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A novel digenic epistatic interaction at two loci regulating spikelet fertility in rice

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Abstract
The seed setting rate (spikelet fertility) is an important determinant of rice yield. In the past few decades, genes that control rice seed set have been cloned, and many quantitative trait loci (QTL) have been identified. However, the epistasis influencing rice seed set remains largely unclear. In this study, a recombinant inbred line population, which consisted of 219 lines developed by crossing the Lemont and Yangdao4 rice cultivars, was grown in five environments to identify the QTL and epistatic loci related to seed set. A total of 26 minor-effect QTL were detected by multiple interval mapping, which explained less than 12.7% of the phenotypic variation individually. A pair of new epistatic loci were detected and confirmed by two-way analysis of variance; the homozygous Yangdao4 allele at the *qSS6.1* locus interacted with the homozygous Lemont allele at the *qSS8.1* locus and resulted in a low seed setting rate. A linear regression analysis and a multiple comparison test suggested that eight alleles at four QTL (*qSS1.3*, *qSS6.3*, *qSS7.1*, and *qSS8.1*) control seed set simultaneously. Marker-assisted selection using these four loci guaranteed a greater than 70% average seed setting rate in all five environments.

Keywords Rice · Seed setting rate · QTL · Epistasis · Recombinant inbred line

Introduction

Panicle number, grain number per panicle, seed setting rate (spikelet fertility) and grain weight are four major factors that determine the final rice yield. The seed setting rate, which is one of the most important components of rice yield, has attracted much research interest over the past few decades.

Rice seed setting is a complicated quantitative trait that is influenced by the male gamete fertility, female gamete fertility, extent of anther dehiscence, and environmental conditions, such as temperature (Liu et al. 2004). Several genes that control the seed setting rate have been characterized in rice, including *PTB1*, *THIS1*, *GSD1*, *GSL5*, *OsSPX1*, and *Laccase-13* (*OsLAC13*). *PTB1*, which encodes a RING-type E3 ubiquitin ligase, positively regulates rice seed setting by promoting pollen tube growth (Li et al. 2013). *THIS1*, which encodes a class III lipase, regulates both tillering and seed set (Liu et al. 2013). *GSD1*, which encodes a putative remorin protein, causes impaired transport of carbohydrates from photosynthetic sites to phloem, influencing seed setting (Gui et al. 2014). *GSL5*, which encodes callose synthase, plays a vital role in microspore development and is essential for male fertility, which influences spikelet fertility (Shi et al. 2015). Zhang et al. (2016) reported that down-regulation of *OsSPX1*, a rice SPX domain gene, reduced the seed setting rate. *Laccase-13* (*OsLAC13*) is a member of the laccase family. Overexpression of *OsLAC13* induced mitochondrial damage and suppressed sugar transportation in anthers, which affected seed setting (Yu et al. 2017). Xiong et al. (2019) reported that *OsMND1*played a critical role in stabilizing meiosis and increased the seed set rate in polyploid rice.

A large number of hybrid sterility loci that result in low spikelet fertility have been previously detected in rice since the interspecific and intersubspecific hybrid sterility are common. The *indica–japonica* hybrid sterility loci that have been previously detected include the following: *S5* (Chen et al. 2008), S7 (Yu et al. 2016), *S8* (Wan et al. 1993), *S24* (Kubo et al. 2000), *S31* (Zhao et al. 2006), *Sa* (Long et al.

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2008), and *Sc* (Shen et al. 2017). The interspecific hybrid sterility loci that have been previously detected in hybrids between Asian cultivated rice (*Oryza sativa* L.) and African cultivated rice (*Oryzaglaberrima*Steud) include the following: *S1* (Sano et al. 1979), *S2* (Sano et al. 1979), *S3* (Sano 1983), *S18* (Doi et al. 1998), *S19* (Taguchi et al. 1999), *S20* (Doi et al. 1999), *S21* (Doi et al. 1999), *S29* (Zhu et al. 2005), *S33* (Ren et al. 2005), *S34* (Zhang et al. 2005), and *S37* (Shen et al. 2015). The sterility loci that have been previously detected between *O. sativa* and *Oryza glumaepatula* include the following: *S12* (Sano 1994), *S22* (Sobrizal et al. 2000a), *S23* (Sobrizal et al. 2000b), *S27* (Sobrizal et al. 2001), *S28* (Sobrizal et al. 2002), and *S56(t)* (Zhang et al. 2018). The *S51(t)*, *S52(t)*, *S53(t)*, *S54(t)*, and *S55(t)* sterile loci have also been previously detected between *Oryza sativa* and *Oryza meridionalis* (Li et al. 2018).

Epistasis has been previously reported to be involved in the regulation of final seed setting. Kubo and Yosimura (2005) reported that epistasis at three loci (*hsa1-IR* on chromosome 12, *hsa2-As* on chromosome 8, and *hsa3-As* on chromosome 9) influenced spikelet fertility (the female gametes carrying the *hsa1-IR*, *hsa2-As*, and *hsa3-As* alleles aborted in a *hsa1-IR* homozygous plant, leading to seed sterility). Kubo et al. (2011) reported that the *S24* loci (on chromosome 5) interacted with the *EFS* loci (on chromosome 2) and caused male gamete sterility, which resulted in low spikelet fertility.

