



Quantitative trait loci mapping of the stigma exertion rate and spikelet number per panicle in rice (*Oryza sativa* L.)

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ABSTRACT. The stigma exertion rate is a polygenic inherited trait that is important for increased seed yield in hybrid rice breeding. To identify quantitative trait loci (QTL) associated with high stigma exertion rate, we conducted QTL mapping using 134 recombinant inbred lines derived from XieqingzaoB and Zhonghui9308, which have high and low stigma exertion rates, respectively. A total of eight QTLs (*qSES6*, *qSSE11*, *qDSE1a*, *qDSE1b*, *qDSE10*, *qDSE11*, *qTSE1*, and *qTSE11*) for single stigma exertion, double stigma exertion, and total stigma exertion were detected. The locations of *qSSE11* and *qTSE11* have not been previously reported, and the *qDSE11* allele from parent XQZB exhibited a positive additive effect. In addition, three QTLs (*qSNP1*, *qSNP3a*, and *qSNP3b*), for spikelet number per panicle were identified. Of note, one QTL (*qSNP1*) was detected in two different environments (Hainan and Zhejiang). To evaluate the advantage of exerted stigma

for cross-pollination, single, dual, and total stigma exertion should be considered separately for future genetic improvement in the production of rice hybrid seeds. In addition, this study provides information for fine mapping, gene cloning, and marker assisted selection, with emphasis on the latter.

Key words: Rice; Stigma exertion rate; Spikelet number per panicle; QTL mapping

INTRODUCTION

Rice (*Oryza sativa* L.) is an important staple cereal food, feeding more than half of the world's population. Increase of rice production is vital to population explosion and global food security (Lou et al., 2014). Hybrid rice that has a yield advantage of 10-20% over conventional varieties was developed and commercially grown. Together with the application of modern cultivation technology, the rice yield has risen to about 6 tons per hectare. This has contributed to self-sufficiency of food supply in China (Cheng et al., 2007). However, how to improve the efficiency of hybrid seed production remains a major unresolved issue. Stigma exertion rate is a key-contributing factor in hybrid seed production. A high stigma exertion rate is expected to trap more pollen and to improve cross-pollination and the efficiency of hybrid seed production in rice. Exerted stigmas remain viable for approximately 4 days and continue to accept pollination (Long and Shu, 2000; Tian et al., 2004).

Yan et al. (2009) performed association mapping on the stigma exertion rate in a minicore of 90 accessions using 109 DNA markers and found that a simple sequence repeat (SSR) marker RM5, played a major role indicative of high correlation with both dual (DSE) and single stigma exertion (SSE) rates. Using two segregating populations derived from *indica* cultivar Guangluai-4 and a wild cultivar W1943 (*O. rufipogon*), Li et al. (2014) demonstrated that the stigma exertion rate is a complex quantitative trait, which is governed by polygenes. Furthermore, those authors performed genetic mapping and validated quantitative trait loci (QTL) associated with stigma exertion rate in rice, and a population of recombinant inbred lines was developed from two superior rice cultivars, Zhongguo Xiangdao (ZX) and Chuanxiang 29B (CX29B), and a total of 11 QTLs for the stigma exertion rate were detected over 2 years; two QTLs on chromosome 1 and four QTLs on chromosome 6 formed two QTL cluster regions, qSe1 and qSe6, respectively. Lou et al. (2014) used the F₂ population derived from two *indica* cytoplasmic male sterility maintainers, Huhan1B and K17B, to map the QTLs that influence stigma exertion in rice. QTLs that influence SSE, DSE, and total stigma exertion (TSE) rates were detected using a linkage map of 92 SSR markers. One, three, and one QTLs were detected for SSE, DSE, and TSE, respectively, on chromosomes 5, 6, and 7.

