs population derived from the cross between the two parents would be suitable for mapping of the QTLs for yield-related traits. The BILs showed tremendous continuous variation and large transgressive segregation in both directions for all traits except panicle grain sterility in only one direction (Fig. 1).

Construction of framework map using SSR markers

In all, 114 SSR primer pairs of 235 tested on IR64 and

Table 1. Phenotypic and variance analysis of yield related traits in rice investigated under field conditions.

Standard deviation	BIL Population			Parental mean		Traits
	Mean	Max	Min	Tarome molaei	IR64	
2.45	25.66	32.17	19.5	32a	24b	Panicle length
46.8	129.7	324.7	75	162.7a	147.3b	Panicle grain number
.08	.17	.41	.05	.48a	.25b	Panicle grain sterility

a, b: indicates differences between the two parents significant at a level of $p < 0.01$.

Tarome molaei, showed polymorphism between the parental DNAs. A linkage map was constructed from BILs population using 114 of these markers giving an evenly spaced coverage of the genome, with an average marker density of one per 14.8 cM, spanning 1692.6 cM across all 12 rice chromosomes (Fig. 2). Map order was in agreement with that provided by McCouch et al. (2002). Segregation distortion in the population, tested by the χ^2 statistic, affected 20 loci mapping to chromosomes 1, 2, 4, 5, 6, 9, 10, 11, and 12. Such distorted segregations in mapping populations have been frequently reported (Harushima et al. 2002; Xu et al. 1997).

Association of molecular markers with quantitative traits and QTL analysis

Using data on segregation of each of the 114 molecular markers and that of the three quantitative traits, i.e. panicle length, panicle grain number and panicle grain sterility, χ^2 -test for independence of attributes was carried out to identify molecular markers showing association independently with each of the three traits. From three to nine QTLs (Table 2) were detected for each of the three studied traits using the advanced BILs by QTL Cartographer ver. 2.5 programme (Wang et al. 2004).

Panicle length

A total of eight QTLs were detected for panicle length on chromosomes 2, 4, 11, and 12. The phenotypic variations explained by each QTL localized in the regions RM485-RM236 and RM279-RM555 of chromosome 2 were in the range of 5-10 with LOD scores 8 and 8.5 (Table 2). In these QTLs, IR64 alleles increased the Panicle length in the range of 0.41-1.99 cm. In the regions RM241-RM348 and RM349-RM280 on chromosome 4, variations of each QTL were explained in the range of 7.5-13 with LOD scores 2.95 and 4.23, also the IR64 (as recurrent parent) alleles increased the Panicle length in the range of 0.55-0.91 cm. In the regions RM167-RM120 and RM21-RM206 on the chromosome 11, variations of each QTL were explained in the range of 2-11 with LOD scores 3.11 and 5.7. In these QTLs, Tarome molaei alleles increased the Panicle length in the range of 1.03-1.7 cm. Finally, two QTLs on the chromosome 12 were explained variations in the range of 5-9, and LOD scores were 4.96 and 5.4. the QP112a and QP112b on chromosome 12 of IR64 had negative and positive additive effect on panicle length, respectively. Therefore, the IR64 alleles contributed to increasing panicle length in both QTLs on chromosomes 2 and 4, and one QTL on chromosomes 12, whereas the Tarome