To identify the digenic epistatic loci that regulate rice seed setting, a recombinant inbred line mapping population, which was developed by crossing the Lemont and Yangdao4 rice cultivars, was used for QTL analysis in the present study. We detected a total of 26 QTL in five mapping environments and identified a pair of new epistatic loci that are involved in the regulation of rice seed setting. These results broaden the understanding of the regulation mechanisms of rice seed setting.

Table 1 Variation in the seed setting rate (%) of the Lemont/Yangdao4 recombinant inbred line mapping population and the parental varieties grown in five environments

Mapping environment/ sowing date	Parental cultivar		Recombinant inbred line population		
	Lemont	Yangdao4	Range	Mean ± SD	CV%
May 23rd 2018	91.1	73.9	15.7–95.5	71.2 ± 15.9	22.3
June 2nd 2018	86.4	82.3	17.2–95.8	75.9 ± 14.4	18.9
May 29th 2017	84.8	81.4	12.5–94.9	63.1 ± 16.1	25.6
June 7th 2017	89.0	92.8	11.1–97.3	71.1 ± 17.7	24.8
June 30th 2017	87.4	69.6	0–91.5	62.1 ± 18.9	30.5

SD standard deviation, *CV* coefficient of variation

Materials and methods

Recombinant inbred line mapping population

A recombinant inbred line (RIL) mapping population (consisting of 219 lines) was developed by crossing the Lemont rice cultivar, an American *japonica* cultivar, with the Yangdao4 rice cultivar, a Chinese *indica* cultivar, using the single seed descent method (Zeng et al. 2019). The RIL population was sown in five mapping environments at the farm of the China National Rice Research Institute in Fuyang, Hangzhou (119°95′E, 30°07′N) for QTL analysis on the following dates: (1) F_{13} in May 29th, 2017, (2) F_{13} in June 7th, 2017, (3) F_{13} in June 30th, 2017, (4) F_{15} in May 23rd, 2018, and (5) F_{15} in June 2nd, 2018.

Each line of the RIL population was planted as a plot in all QTL-mapping environments. Each plot was planted in a randomized block design. Eighteen individual plants were grown for each line. The 18 plants were planted in three rows of six, with 20 cm separations between the rows and 17 cm separations between the plants of each row.

Measurement of seed setting rate

At the mature stage, 5 panicles were randomly selected from 18 individual plants of each line and were measured. The average seed setting (%) of the 5 panicles was used in the QTL analysis.

QTL analysis and statistical analysis

The genetic linkage map used in the present study consisted of 208 polymorphic markers, representing a total of 2228.0 cM, with an average of 11.4 cM between adjacent markers (Zeng et al. 2019). A multiple interval mapping (MIM) method was used for the QTL analysis and detection of the digenic epistatic loci with Windows QTL Cartographer 2.5 software as previously described by Zeng et al. (2019). Briefly, the MIM model built for QTL analysis consisted of two steps. First, the MIM forward search method was used to create an initial MIM model; the Bayesian information criterion (BIC)-based model selection criteria (BIC-M0 in the software) was used, with a MIM search walk speed of 1 cM. Second, several MIM model refinement rounds were performed to build the final MIM model: (1) searching repeatedly for QTLs with main effects until no main effect QTLs were found, (2) searching repeatedly for pairs of epistatic QTLs until no epistatic QTLs were found, (3) testing for the epistatic QTLs and excluding all the epistatic QTLs that were not statistically significant, (4) testing for the main effects of QTLs and excluding all the main effect QTLs that