Zhang et al. (2009) studied a population of recombinant inbred lines (RILs) derived from two *indica* rice varieties, Zhenshan 97 and HR5, that were used to map QTLs for spikelet number per panicle (SNP). One major QTL (*qSNP7*) and three minor QTLs (*qSNP1*, *qSNP2*, and *qSNP3*) were identified on chromosomes 7, 1, 2, and 3, respectively. Deshmukh et al. (2010) identified candidate genes for SNP in rice (*O. sativa* L.) and reported that a large number of well-filled SNPs is an important yield component trait in rice. Additionally, those authors generated a set of RILs derived from a cross between Pusa 1266 (high SNP) and Pusa Basmati 1 (low SNP), and identified four QTLs on chromosomes 3 and 4.

In the present study, four flowering-related traits, including SSE, DSE, TSE, and SNP, were measured in two different environments (Hainan and Zhejiang) in an RIL population. Here, we identify the QTLs influencing stigma exertion rate and SNP. These results will be useful in facilitating the development of male sterile lines with a high stigma exertion rate and an increase in the SNP, which would be of great value in hybrid rice seed production.

MATERIAL AND METHODS

Materials and field experiments

RIL populations consisting of 134 lines were derived from combinations of XieqingzaoB (XQZB) and Zhonghui9308 (ZH9308). XQZB and ZH9308 have high and low stigma exertion rates, respectively. RIL populations and their parents were cultivated at Lingshui, Hainan Island on December 27, 2014 and in Fuyang field of the China National Rice Research Institute, Hangzhou, Zhejiang Province, China on June 18, 2015. Plants were established in six rows, with eight plants per row, and spaces of 30 x 20 cm within and between rows, respectively. Standard cultivation practices were followed.

Trait evaluation

The stigma exertion rate was subdivided into three traits: SSE, DSE, and TSE (Figure 1). At 5-7 days after heading, five normal panicles in each line were sampled from different plants. After the lower side spikelets of the panicle flowered, the panicles were used to determine the stigma exertion rate, as follows.

$$\text{SSE (\%)} = [\text{SSE} / (\text{SSE} + \text{DSE} + \text{no stigma exertion})] \times 100$$

$$\text{DSE (\%)} = [\text{DSE} / (\text{SSE} + \text{DSE} + \text{no stigma exertion})] \times 100$$

$$\text{TSE (\%)} = \text{SSE (\%)} + \text{DSE (\%)}$$

$$\text{SNP} = \text{SSE} + \text{DSE} + \text{no stigma exertion}$$

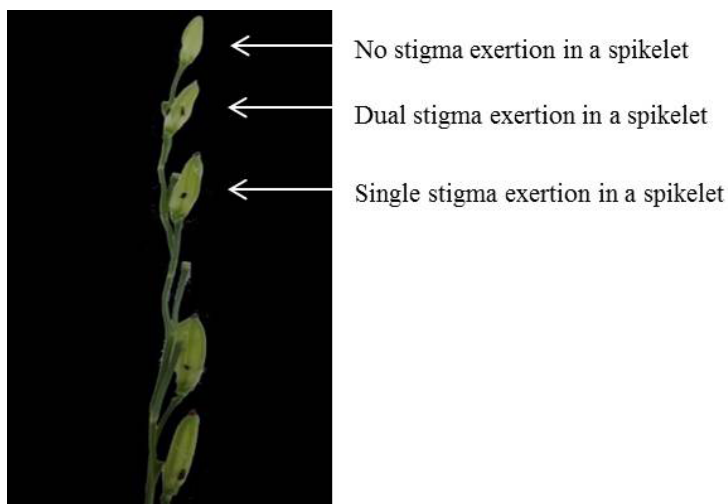


Figure 1. Example of single, dual, and no stigma exertion in a spikelet investigated in this study.

Construction of a genetic map and QTL analysis

A total of 198 SSR markers, which were distributed across the rice genome according to previously reported linkage maps, and known polymorphisms between the parents, were used to determine the genotype of the RIL populations (Liang et al., 2012). The genetic linkage map was constructed using the MAPMAKER/Exp version 3.0 program (Lincoln et al., 1992). The entire genome spanned 1814.50 cM with an average interval of 9.16 cM between adjacent markers.