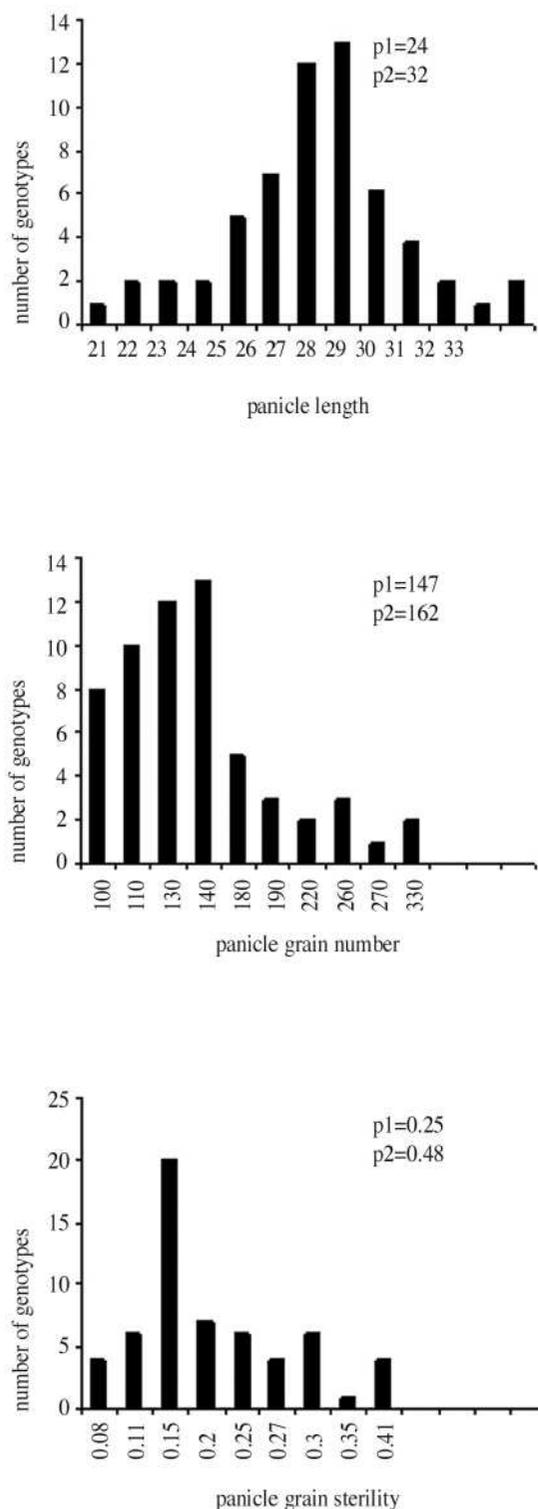


Fig. 1. Frequency distribution of traits in the BILs population of rice. The mean trait values of both parents (P_1 = IR64, P_2 = Tarome molaei) were indicated.

molaei alleles in both QTLs on chromosomes 11 and one QTL on chromosomes 12 contributed to increasing panicle length. In previous research, QTLs for panicle length were reported

between RM210 and RM256 on chromosome 8 in three populations (Marri et al. 2005; Thomson et al. 2003; Xiao et al. 1998) and between markers RM288 and RM205 on chromosome 9 in three populations (Marri et al. 2005; Septiningsih et al. 2003a, b; Thomson et al. 2003).

Panicle grain number

A total of three QTLs for panicle grain number were detected on chromosomes 1 and 12 (Table 2). The phenotypic variations explained by each QTL in the regions RM237-M473A and RM431-RM14 on chromosome 1 were in the range of 15-21.4 grain, and the LOD scores were 3.33 and 3.43 (Table 2). The IR64 allele, QGN1b, increased the grain number about 20.1 grain, but the Tarome molaei allele, QGN1a, increased the grain number about 32 grain. Also, one QTL detected on chromosomes 12 explained 27.8 of phenotypic variations with LOD score 4.06. In this QTL, Tarome molaei allele, QGN12, increased the grain number about 45 grain. For grains per panicle reported 17 QTLs on chromosomes 1, 2, 3, 4, 5, 6, 9, 11, and 12 (Brondani et al. 2002; Marri et al. 2005; Moncada et al. 2001; Septiningsih et al. 2003a; Thomson et al. 2003; Xiao et al. 1998; Yoon et al. 2006).

Panicle grain sterility

A total of nine QTLs associated with panicle grain sterility were detected on chromosomes 1, 2, 5, 6, 9, 10, and 11 (Table 2). Among them, QTLs located at the chromosomes 5 and 11 had the highest positive and negative additive effect on panicle grain sterility, respectively. The phenotypic variations explained by all detected QTLs was 81 percent. Two of the nine detected QTLs for this trait, the QTLs QS2a and QS2b with $R^2 = 31.9$ and 23.9, were selected as main QTLs.

Discussion

Grain yield in rice is composed of the number of grains and panicle grain sterility. Identification and location of genes controlling this trait is extremely difficult because of the large influence of environmental factors including the depth of flooding water, plant density, and weather conditions such as light and temperature. In our experiment, the Tarome molaei alleles at the chromosomes 1 and 12 increased the grain number of rice but another QTL on chromosome 1 of IR64 increased the grain number of rice. About panicle grain sterility, five QTLs of IR64 alleles and four QTLs of Tarome molaei alleles decreased the panicle grain sterility of rice. These results would suggest the possibility that the genes from the IR64 and Tarome molaei alleles could increase the grain number and decrease the panicle grain sterility of rice.