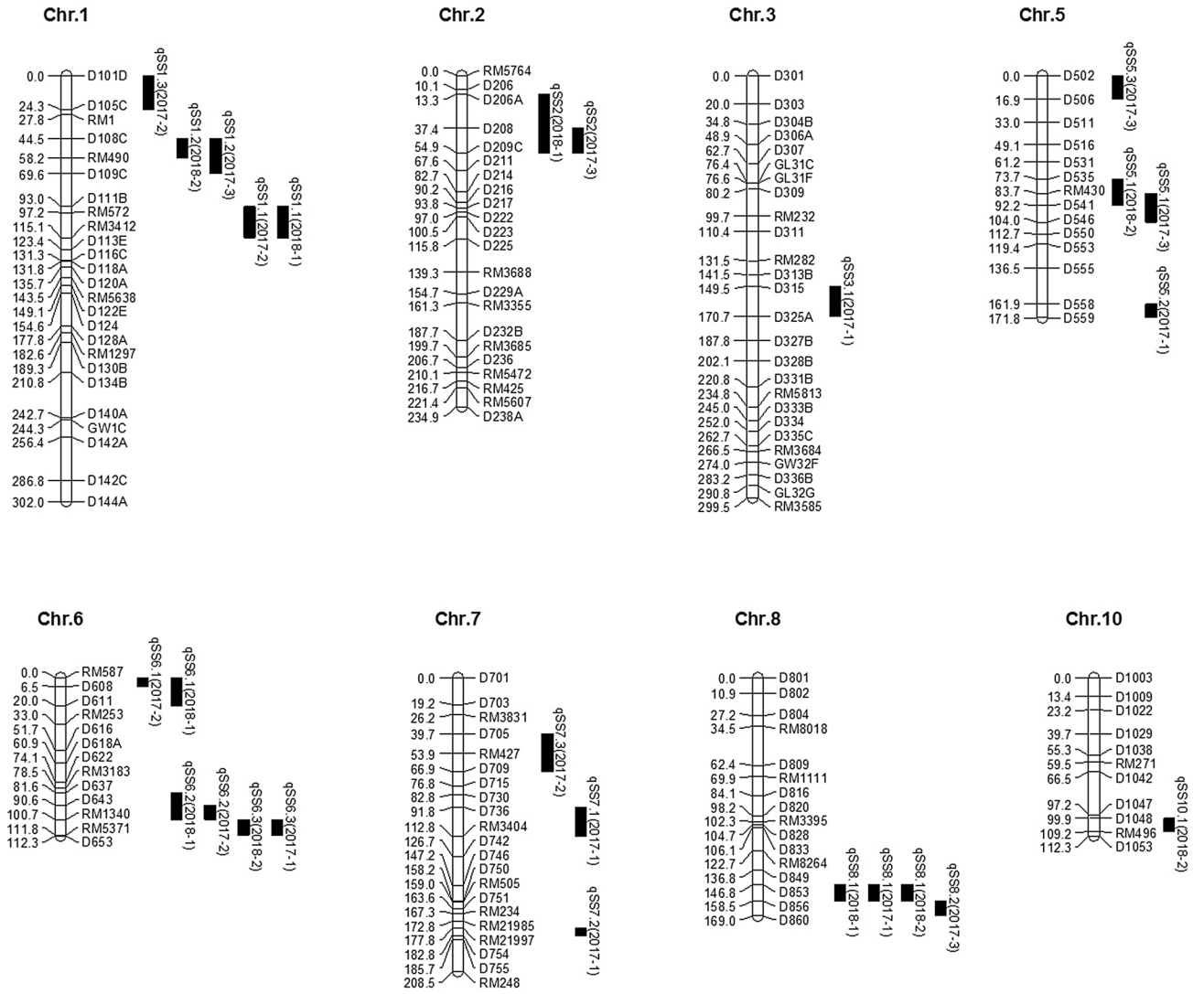

Fig. 1 QTL responsible for the seed setting rate detected by multiple interval mapping in the Lemont/Yangdao4 recombinant inbred line population grown in five mapping environments. Digits left of the chromosome bars indicate genetic distance (cM) of the corresponding markers. Data within the parentheses following the QTL name at the right of the chromosome bars indicate the mapping environment (2017-1, sown on May 29th, 2017; 2017-2, sown on June 7th, 2017; 2017-3, sown on June 30th, 2017; 2018-1, sown on May 23rd, 2018; and 2018-2, sown on June 2nd, 2018)

were not statistically significant, (5) optimizing positions of both main and epistatic QTLs.

The DNA extraction and PCR protocols followed Ye et al. (2017). QTL nomenclature was as described by Wang et al. (2018). The two-way analysis of variance (ANOVA), linear regression analysis, and Duncan's multiple-range test were performed using the SAS 8.01 software (SAS Institute, Cary, NC, USA).

Results

Phenotypic variation of the Lemont/Yangdao4 recombinant inbred line population

The variation in the seed setting rate of the RIL population planted in five environments is listed in Table 1. This population had a higher average seed setting when planted in 2018 compared with those planted in 2017, suggesting that the 2018 environments were more favorable for achieving a higher seed setting than the 2017 environments. The Lemont cultivar had higher seed setting than the Yangdao4 cultivar in four environments. But the Yangdao4 cultivar

Table 2 QTL for the seed setting rate detected by multiple interval mapping using the Lemont/Yangdao4 recombinant inbred line population grown in five mapping environments

Mapping environment /Population	Chr.	QTL name	LOD	Marker interval	Nearest marker	Additive effect[a] (%)	R^2 (%)
May 23rd 2018/F_{15}	1	*qSS1.1*	1.3	D111B–RM3412	RM572	2.6	2.2
	2	*qSS2*	1.5	D206A–D209C	D208	2.8	2.9
	6	*qSS6.1*	2.2	RM587–D611	D608	3.3	3.4
	6	*qSS6.2*	2.4	D637–RM1340	D643	− 3.6	5.4
	8	*qSS8.1*	1.3	D853–D856	D853	− 2.9	3.8
June 2nd 2018/F_{15}	1	*qSS1.2*	2.5	D108C–RM490	RM490	− 3.5	5.5
	5	*qSS5.1*	2.1	D535–D541	RM430	− 2.9	4.2
	6	*qSS6.3*	2.6	RM1340–RM5371	RM5371	− 3.6	7.6
	8	*qSS8.1*	2.4	D853–D856	D856	− 3.3	4.8
	10	*qSS10.1*	2.2	D1048–RM496	RM496	3.2	4.0
May 29th 2017/F_{13}	3	*qSS3.1*	1.6	D315–D325A	D315	3.3	2.2
	5	*qSS5.2*	3.3	D558–D559	D559	− 4.0	5.6
	6	*qSS6.3*	4.4	RM1340–RM5371	RM5371	− 4.6	8.5
	7	*qSS7.1*	4.8	D736–RM3404	D736	− 6.4	12.7
	7	*qSS7.2*	4.9	RM21997–D754	D754	4.8	5.2
	8	*qSS8.1*	5.1	D853–D856	D856	− 5.6	10.6
June 7th 2017/F_{13}	1	*qSS1.3*	2.3	D101D–D105C	D101D	− 5.1	8.7
	1	*qSS1.1*	1.4	D111B–RM3412	RM572	2.9	1.6
	6	*qSS6.1*	1.7	RM587–D608	RM587	3.3	3.3
	6	*qSS6.2*	2.5	D643–RM1340	D643	− 4.1	6.4
	7	*qSS7.3*	3.0	D705–D709	RM427	− 4.3	5.8
June 30th 2017/F_{13}	1	*qSS1.2*	1.8	D108C–D109C	RM490	− 3.7	3.4
	2	*qSS2*	1.5	D208–D209C	D208	3.6	3.2
	5	*qSS5.3*	2.5	D502–D506	D502	− 4.7	5.9
	5	*qSS5.1*	2.4	RM430–D546	D541	− 4.3	5.2
	8	*qSS8.2*	2.4	D856–D860	D860	− 4.6	5.6