The chromosomal locations of putative QTLs were determined by exclusive composite interval mapping with QTL IciMapping 4.0 (Wang, 2009). A locus with a threshold log of the odds (LOD) value ≥ 2.0 and $P \leq 0.05$ was declared as a putative QTL. The contribution rate (H^2) was estimated as percentage of variance explained by each locus or epistatic pair in proportion to the total phenotypic variance. QTLs were named according to conventions of nomenclature (McCouch, 2008) and in alphabetic order for QTLs on the same chromosome.

RESULTS

Stigma exertion rate in the RIL and parent populations

Phenotypic analysis indicated that the SSE, DSE, and TSE rates of the ZH9308 and XQZB parents were higher in plants grown in Zhejiang compared with those grown in Hainan; however, the differences were only statistically significant for the DSE rate in the XQZB parent (Table 1). The SSE, DSE, and TSE in the RIL population exhibited increased values in Zhejiang compared with those in Hainan, and SSE and TSE were significantly different. However, the DSE did not significantly differ between the two locations. The height values for SSE, DSE, and TSE in the RIL population were 52.28, 12.92, and 64.76% in Hainan and 47.86, 22.91, and 65.95% in Zhejiang, respectively. SSE (Figure 2A) and TSE (Figure 2C) exhibited continuous variation, which followed a normal distribution in both environments. However, DSE (Figure 2B) deviated significantly from the normal distribution. In addition, transgressive segregation was observed for all traits.

Table 1. Performance of the trait stigma exertion and spikelet number per panicle in the parent and recombinant inbred line (RIL) population.

Traits	ZH9308 \pm SE		XQZB \pm SE		RILs (mean) \pm SE		RILs (range)	
	Hainan	Zhejiang	Hainan	Zhejiang	Hainan	Zhejiang	Hainan	Zhejiang
SSE	9.36 \pm 0.58	11.07 \pm 0.94	23.99 \pm 3.10	25.59 \pm 2.23	14.99 \pm 0.93**	18.22 \pm 0.90**	0.95-52.28	0-47.86
DSE	1.19 \pm 0.31	1.35 \pm 0.24	2.03 \pm 0.86*	5.05 \pm 0.56*	3.01 \pm 0.25	3.56 \pm 0.35	0-12.92	0-22.91
TSE	10.55 \pm 0.55	12.42 \pm 0.93	26.01 \pm 3.09	30.64 \pm 2.07	18.00 \pm 1.16*	21.71 \pm 1.20*	0.95-64.76	0-65.95
SNP	252.20 \pm 7.41	251.20 \pm 6.03	121.40 \pm 3.59***	118.80 \pm 2.58***	154.59 \pm 3.08***	184.46 \pm 3.42***	94.60-288.20	91-315.40

*Significant at the 0.05 level; **significant at the 0.01 level; ***significant at the 0.001 level (analyzed by the Student *t*-test); SSE = single stigma exertion rate; DSE = dual stigma exertion rate; TSE = total stigma exertion rate; SNP = spikelet number per panicle; SE = standard error.

SNP in RIL and parent populations

In both environments, the average SNP was increased in ZH9308 plants compared with XQZB plants (Table 1). SNP of the ZH9308 parent was increased in Hainan compared with Zhejiang; however, the difference was not significant. The XQZB parent had a higher SNP in Hainan than in Zhejiang, and this difference was significant.