In the past, QTLs have been mapped for nine yield related traits in rice and from three wild species, *O. rufipogon*, *O. glumaepatula*, and *O. grandiglumis*. More QTLs were identified on chromosomes 1, 2, 3, and 4. Accordingly, 10 of 20 detected QTLs for yield related traits were also on chromosomes 1, 2, 3,

QTLs for Grains in Rice

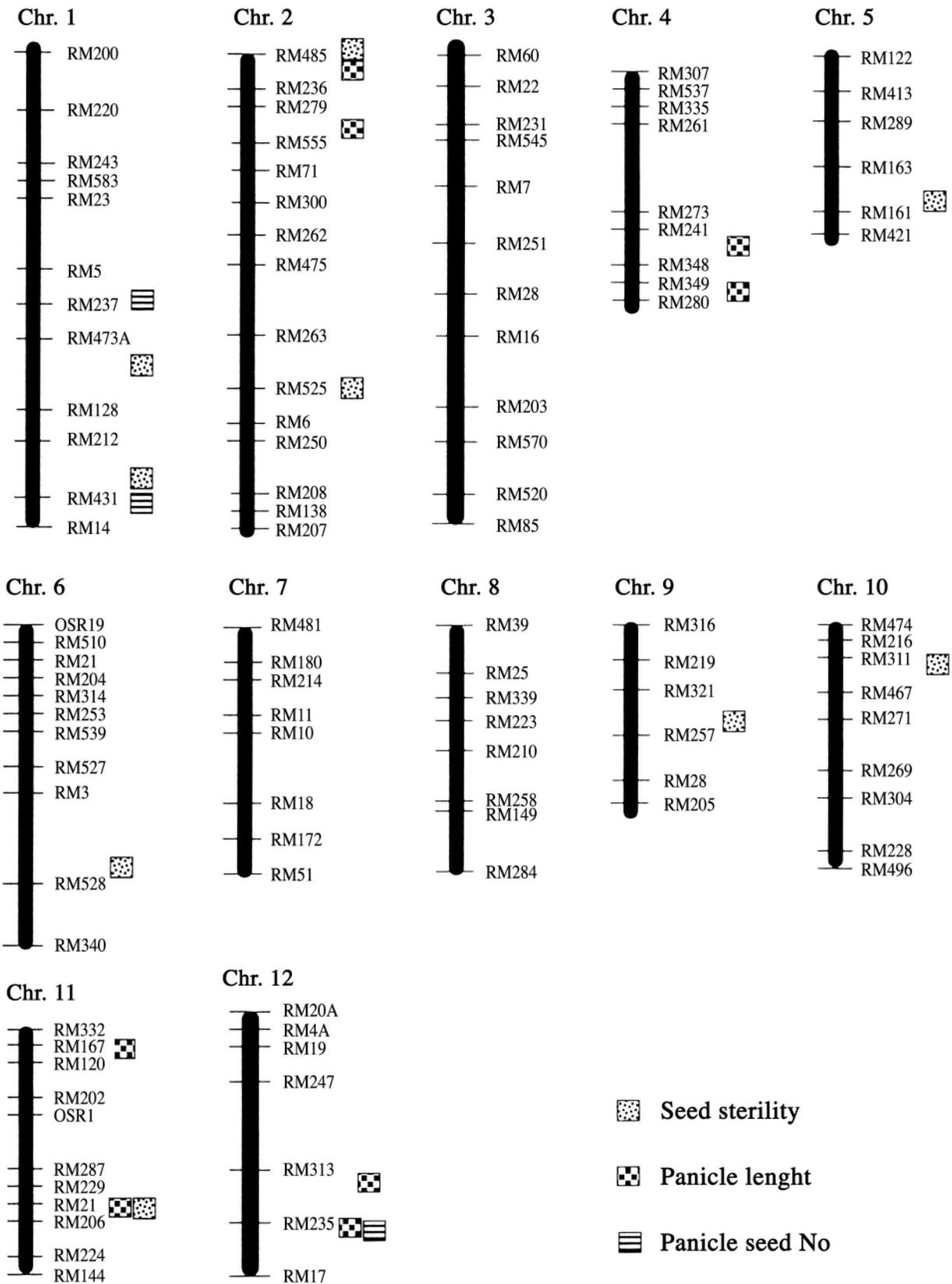


Fig. 2. SSR linkage map of rice based on fifty-nine advanced BILs (BC₂F₃) population. The abbreviation RM is used for rice microsatellite markers developed by McCouch et al. (1997a).

Table 2. Map position and putative QTLs for yield- related traits detected in advanced BILs (BC₂F₃) population of rice.