A positive additive effect indicated that the Lemont allele increased the seed setting rate, while a negative additive effect indicated that the Yangdao4 allele increased the seed setting rate

Table 3 Two-way ANOVA performed to confirm the epistatic loci between *qSS6.1* and *qSS8.1*. D608 and D853, the nearest markers, were used to represent the *qSS6.1* and *qSS8.1* loci, respectively

Environment	Source	DF	Type I SS	Mean Square	F value	P
May 23rd 2018	D608	1	0.9	0.9	0	0.95
	D853	1	0.8	0.8	0	0.96
	D608*D853	1	3304.3	3304.3	13.3	0.0003**
June 2nd 2018	D608	1	3.0	3.0	0.01	0.90
	D853	1	52.0	52.0	0.3	0.61
	D608*D853	1	2011.0	2011.0	9.9	0.0018**
May 29th 2017	D608	1	18.8	18.8	0.07	0.79
	D853	1	12.8	12.8	0.05	0.82
	D608*D853	1	3346.1	3346.1	13.2	0.0003**
June 7th 2017	D608	1	3.8	3.8	0.01	0.91
	D853	1	6.0	6.0	0.02	0.89
	D608*D853	1	2335.2	2335.2	7.5	0.0065**
June 30th 2017	D608	1	48.2	48.2	0.1	0.71
	D853	1	2.7	2.7	0.01	0.93
	D608*D853	1	595.8	595.8	1.7	0.19

$^{**}P < 0.01$

Table 4 Duncan's multiple-range test was used to study the epistatic effect between the *qSS6.1* and *qSS8.1* loci

Group	Genotype at *qSS6.1*	Genotype at *qSS8.1*	Average seed setting rate ± SD (%)					Number of lines
			May 23rd 2018	June 2nd 2018	May 29th 2017	June 7th 2017	June 30th 2017	
1	l/l	l/l	73.5 ± 13.5 a	77.9 ± 11.8 a	63.7 ± 12.6 a	72.9 ± 14.2 a	64.8 ± 14.7 a	33
2	l/l	y/y	74.1 ± 12.3 a	78.2 ± 13.8 a	66.5 ± 14.7 a	73.5 ± 16.5 a	64.1 ± 20.2 a	64
3	y/y	l/l	60.6 ± 20.0 b	68.7 ± 15.4 b	53.4 ± 17.7 b	62.5 ± 22.3 b	57.3 ± 20.4 a	37
4	y/y	y/y	72.4 ± 16.0 a	75.9 ± 14.7 a	63.0 ± 16.9 a	71.2 ± 16.9 a	62.3 ± 17.3 a	63

Digits followed by the same letter in the same column do not vary significantly at $P < 0.05$.

show higher seed setting than the Lemont cultivar when planted in June 7th, 2017, indicating that the seed setting was easily influenced by the environment.

QTL responsible for seed setting rate detected by MIM

A total of 26 QTL were detected by MIM in five QTL-mapping environments. These QTL were distributed on chromosomes 1, 2, 3, 5, 6, 7, 8, and 10 (Fig. 1). All of the detected QTL explained less than 12.7% of the phenotypic variation individually. Twenty-four of the QTL explained less than 8.7% of the phenotypic variation individually (Table 2). These results indicated that the seed setting rate was controlled by minor-effect QTL. We examined the QTL positions and found that the 26 detected QTL were localized in 17 chromosome regions (Fig. 1).

Digenic epistatic loci detected by MIM

MIM was used to search for digenic epistatic loci in the Lemont/Yangdao4 RIL population, which was grown in five environments. A pair of digenic epistatic loci (*qSS6.1*/*qSS8.1*) was detected in the May 23rd, 2018 environment using MIM. The LOD value, epistatic effect, and epistatic effect explained by the digenic loci were 1.9, 3.4, and 3.2%, respectively. We did not identify any other digenic epistatic loci.

Confirmation of the digenic epistatic loci using two-way ANOVA

We used two-way ANOVA to further test the epistatic loci between *qSS6.1* and *qSS8.1*. The nearest markers D608 and D853 were chosen to represent the *qSS6.1* and *qSS8.1* loci, respectively. Two-way ANOVA showed that there were highly significant interactions ($P < 0.01$) between the *qSS6.1* and *qSS8.1* loci in four of the five environments. The interaction between the *qSS6.1* and *qSS8.1* loci was not significant when the RIL population was planted in the June 30th, 2017 environment (Table 3).