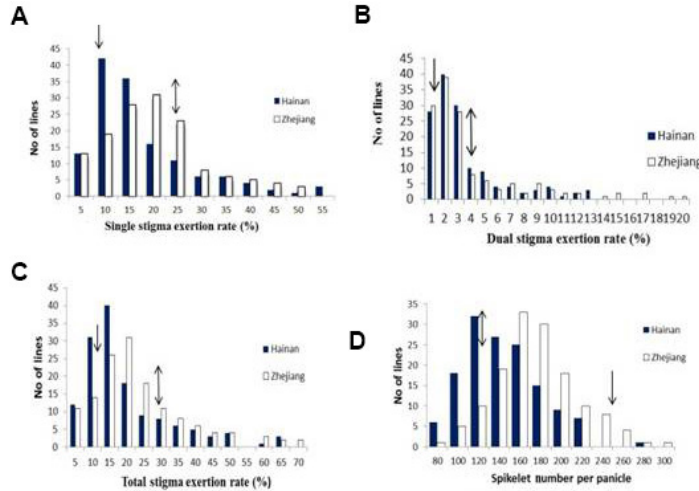


Figure 2. Frequency distribution of **A.** single exerted stigma (SSE), **B.** dual exerted stigma (DSE), **C.** total exerted stigma (TSE), and **D.** spikelet number per panicle (SNP) in the recombinant inbred line (RIL) population from Hainan and Zhejiang. Single arrows and double-headed arrows indicate the average values from ZH9308 and XQZB parent lines, respectively.

The RIL populations exhibited average SNP values of 154.59 and 184.46 in Hainan and Zhejiang, respectively, and these values significantly differed. SNP ranged from 94.60 to 288.20 and from 91 to 315.40 in Hainan and Zhejiang, respectively. Furthermore, this trait exhibited a normal distribution (Figure 2D).

The SSE, DSE, and TSE traits were significantly correlated with each other in an environmentally dependent manner. No correlation was observed between SNP and stigma exertion. The highest phenotypic correlation was observed between HSSE and HTSE ($r = 0.996$) followed by HDSE and HTSE ($r = 0.942$), and HSSE and HDSE ($r = 0.908$) in Hainan as well as ZJSSE and ZJTSE ($r = 0.988$) and ZJDSE and ZJTSE ($r = 0.926$), and ZJSSE and ZJDSE ($r = 0.866$) in Zhejiang (Table 2). These results indicated that the lines with higher values for SSE were more likely to exhibit increased values for DSE, which would ultimately increase the TSE.

Table 2. Correlation (Pearson) coefficients among the traits of 134 RIL populations derived from XQZB and ZH9308 parent lines in Hainan and Zhejiang.

	HSSE	HDSE	HTSE	HSNP	ZJSSE	ZJDSE	ZJTSE	ZJSNP
HSSE	1							
HDSE	0.908**	1						
HTSE	0.996**	0.942**	1					
HSNP	0.073	0.109	0.083	1				
ZJSSE	0.894**	0.809**	0.890**	0.011	1			
ZJDSE	0.801**	0.781**	0.809**	0.138	0.866**	1		
ZJTSE	0.891**	0.827**	0.891**	0.039	0.988**	0.926**	1	
ZJSNP	0.060	0.058	0.061	0.727**	0.061	0.095	0.068	1

**Correlation is significant at the 0.01 level (two-tailed); HSSE = Hainan single stigma exertion rate; HDSE = Hainan dual stigma exertion rate; HTSE = Hainan total stigma exertion rate; HSNP = Hainan spikelet number per panicle; ZJSSE = Zhejiang single stigma exertion rate; ZJDSE = Zhejiang dual stigma exertion rate; ZJTSE = Zhejiang total stigma exertion rate; ZJSNP = Zhejiang spikelet number per panicle.

