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MAPPING QTLs INFLUENCING STIGMA EXERTION IN RICE

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Abstract

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Stigma exertion plays an important role in the efficient improvement of commercial seed production in hybrid rice. To understand the genetic basis of stigma exertion, a quantitative trait loci (QTLs) analysis was conducted using the F_2 population derived from two *indica* cytoplasmic male sterility (CMS) maintainers Huhan1B and K17B. QTLs influencing percentage of single exerted stigma (PSES), percentage of dual exerted stigma (PDES) and total exerted stigma (PES) were detected using a linkage map of 92 SSR markers. A total of 1, 3 and 1 QTLs were detected for PSES, PDES and PES, respectively. There were one to five pairs of epistatic QTLs affected stigma exertion. The contribution rate of additive and epistatic effects were in a low magnitude for most cases (0.83%-13.77%) while one QTL (*qPES-6*) and one pair of epistatic QTL explained 18.82% and 25.44% of total variance. The interval of RM3575-RM3351 on chromosome 5 contained two QTLs for PSES and PDES with reverse function, while RM8225-RM225 on chromosome 6 influencing PDES and PES functioned in the same way. If *qPSES-5* and *qPDES-5* were two tightly linked QTLs, recombination between these loci could improve both PSES and PDES. Otherwise the application of *qPDES-5* to improve PDES could be selected by MAS in rice breeding practice.

Key words: rice, stigma exertion, QTL mapping

Abbreviations: QTL - quantitative trait locus; RILs - recombinant inbred lines; DH - double-haploid; PSES - percentage of single exerted stigma (%); PDES - percentage of dual exerted stigma (%); PES - percentage of total exerted stigma (%); CMS - cytoplasmic male sterility; SSR - single sequence repeat; PCR - polymerase chain reaction; LOD - log of odd; H^2 - Contribution rate (%); A - additive effect; D - dominant effect; AA - additive-by-additive interaction; DA - dominant-by-additive interaction; MAS - marker-assisted selection

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. The development and commercial release of hybrid rice, that is, successful application of heterosis, breaks the ceiling of rice yield potential and has been a key contribution to meeting the challenge presented by an ever increasing population. Being an autogamous crop, it is difficult to reliably produce an acceptable quantity of hybrid rice seed (Azzini and Rutger, 1982). Therefore, improvement of the hybrid seed production efficiency is an essential prerequisite for successful implementation of hybrid rice. Virmani (1994) reported that cross-pollination efficiency was affected by some floral traits (e.g. flowering behavior, pollen longevity, stigma exertion and spikelet opening angle). Among them, stigma exertion is especially emphasized as a component increasing the opportunity of pollination (Kato and Namai, 1987). Maternal parent with high percentage of exerted stigma is expected not only to catch more pollen from paternal parent, but also to overcome the barrier of flowering asynchronization between maternal parent and paternal parent. As a key character for a high yielding of both male sterile line and the hybrid seed production, stigma exertion receive consistent attention from rice researchers (Virmani and Athwal, 1973;

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Virmani et al., 1994; Yuan et al., 1981; Yuan and Fu 1995; Li et al. 2001; Uga et al. 2003a, b; Xu et al., 2003; Miyata et al., 2007; Sidharthan et al., 2007; Yan et al., 2009).

Virmani and Athwal (1973) hypothesized that stigma exertion was controlled by polygenes, judging from the continuous phenotypic variation. Traditional selection on stigma exertion based on direct observation in the field is inefficient because the traits are very complex and easily affected by environmental factors. Recent progress in DNA marker technique and the construction of linkage maps have enabled the detection of quantitative trait loci (QTLs) controlling complex genetic traits (e.g. stigma exertion) and applicable to actual breeding. QTL analysis for percentage of exerted stigma and some other floral morphological traits were conducted by using segregating populations. Two QTLs for rate of exerted stigma were identified in backcross population (Li et al., 2001), recombinant inbred lines (RILs) population (Uga et al., 2003b) or double-haploid (DH) population (Li et al., 2003) derived from a cross between *O. sativa* and *O. rufipogon* or *indica* and *japonica* varieties, respectively. Using F_2 population between *japonica* and *indica*, a major QTL *qES3* on chromosome 3 was detected, which explained 31.63% of the total phenotypic variance (Miyata et al., 2007). Yu et al. (2006) reported three or four main effect QTLs for percentage of single exerted stigma (PSES), dual exerted stigma (PDES) and total exserted stigma (PES) under stress or non-stress conditions.