Epistatic effect between *qSS6.1* and *qSS8.1*

To study the mode of interaction between the *qSS6.1* and *qSS8.1* loci, different lines of the RIL population were classified into four groups according to marker genotypes at the two loci. Group 1 contained lines carrying the Lemont alleles at both loci. Group 2 contained lines carrying the Lemont allele at *qSS6.1* and the Yangdao4 allele at *qSS8.1*. Group 3 contained lines carrying the Yangdao4 allele at *qSS6.1* and the Lemont allele at *qSS8.1*. Group 4 contained lines carrying the Yangdao4 alleles at both loci (Table 4). D608 and D853, the nearest markers, were chosen to represent the *qSS6.1* and *qSS8.1* loci, respectively. Duncan's multiple-range test indicated that Group 3 had a significantly lower ($P < 0.05$) seed setting (%) than the other three groups when planted in four mapping environments. Group 3 had a lower seed setting than the other three groups when planted in the June 30th, 2017 environment, but this result was not significant (Table 4). This result clearly showed that the homozygous Yangdao4 allele at the *qSS6.1* locus interacted with the homozygous Lemont allele at the *qSS8.1* locus and resulted in low spikelet fertility (Fig. 2).

Manipulation of seed setting rate by marker-assisted selection

Among the twenty-six QTL that were detected in the present study, only four QTL explained more than 8% of the phenotypic variation: *qSS1.3*, *qSS6.3*, *qSS7.1*, and *qSS8.1* (Table 2). We focused on these four QTL for further analysis. At these four loci, the alleles for increased seed set all originated from the Yangdao4 cultivar, while the alleles for decreased seed set all originated from the Lemont cultivar (Table 2). The number of seed setting-increasing alleles was calculated for each line of the RIL population. Different lines in the RIL population were classified into five different groups according to their number of seed setting-increasing alleles at the four loci: lines carrying 0, 2, 4, 6, and 8 seed setting-increasing alleles (Table 5). The lines