QTL analysis of stigma exertion rate

Eight QTLs for stigma exertion rate were identified with LOD values varying from 2.04 to 2.68 and were distributed on chromosomes 1, 6, 10, and 11 in both environments (Table 3 and Figure 3). The phenotypic variance explained by each QTL for stigma exertion rates ranged from 6.49 to 8.90%. For the SSE rates, two QTLs, *qSSE6* and *qSSE11*, were detected on chromosomes 6 and 11, respectively, QTL *qSSE6* was identified in Hainan, and *qSSE11* was identified in Zhejiang. These QTLs explained 7.47% of the phenotypic variation in Hainan and 8.37% of the phenotypic variation in Zhejiang, and the alleles were derived from the XQZB parent line. The additive effect was 3.05 and 3.18, respectively. For the DSE rates, four QTLs (*qDSE1a*, *qDSE1b*, *qDSE10*, and *qDSE11*) were detected on chromosomes 1, 1, 10, and 11, respectively. The QTL *qDSE10* was identified in Hainan and explained 7.59% of the phenotypic variation, whereas others found in Zhejiang explained 7.92, 8.90, and 7.28% of the phenotypic variation. The alleles *qDSE1a*, *qDSE1b*, and *qDSE10* were inherited from the ZH9308 parent line with negative effects of 1.17, 1.27, and 0.81, respectively. Conversely, the *qDSE11* allele from the XQZB parent line exhibited a positive additive effect of 1.16. Regarding the total stigma exertion rates, two QTLs were detected on chromosome 1 and 11, respectively. The QTLs *qTSE1* and *qTSE11* on chromosomes 1 and 11, respectively, explained 6.49 and 8.03% of the phenotypic variation in Zhejiang. The *qTSE1* and *qTSE11* alleles were derived from the ZH9308 and XQZB parents, respectively. The former had a negative effect of 3.73, whereas the latter had a positive additive effect of 4.18.

Table 3. Putative quantitative trait loci (QTLs) for stigma exertion rate and spikelet number per panicle detected in an RIL population derived from the XQZB and ZH9308 parent lines in Hainan and Zhejiang.

Trait	QTL	Chr	Interval	Distance (cM)	Sources	Hainan-2014-15			Zhejiang-2015		
						LOD	A	PVE (%)	LOD	A	PVE (%)
SSE	<i>qSSE6</i>	6	RM587-RM510	14.00	XQZB	2.04	3.05	7.47			
	<i>qSSE11</i>	11	RM5704-RM4469	34.00	XQZB				2.48	3.18	8.37
DSE	<i>qDSE1a</i>	1	RM7278-RM6324	16.00	ZH9308				2.22	-1.17	7.92
	<i>qDSE1b</i>	1	RM1-RM3746	34.00	ZH9308				2.68	-1.27	8.90
	<i>qDSE10</i>	10	RM474-RM6646	23.00	ZH9308	2.19	-0.81	7.59			
	<i>qDSE11</i>	11	RM167-RM5704	33.00	XQZB				2.14	1.16	7.28
TSE	<i>qTSE1</i>	1	RM1-RM3746	34.00	ZH9308				2.10	-3.73	6.49
	<i>qTSE11</i>	11	RM5704-RM4469	34.00	XQZB				2.38	4.18	8.03
SNP	<i>qSNP1</i>	1	RM1-RM3746	52.00	ZH9308	3.70	-12.17	11.21	3.33	-12.88	10.19
	<i>qSNP3a</i>	3	RM168-RM143	133.00	ZH9308	3.99	-18.22	25.04			
	<i>qSNP3b</i>	3	RM282-RM6283	42.00	ZH9308				3.79	-17.39	17.38

A = additive effect of each QTL; PVE = phenotypic variance explained by each QTL; LOD = logarithm of odds; *qSSE* = QTL for single stigma exertion rate; *qDSE* = QTL for double stigma exertion rate; *qTSE* = QTL for total stigma exertion rate; *qSNP* = QTL for spikelet number per panicle.

QTL analysis of SNP

Three QTLs for SNP with LOD values varying from 3.33 to 3.99 were detected on chromosomes 1 and 3 in both environments (Table 3 and Figure 3). The phenotypic variance explained by each QTL ranged from 10.19 to 25.04%. The QTL located on chromosome 1 was flanked by RM1-RM3746, whereas the QTL on chromosome 3 was flanked by RM168-RM143 and RM282-RM6283. *qSNP1* was identified in both environments and explained

11.21 and 10.19% of the phenotypic variation in Hainan and Zhejiang, respectively. *qSNP3a* was identified in Hainan with 25.04% of the phenotypic variation, and *qSNP3b* was identified in Zhejiang with 17.38% of the phenotypic variation. The alleles of these QTLs were derived from the ZH9308 parent, and all exhibited explained negative effects (Table 3).