Huhan1B is a CMS line with nice grain quality and high level drought tolerance. Hybrid combinations Huyou No. 2 and Hanyou No.3 have been released by Chinese Ministry of Agriculture or Shanghai Municipal Government, respectively. But this CMS line has a shortcoming of low yielding of seed production, probably caused by low percentage of stigma exertion. K17 is a commercial CMS line with high percentage of stigma exertion. Here we identified the QTLs influencing stigma exertion by using the F_2 population between two CMS maintainers (Huhan1B and K17B) to reveal the genetic basis of stigma exertion rate.

Material and Methods

Genetic Population and Field Experiments

One hundred and ninety F_2 plants were developed from a cross between two *indica* CMS lines, Huhan1B and K17B.

The germinated seeds of F_2 plants and parents were sown on November 30 of 2006 in Hainan, China, and the seedlings were transplanted on December 27.

At 7-10 days after heading, three normal panicles from each plant were sampled for counting the spikelet number per panicle (SNP), spikelets with single exerted stigma (SES) and spikelets with dual exerted stigma (DES). PSES and PDES are the ratio of SES and DES to SNP, respectively. PES is equivalent to the sum of PSES and PDES.

DNA Preparation and PCR Amplification

The total genomic DNA was extracted from fresh leaves of 190 F_2 individuals and their parents using the CTAB method as described by Lou et al. (2005). The extracted DNA was dissolved in TE buffer and tested for quality and quantity using a DU 640 nucleic acid and protein analyzer (Beckman Coulter Co.). Then these 192 DNA samples were diluted into 25 ng/μl with sterilized double distilled water and stored at 4°C for the polymerase chain reaction (PCR). PCR amplifications were performed according to Lou et al. (2009) in Thermo Hybaid MBS 0.2S PCR Thermal Cycler (Fisher Scientific International, Hampton, NH, USA). PCR products with large difference were separated on 3% agarose gel and detected by using a UV-GIS detection system (Shanghai Tanon Science and Technology Co., Ltd.). Otherwise, PCR products were separated on 5% denatured polyacrylamide gel electrophoresis and detected by silver staining (Xu et al., 2002).

Linkage Map Construction and Data Analysis

A set of 92 SSR markers, which distributed along rice genome according previously reported linkage maps and revealed polymorphisms between the parents, were used to determine the genotypes of each plant in the F_2 population. The genetic linkage map was constructed by using the program MapMaker/Exp V3.0 (Lincoln et al., 1992) based on the genotype data of the F_2 population, for which the average interval distance between pairs of markers was 10.1 cM.

The chromosomal locations of putative QTLs were determined by composite interval mapping of QTLMapper V2.0 on the basis of mixed model approach (Wang et al., 1999). A locus with a threshold value of $LOD \geq 2.0$ and $P \leq 0.05$ was declared as a putative QTL. 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 following the popular nomenclature (McCouch et al., 1997).

Results

Frequency Distribution and Correlation of Stigma Exertion in F_2 Population

There were distinct differences between parents on PDES and PES, and approximation on PSES (Table 1). In particular, PDES of K17B (52.3%) was nearly three times as many as that of Huhan1B (17.5%). A wide continuous variation was recognized in all traits of F_2 population, indicating quantitative inheritance of these characters. Transgressive segregation with both directions and normal distribution were observed in all of the traits (Table 1, Figure 1).

The pairwise phenotypic correlation coefficients among PSES, PDES and PES were examined (Table 2). Significant correlations were observed. PSES was negatively related with PDES at a significance level of 1% and with PES at the level of 5%. PDES had a very strong positive relation with PES.

QTLs for Stigma Exsertion

A total of 5 QTLs for stigma exertion were identified, distributed on three chromosomes with LOD values varying from 4.54 to 14.06 (Table 3, Figure 2). The phenotypic variance explained by each QTL ranged from 4.77% to 18.82%.

Only one QTL for PSES was mapped to RM3575-RM3351 on chromosome 5, accounting for 10.02% of the variation with an LOD score of 4.54. The allele from K17B had a positive effect, which could increase PSES by 1.79%.