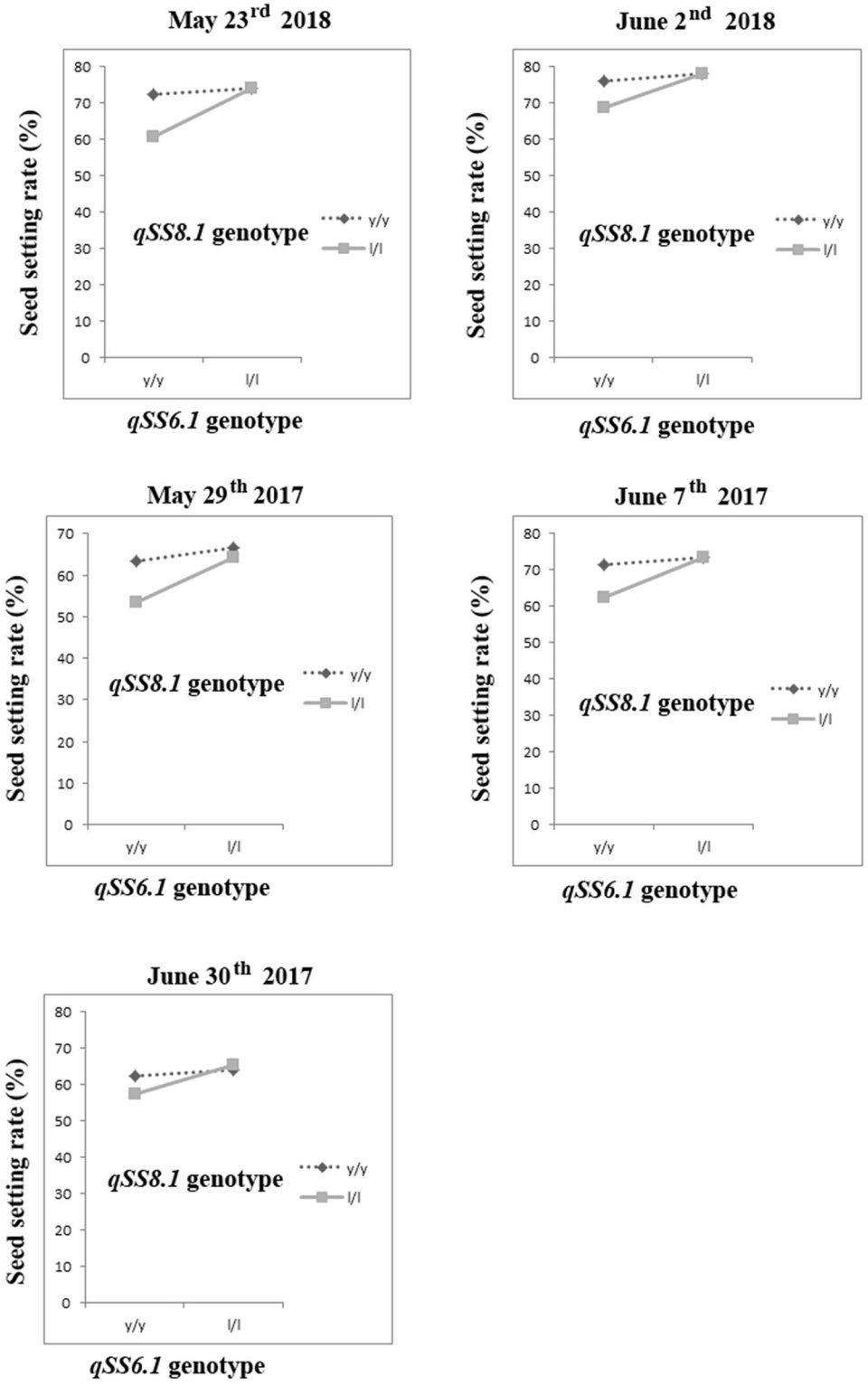

Fig. 2 Interaction effects between the *qSS6.1* and *qSS8.1* loci on the seed setting rate of the Lemont/Yangdao4 recombinant inbred line population planted in five environments (May 29th, 2017, June 7th, 2017, June 30th, 2017, May 23rd, 2018, and June 2nd, 2018). Markers D608 and D853 were used to represent the *qSS6.1* and *qSS8.1* loci, respectively. The 'l' and 'y' symbols indicate the Lemont and Yangdao4 alleles, respectively

Table 5 Pyramiding effect of four QTL. Different lines in the Lemont/Yangdao4 RIL population were classified into five groups according to the number of seed setting-increasing alleles that they carried (0, 2, 4, 6, and 8 seed setting-increasing alleles) at the four loci (*qSS1.3*, *qSS6.3*, *qSS7.1*, and *qSS8.1*)

Number of seed setting-increasing alleles	Number of lines	Average seed setting (%) ± SD				
		May 23rd 2018	June 2nd 2018	May 29th 2017	June 7th 2017	June 30th 2017
0	3	47.8 ± 16.7	54.0 ± 24.8	37.9 ± 23.0	45.8 ± 18.2	43.9 ± 16.0
2	31	63.8 ± 18.2	70.5 ± 13.9	55.0 ± 15.1	62.5 ± 21.7	57.9 ± 18.4
4	48	71.5 ± 11.5	74.7 ± 15.6	59.8 ± 14.6	69.1 ± 17.8	58.5 ± 18.9
6	60	70.6 ± 15.6	78.0 ± 10.5	65.0 ± 14.6	71.4 ± 14.1	63.0 ± 18.7
8	34	78.2 ± 14.4	83.1 ± 11.5	71.3 ± 13.1	79.2 ± 14.3	70.1 ± 15.6

The 0-allele group indicates that none of the four loci were selected, while the 8-allele group indicates that all of the four loci were selected

See Fig. 3 for the differences among the five different groups compared using Duncan's multiple-range test

carrying more seed setting-increasing alleles had higher average seed setting. This trend was consistent in all five environments (Table 5).

Duncan's multiple-range test showed that lines carrying 4, 6, or 8 seed setting-increasing alleles at the four loci did not differ significantly at $P = 0.05$ (Fig. 3); however, marker-assisted selection using markers selecting for these four QTL was able to guarantee an average seed setting rate over 70% in all of the mapping environments (Table 5).

Linear regression analysis using data from the different lines also confirmed a linear relationship between seed setting and the number of seed setting-increasing alleles each line carried at the four loci. This yielded the following five equations in five QTL-mapping environments: $F = 16.8$, $P < 0.0001$, when the RIL population was sown on May 23, 2018; $F = 23.1$, $P < 0.0001$, when sown on June 2, 2018; $F = 31.2$, $P < 0.0001$, when sown on May 29, 2017; $F = 20.9$, $P < 0.0001$, when sown on June 7, 2017; and $F = 11.6$, $P = 0.0008$, when sown on June 30, 2017 (Fig. 3).

Discussion

The seed setting rate of the Lemont/Yangdao4 RIL population varied even though the sowing dates in each year were similar. For example, the average seed setting of the population sown on June 7, 2017, was 8% higher than that sown only 9 days earlier on May 29, 2017. Additionally, the average seed setting of the population sown on June 30, 2017, was 9% lower than that sown 23 days earlier on June 7, 2017. These results are consistent with the previous finding that the rice seed setting rate is easily influenced by the environment (Yu et al. 2017).

Although epistasis is one of the genetic factors that influences rice seed setting, reports about the identification of epistatic QTL controlling seed setting are rare (Kubo and Yosimura 2005; Kubo et al. 2011). In the present study, we identified a pair of new epistatic QTL between *qSS6.1* (on chromosome 6) and *qSS8.1* (on chromosome 8), which has not been reported in previous studies. Two-way ANOVA confirmed that the interaction between the *qSS6.1* and *qSS8.1* loci was significant in four mapping environments. The interaction between the *qSS6.1* and *qSS8.1* loci was not statistically significant when this RIL population was planted on June 30, 2017. This result indicated that the environment has an effect on this epistatic loci, but this epistatic locus was stable in the majority of mapping environments tested.