LOD ^a (%)	R ² ** ^c	ADDITIVE ^b EFFECT	QTL	MARKER INTER- VALS	CHROMO- SOME	Traits
8.5	5	1.99	QPI2a	RM485-RM236	2	Panicle length
8	10	.41	QPI2b	RM279-RM555	2	
2.95	7.5	.55	QPI4a	RM241-RM348	4	
4.23	13	.91	QPI4b	RM349-RM280	4	
3.11	2	-1.7	QPI11a	RM167-RM120	11	
5.7	11	-1.03	QPI11b	RM21-RM206	11	
5.4	9	-.57	QPI12a	RM13-RM235	12	
4.96	5	1.53	QPI12b	RM235-RM17	12	
3.43	21.4	-32	Q PGN1a	RM237-M473A	1	Panicle grain number
3.33	15	20.1	Q PGN1b	RM431-RM14	1	
4.06	27.8	-45	Q PGN12	RM235-RM17	12	
2.5	4.8	-.38	QS1a	RM473A-M128	1	panicle grain sterility
5.1	.6	-3.3	QS1b	RM212-RM431	1	
7.2	31.9	-1.52	QS2a	RM485-RM236	2	
5.9	23.9	5	QS2b	RM525-RM6	2	
3.4	10.7	5.7	QS5	RM163-RM161	5	
5.2	2	-1.52	QS6	RM3-RM528	6	
4.3	1.6	1.1	QS9	RM321-RM257	9	
6.4	3.6	-1.34	QS10	RM311-RM467	10	
7.8	2	-6.8	QS11	RM21-RM206	11	

a: LOD score (threshold 3.0).

b: Estimated effect of replacing Tarome molaei allele by IR64 alleles.

c: Proportion of the phenotypic variability explained by the marker.

and 4. For grains per panicle reported 17 QTLs on chromosomes 1, 2, 3, 4, 5, 6, 9, 11, and 12 (Brondani et al. 2002; Marri et al. 2005; Moncada et al. 2001; Septiningsih et al. 2003a; Thomson et al. 2003; Xiao et al. 1998; Yoon et al. 2006), but we report two new QTLs on chromosome 1 and one new QTL on chromosome 12. In rice cultivars, as many as 42 out of 330 QTLs reported for spikelet sterility map to chromosome 10 (<http://www.gramene.org>); we also report one out of nine QTLs for panicle grain sterility on chromosome 10. Several studies showed that yield and its related traits loci were commonly located at two marker intervals on chromosome 2. One interval was between markers RM250 and RM207 where QTLs for yield, grains per panicle, grains per plant, panicles per plant, grain weight, and grain number were located (Marri et al. 2005; Moncada et al. 2001; Septiningsih et al. 2003a, b; Xiao et al. 1998). The other region was between RM262 and RM263 where QTLs for yield per panicle, grains per panicle, grain weight, spikelets per panicle, panicles per plant, and number of tillers per plant, were mapped in two populations (Brondani et al. 2002; Marri et al. 2005). QTLs for panicle length and grains per panicle were collected between RM210 and RM256 on chromosome 8 in three populations (Marri et al. 2005; Thomson et al. 2003; Xiao et al. 1998). In other studies, QTLs for yield, panicle length, spikelets per panicle, grains per panicle, and grain weight were co-localized between markers RM288 and RM205 on chromosome 9 in three populations

(Marri et al. 2005; Septiningsih et al. 2003a, b; Thomson et al. 2003). In our review of research, for three target traits (i.e. panicle length, panicle grain number, and panicle grain sterility), we did not find QTLs the same as those previously reported QTLs (Table 2) and at least some of these could be new QTLs.

In general, QTL mapping studies conducted on data collected from a relatively small population size under a single environment are likely to detect the loci with large effects and miss the loci with small effects (Edwards et al. 1992; Tanksley 1993). Therefore, the number of the putative QTLs identified in this study should be considered a minimum of all those segregating in the population. There is substantial interest in using QTL mapping to understand the genetic basis of variation in plant growth and morphology, and a wide variety of traits are amenable to QTL analysis. Most of the studies discussed have taken advantage of recent improvements in marker technology and statistical analysis to improve mapping sensitivity and resolution. There are no clear candidate genes for a substantial number of the growth QTL identified, suggesting that natural variation and QTL mapping may provide new insight into growth processes. This, of course, will require the cloning of the underlying QTL. Several QTLs have been cloned by map-based cloning in Arabidopsis, rice, and tomato, suggesting that QTL cloning is becoming quite feasible (El-Assal et al. 2001; Takahashi et al. 2001; Yano et al 2000).

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