Three QTLs on chromosome 5, 6 and 7 were identified for PDES, collectively explained 25.07% of the phenotypic variance. The largest effect QTL, *qPDES-6*, flanked by RM111 and RM225 on chromosome 6, accounted for 13.77% of the variation, with a LOD value of 14.06. Additive effect and dominant effect of this QTL were -7.93% and 4.11%, respectively. The other two QTLs included one mapped to RM3575-rm3351 (*qPDES-5*) on chromosome 5 and another located in RM432-RM560 (*qPDES-7*) on chromosome 7. These QTLs accounted for 6.53% and 4.77% of the variance with LOD values of 8.46 and 4.72. The K17B allele at each locus could decrease or increase PDES by 5.46% and 4.67%, respectively.

A major QTL *qPES-6* was associated with PES. This locus in the interval of RM8225-RM111 on chromosome 6 explained 18.82% of the phenotypic variation, with additive effect of -5.06% and dominance deviation of 4.34%.

Digenic Interaction

Significant epistatic QTLs are summarized in Table 4. In total, 9 QTL pairs had significant epistatic effect on all traits,

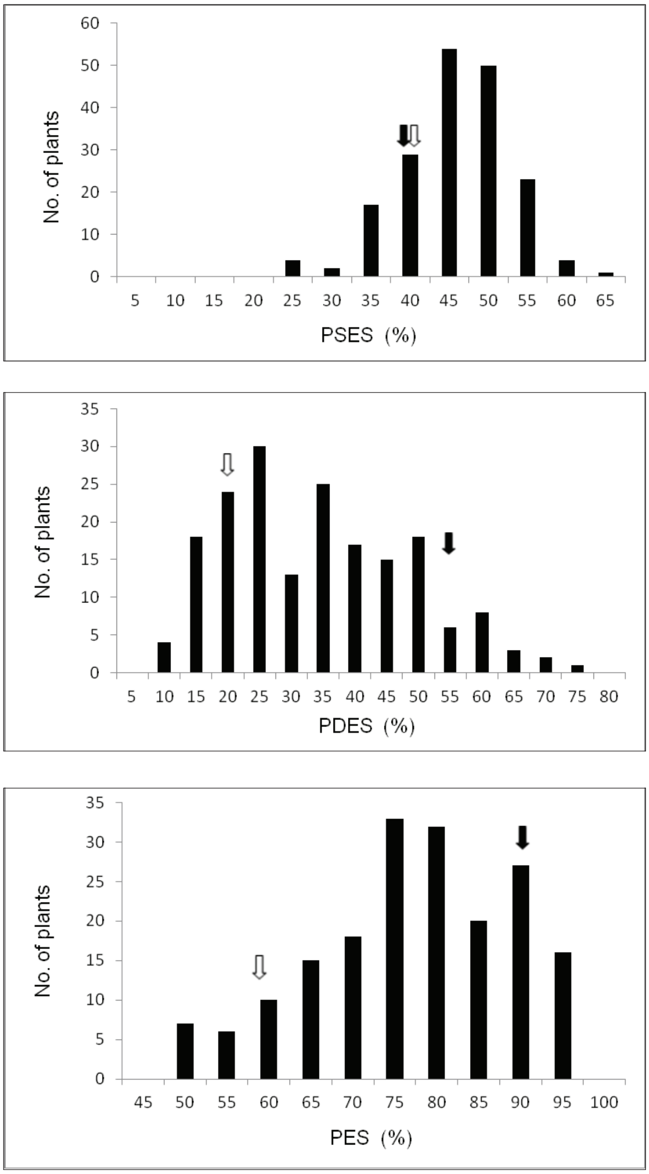

Fig. 1. Phenotypic distribution of PSES, PDES and PES in F_2 population of the cross between Huhan1B and K17B. Mean values of Huhan1B (open arrow) and K17B (closed arrow) were shown above

Table 1
Performance of stigma exertion of the parents and F_2 population

Traits	Huhan1B	K17B	F_2 population			
			Mean	Range	Kurtosis	Skew
PSES, %	38.8±3.2	36.4±4.6	43.1±6.8	22.4-60.2	0.43	-0.49
PDES, %	17.5±3.1	52.3±5.5	31.6±14.5	6.2-70.4	-0.46	0.47
PES, %	56.3±4.4	88.7±3.6	74.7±11.7	45.4-94.8	-0.44	-0.46

covering all chromosomes except chromosome 4 and 12. The contribution rate of the epistatic QTLs ranged from 5.25% to 25.44%. Among these epistatic QTLs, five pairs of loci influenced PSES, three affected PDES and one had effect on PES. But only two pairs for PDES were involved in major QTL affecting all for PDES, accounting for 6.95% and 5.25% of the phenotypic variation, respectively.