The locations of the seed setting QTL identified in the present study were compared with those from previous studies. The *qSS1.3* locus was co-localized with the *S-d* loci (Li et al. 2008). The location of the *qSS2* locus overlapped with the location of the *S32(t)* locus previously reported by Li et al. (2007). The *f5-Du* (Wang et al. 2006) and *S31* (Zhao et al. 2007) loci were localized in the marker interval of the *qSS5.3* locus. The *qSS7.1* locus was co-localized with the *S30(t)* locus (Zhu et al. 2005) (Table 6).

The regulation of rice seed setting is complicated because (1) it is easily influenced by the environment, (2) the majority of QTL controlling seed setting have only minor effects, which we have demonstrated in the present study, and (3) epistasis is an additional factor that influences seed setting. However, the results in the present study suggest that marker-assisted selection can still be used to manipulate the seed setting rate by QTL pyramiding. We provided direct evidence that pyramiding four QTL (*qSS1.3*, *qSS6.3*, *qSS7.1*, and *qSS8.1*) simultaneously can guarantee a greater than 70% seed setting rate in all five growing environments. The four nearest markers (D101D, RM5371, D736, and D856) to the four QTL can be used directly in molecular breeding in the Lemont/Yangdao4 segregating populations.

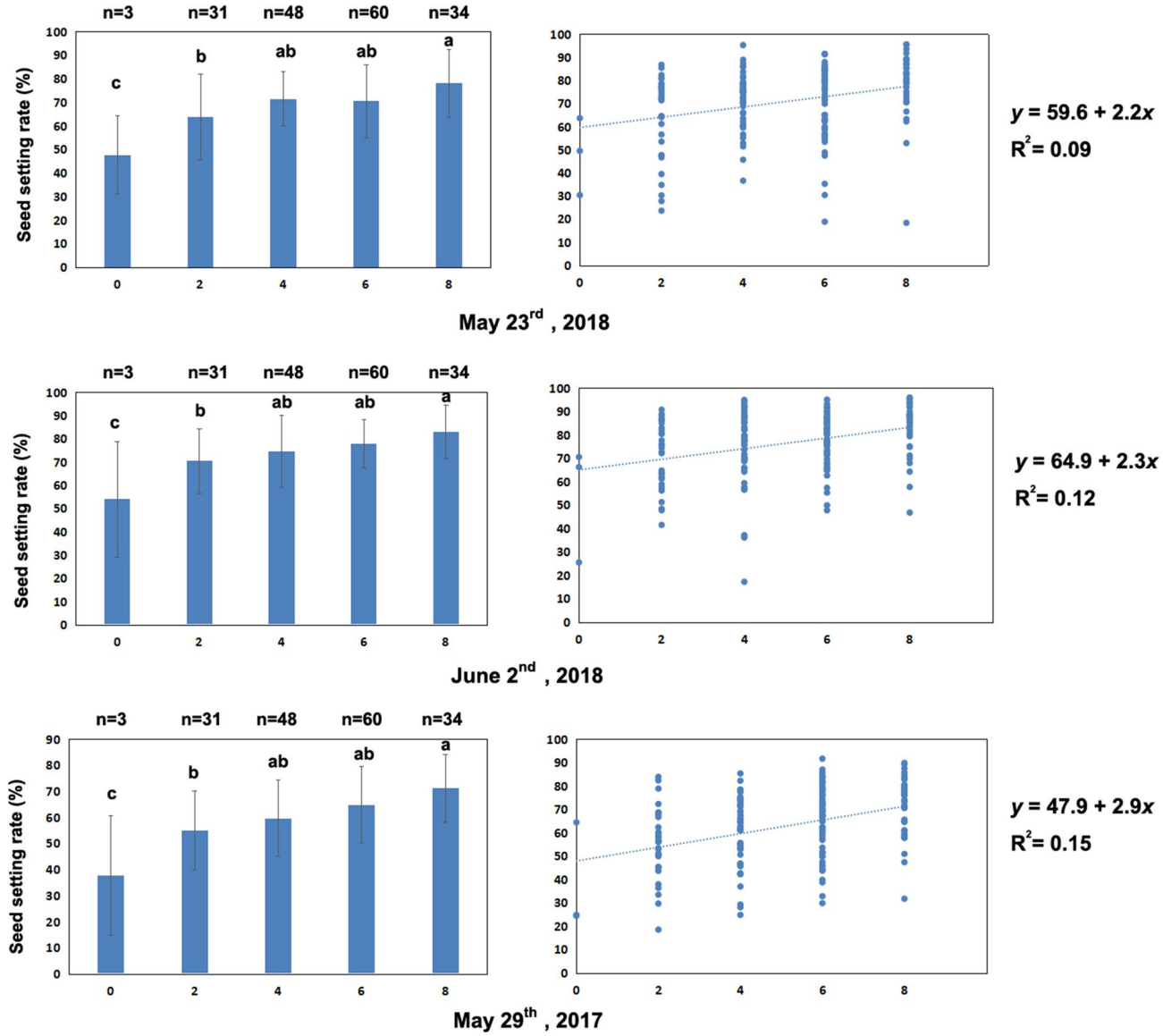

Fig. 3 Eight alleles at four quantitative trait loci (*qSS1.3*, *qSS6.3*, *qSS7.1*, and *qSS8.1*) controlled seed setting. The Yangdao4 alleles at these four loci increased seed setting, while the Lemont alleles decreased it. At these four loci, lines carrying more seed setting-increasing alleles had a higher seed setting rate than lines carrying more seed setting-decreasing alleles. The X-axis indicates the number of seed setting-increasing alleles carried at the four loci. The Y-axis indicates the seed setting rate (%). In the histograms (left), different lines in the RIL population were classified into five groups according to the number of seed setting-increasing alleles each line carried at the four loci. Duncan's multiple-range test ($P < 0.05$) was performed to compare the differences among the five groups. In the scatter diagrams (right), linear regression analysis using data of different lines confirmed that the plants carrying more seed setting-increasing alleles had a higher seed setting and yielded five equations in the five QTL-mapping environments