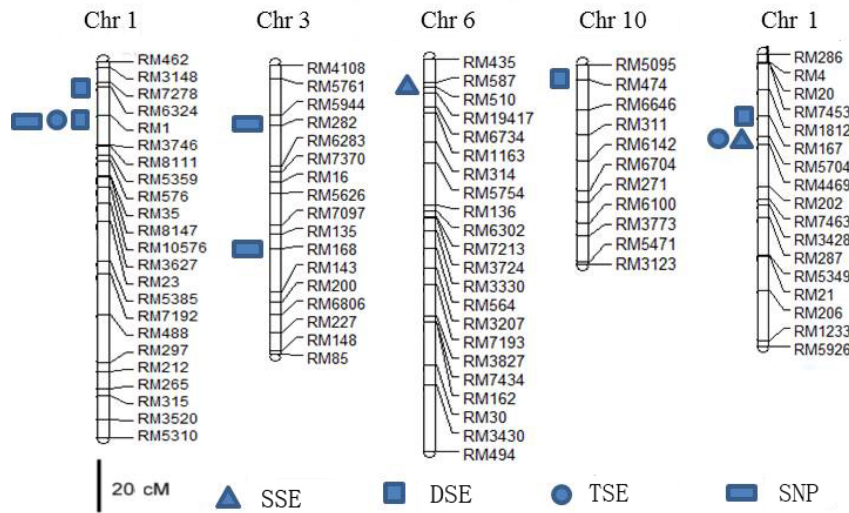


Figure 3. Chromosomal locations of QTL for the percentage of SSE, DSE, TSE, and SNP in RIL populations from the cross between XQZB and ZH9308. Triangle, square, circle, and rectangle represent putative regions of QTLs for SSE, DSE, TSE, and SNP, respectively.

Data were collected in March, April, and May 2015, in Hainan and in August, September, and October 2015 in Zhejiang. The temperatures of the two environments in Hainan and Zhejiang during the data collection periods of March-August, April-September, and May-October were significantly different (Table 4).

Table 4. Comparative study of monthly temperatures in two environments in Hainan and Zhejiang.

Hainan temperature (°C), 2015			Zhejiang temperature (°C), 2015		
Month	Average ± SE	Ranges	Month	Average ± SE	Ranges
March	25.26 ± 0.17**	24.00-27.50	August	27.89 ± 0.42***	24.00-33.00
April	26.42 ± 0.35***	21.50-28.50	September	23.78 ± 0.34***	20.00-27.50
May	30.00 ± 0.14***	29.00-31.50	October	19.39 ± 0.38***	13.00-23.50

Significant at the 0.01 level. *Significant at the 0.001 level (analyzed by the Student *t*-test) SE = standard error.

Analysis of variance (ANOVA) based on a fixed-effect model revealed highly significant variance between the two environments (Hainan and Zhejiang) in the RIL population (Table 5). Increased values were observed in Zhejiang compared with Hainan for all traits except for SNP (Table 1). Clearly, environmental factors in Zhejiang favored the stigma exertion rate.

Table 5. Results of ANOVA based on a fixed-effect model of the 134 RIL populations in two environments.

Trait	Sources of variation	d.f.	MS	F-value	P value
SSE	Line	133	22.47	17.80***	2E-48
	Environments	1	701.49	56.15***	8.39E-12
	Residual	133	12.50		
DSE	Line	133	22.32	6.63***	8.94E-25
	Environments	1	20.28	6.02**	0.015
	Residual	133	3.36		
TSE	Line	133	371.31	17.28***	1.16E-47
	Environments	1	920.39	42.85***	1.17E-09
	Residual	133	21.47		
SNP	Line	133	2553.39	6.22***	2.01E23
	Environments	1	59779.13	145.70***	4.08E23
	Residual	133	410.27		

Significant at the 0.01 level; *significant at the 0.001 level; Line, the RIL population; Environments, Hainan and Zhejiang; MS, means square.