Table 2
Correlation coefficients among the traits of stigma exertion in F_2 population derived from Huhan1B and K17B

	PSES	PDES	PES
PDES	-0.601**		
PES	-0.167*	0.888**	

Table 3
Putative QTL and their additive and dominance effects for percentage of exserted stigma in the F_2 rice population

Trait	QTL	Chr.	Interval	LOD	Ai[a]	Di[b]	H^2(Ai)[c]	H^2(Di)[c]	H^2(A)[d]
PSES	*qPSES-5*	5	RM3575- RM3351	4.54	-1.79		10.02		10.02
PDES	*qPDES-5*	5	RM3575- RM3351	8.46	5.46		6.53		25.07
	qPDES-6	6	RM111- RM225	14.06	-7.93	4.11	13.77	1.85	
	qPDES-7	7	RM432- RM560	4.72	-4.67		4.77		
PES	*qPES-6*	6	RM8225- RM111	7.44	-5.06	4.34	18.82	6.72	18.82

[a] Ai is the additive effects of QTL. Positive values of additive effects indicate that the Huhan1B genotype have a positive effect on that trait.

[b] Di is the dominant effects of QTL. Positive values of dominant effects indicate that the Huhan1B genotype have a positive effect on that trait.

[c] H^2(Ai) and H^2(Di) are the percentage of the phenotypic variation explained by Ai and Di.

[d] H^2(A) is the collective percentage of the phenotypic variation explained by Ai for the trait.

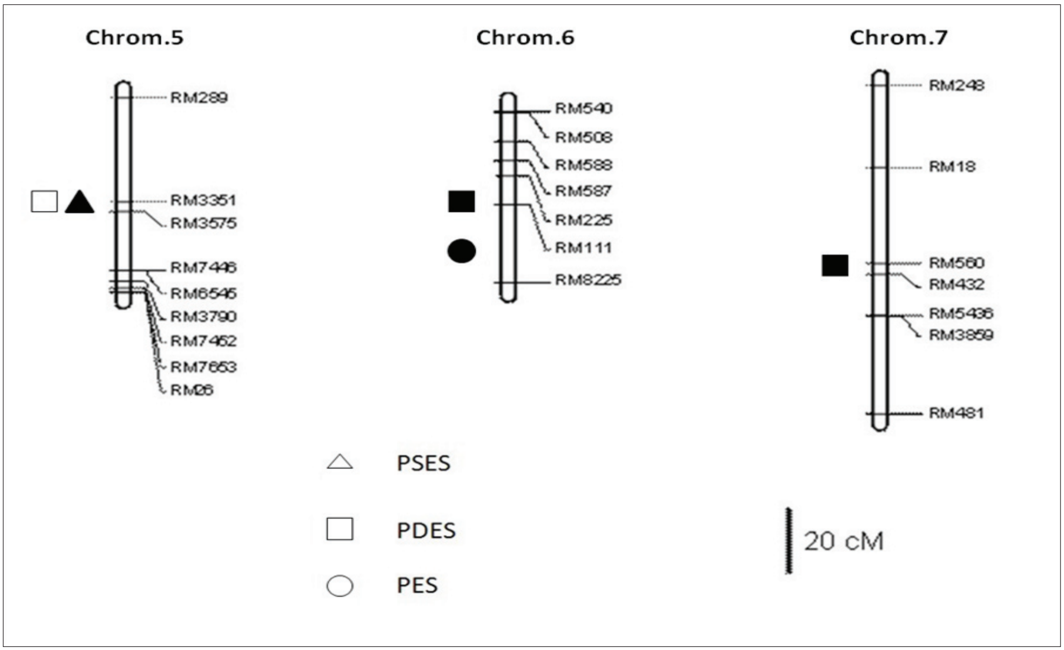

Fig. 2. Chromosomal locations of QTLs for percentage of single exserted stigma (PSES), dual exserted stigma (PDES) and total exserted stigma (PES) in F_2 population from the cross between Huhan1B and K17B. Triangle, square and rotundity represent putative regions of QTLs for PSES, PDES and PES, respectively. White figure indicates that the increasing effect is from Huhan1B; black figure indicates that the increasing effect is from K17B