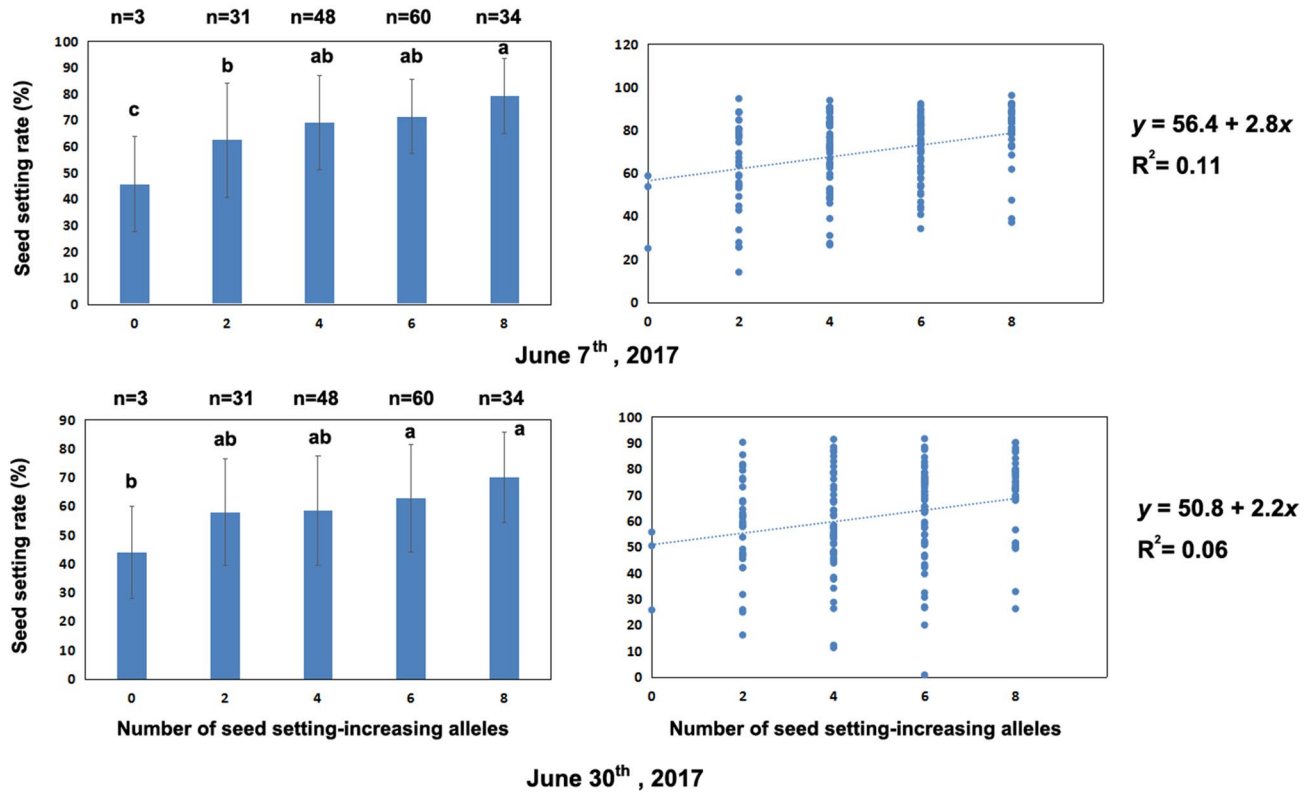

Fig. 3 (continued)

Table 6 Comparison of QTL positions between the present and previous studies

Present study				Previous study		
Chr.	QTL name	No. of collocated QTL detected at the QTL [a]cluster	Marker interval (physical position)	QTL name	Marker interval (physical position)	References
1	*qSS1.3*	1	D101D–D105C 1.072–4.348 Mb	*S-d*	PSM93–IND10 3.037–3.104 Mb	Li et al. (2008)
2	*qSS2*	2	D206A–D209C 2.372–5.250 Mb	*S32(t)*	RM236–RM12475 2.105–2.775 Mb	Li et al. (2007)
5	*qSS5.3*	1	D502–D506 0.549–2.586 Mb	*f5-Du*	WFPM3–WPRO-1 1.279–1.348 Mb	Wang et al. (2006)
5	*qSS5.3*	1	D502–D506 0.549–2.586 Mb	*S31*	Indel193–Indel212 1.291–1.488 Mb	Zhao et al. (2007)
7	*qSS7.1*	1	D736–RM3404 17.328–20.107 Mb	*S30(t)*	RM432–RM11 18.960–19.258 Mb	Zhu et al. (2005)

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