DISCUSSION

Stigma exertion rate is a female parental trait important for improving hybrid seed production in rice. In this study, we report QTL for stigma exertion rate that were detected in two different environments. The exerted stigma is fragile and can be easily damaged by environmental conditions such as wind, water stress, and physical interruption, during the flowering period (Yan et al., 2009). In this study, we made several improvements to previously used methods when measuring the stigma exertion rate. In some previous studies, panicles were collected at flowering to avoid damage to exerted stigmas and to improve the accuracy of the collected data (Uga et al., 2003; Yan et al., 2009), whereas in other studies, panicles were collected at approximately 7-10 days post-flowering (Miyata et al., 2007; Takano-kai et al., 2011). Although some exerted stigmas are vulnerable, we noted that the pistil style in the flower remains discernible until the grain-filling period. In the present study, we sampled five main panicles from the parent and five from each line of the RIL population at 5-7 days after heading, when the lower spikelets of the panicle had flowered. The stigma exertion rate is affected by temperature. According to our observations, a temperature of 18°-25°C and conditions of high light intensity are favorable for stigma exertion. The temperatures of the two environments in Hainan and Zhejiang significantly differed during the flowering period (Table 4). ANOVA, based on a fixed-effect model, revealed highly significant variance between the RIL populations in the two environments (Table 5). Increased values were observed for all traits in Zhejiang compared with Hainan, with the exception of SNP (Table 1). These data clearly demonstrate that the environmental factors in Zhejiang favored a high stigma exertion rate. The identified QTLs that were related to the stigma exertion rate were not stable in both environments due to different environmental factors, such as temperature, wind, water stress, and physical interruption; whereas the QTL related to SNP (qSNP1) was stable in both environments.

In this study, a total of eight QTLs for stigma exertion rate were detected in both environments (Hainan and Zhejiang). We identified two markers for stigma exertion on chromosome 1 (RM7278 and RM1) that were associated with *qDSE1a*, *qDSE1b*, and *qTSE1*; one marker on chromosome 6 (RM587) that was associated with *qSSE6*; one marker on chromosome 10 (RM474) that was associated with *qDSE10*; and two markers on chromosome 11 (RM5704 and RM167) that were associated with *qSSE11*, *qDSE11*, and *qTSE11*. Numerous

studies have described QTLs associated with SSE, DSE, and TSE in rice. Specifically, five QTLs were found to be distributed on chromosomes 2 and 3 (Li et al., 2003); nine QTLs were found on chromosomes 1, 2, 5, and 8 (Yu et al., 2006); fifteen on chromosomes 1, 5, 6, 7, 8, 9, 10, and 11 (Yan et al., 2009); six on chromosomes 1, 2, 5, and 8 (Songpig et al., 2009); 11 on chromosomes 1, 3, 6, 7, 9, 10, and 12 (Li et al., 2014); and five on chromosomes 5, 6, and 7 (Lou et al., 2014).

In this study, we demonstrated that the QTL *qDSE1a* (distance 16.0 cM) between SSR markers RM7278 and RM6324 on chromosome 1 is related to DSE (Figure 3). Li et al. (2014) also identified one QTL that controls DSE in rice, *qDSE-1* (distance 15.5 cM), close to the SSR marker RM10105 on the same chromosome and in the same region. The chromosome regions harboring *qDSE-1* and *qDSE1a* overlap, suggesting that these two QTLs may be identical. This finding confirms the accuracy of the stable QTLs identified in the present study. We identified QTL *qSSE6* (distance 14.0 cM), which is related to SSE and is located between SSR markers RM587 and RM510. Yan et al. (2009) identified one QTL controlling SSE in rice, *qSSE-6* (distance 0.5 cM), which is located 13.5 cM from the SSR marker RM133 on chromosome 6. In the present study, we identified QTL *qDSE10* (distance 23.0), which is related to DSE and is located between SSR markers RM474 and RM6646. Li et al. (2014) identified one QTL controlling DSE in rice, *qDSE-10* (distance 40.0 cM), which is close to the SSR marker RM271 located 17 cM away on chromosome 10. We identified the QTL *qDSE11* (distance 33.0 cM), which is related to DSE and is located between SSR markers RM167 and RM5704. Yan et al. (2009) also identified one QTL controlling DSE in rice, *qSSE-11* (distance 8.7 cM), which is close to the SSR marker RM7203 located 24.7 cM away on chromosome 11. To our knowledge, the *qSSE11* and *qTSE11* regions have not been previously reported.

In this study, we detected a total of three QTLs for SNP in two environments (Hainan and Zhejiang) on chromosomes 1 and 3, which are related to SSR markers (RM1, RM168, and RM282). The QTLs *qSNP1*, *qSNP3a*, and *qSNP3b* were associated with the three markers. Markers associated with SNP have previously been reported. Six QTLs are distributed on chromosomes 2, 3, 4, 5, 9, and 11 (Yu et al., 2006; Songpig et al., 2009); four QTLs are distributed on chromosomes 1, 2, 3, and 7 (Zhang et al., 2009); four on chromosomes 3 and 4 (Deshmukh et al., 2010); three on chromosomes 1, 2, and 7 (He et al., 2010); and two on chromosomes 3 and 6 (Liang et al., 2012).

In the present study, one QTL related to SNP (*qSNP1*) was identified between SSR markers RM1 and RM3746 on chromosome 1. Zhang et al. (2009) and He et al. (2010) also reported one QTL related to SNP (*qSNP-1*) on the same chromosome. We identified two QTLs related to SNP (*qSNP3a* and *qSNP3b*) on chromosome 3. Previous studies (Yu et al., 2006; Songpig et al., 2009; Deshmukh et al., 2010; Liang et al., 2012) have also reported two QTLs related to SNP on the same chromosome.

For the commercial use of hybrid rice cultivation as hybrid rice seed production, maternal lines must have exerted stigma that trap more pollen dispersed from the male parent, thus overcoming the barrier of pollination and increasing the yield of hybrid seeds. QTLs with large effects have been identified as valuable resources for the genetic improvement of quantitative traits (Jiang et al., 2004; Toojinda et al., 2005). In this study, the interval flanked by the SSR markers RM5704 and RM4469 on chromosome 11 had an important effect. If *qSSE11* and *DSE11* were two tightly linked QTLs, then the combination between these loci could improve both SSE and DSE. Otherwise, DSE is a more favored parameter than SSE, due to the exertion of dual stigmas, which doubles the probability of outcrossing for the production of hybrid seed compared with the exertion of one out of two stigmas in rice. Therefore, the

application of *qDSE11* to improve DSE could be selected by marker-assisted selection (MAS) in rice breeding. A significant positive correlation was observed among SSE, DSE, and TSE.

In summary, we identified six SSR markers for stigma exertion distributed on chromosomes 1, 6, 10, and 11; five markers in different regions identified in previous studies on chromosomes 6, 10, 11, and one marker on chromosome 1 in a similar region to that identified in previous studies. In addition, we identified three markers for SNP that were distributed on chromosomes 1 and 3, consistent with previous reports. The addition of new regions increases the descriptive power of QTLs associated with stigma exertion and SNP. The SSE rate is sufficient for hybrid rice seed production. To evaluate the advantage of exerted stigma for cross-pollination, SSE, DSE, and TSE should be considered separately for future genetic improvement for the high production of rice hybrid seeds; this study also provides information for fine mapping, gene cloning, and MAS, with emphasis on the latter.

Conflicts of interest

The authors declare no conflict of interest.

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