Table 4

Epistatic QTL for percentage of exserted stigma in the F_2 rice population $p<0.01$

Trait	Ch-In i	Interval i	QTL	Ch-In j	Interval j	QTL	LOD	Ai [a]	H^2(Ai) [c]	Aj [a]	H^2(Aj) [c]	AAij [b]	H^2(AAij) [c]	H^2(AA) [d]	DAij [b]	H^2(DAij) [c]	H^2(DA) [d]
PSES	1-4	rm297-rm246		9-2	rm553-rm3808		7.29	3.27	7.48			-4.34	11.34	60.79			
	2-3	rm5984-rm423		2-6	rm526-rm7511		4.57					3.64	7.98				
	2-4	rm423-rm5340		2-1	rm208-rm530		11.3					-6.5	25.44				
	3-2	rm442-rm468		8-2	rm5556-rm6208		7.42					-2.68	4.32				
	10-1	rm228-rm3773		11-1	rm209-rm229		9.01					-4.41	11.71				
PDES	1-6	rm306-rm5422		7-3	rm432-rm560	*qPDES-7*	9.47			-1.13	2.01	5.75	6.95	25.05			
	6-5	rm588-rm508		8-2	rm5556-rm6208		13.3					7.64	12.85				
	7-3	rm432-rm560	*qPDES-7*	10-1	rm228-rm3773		7.56	-1.11	1.97			5.06	5.25				
PES	5-5	rm3575-rm3351		11-1	rm209-rm229		8.39			3.46	5.46	3.93	6.52	6.52	-0.3	0.83	0.83

[a] Ai and Aj are the additive effects of the test points i and j, respectively. Positive values of Ai and Aj imply that the Huhan1B genotype has a positive effect on that trait.

[b] AAij and DAij are the effects of additive-by-additive and dominant-by-additive interaction between points i and j, respectively. A positive value indicates that the parental two-locus genotypes have a positive effect and that the recombinants have a negative effect.

[c] H^2(Ai), H^2(Aj), H^2(AAij) and H^2(DAij) are the percentage of the phenotypic variation explained by Ai, Aj, AAij and DAij, respectively.

[d] H^2(AA) and H^2(DA) are the collective percentage of the phenotypic variation explained by AAij and DAij, respectively, for the trait.

Discussion

QTLs Identified for Stigma Exsetion in Rice

Due to the increase of possibility of cross-pollination, stigma exertion is a key trait to determine the yield potential of both CMS line and hybrid F_1 seed production. In this study, we constructed a set of F_2 population derived from a cross between two commercial CMS maintainers, Huhan1B and K17B, and identified QTLs controlling stigma exertion. A total of 5 QTLs and 9 epistatic QTL pairs were found to associate with the stigma exertion in rice. Two QTLs, *qPDES-6* and *qPES-6*, have not been reported in previous researches (Li et al., 2001; Uga et al., 2003b; Miyata et al., 2007; Yan et al., 2009). One QTL cluster was detected for PSES and PDES in the interval RM3575-RM3351 on chromosome 5 where the Huhan1B allele increased PDES, but decreased PSES. This region also contained *qPSES-5* reported by Yu et al. (2006) and was very close to two QTLs, namely *qPEST-5* and *qRES-5*, mapped by Li et al. (2001) and Uga et al. (2003b). Yan et al. (2009) identified one marker associated with dual and total stigma exertion on chromosome 5 (RM178), which was also adjacent to the cluster. Another QTL cluster located in the interval of RM8225-RM225 on chromosome 6 including *qPDES-6* and *qPES-6*, which shared the same marker RM111. The QTLs in this cluster explained 13.77% of phenotypic variation for PDES and 18.82% for PES. The K17B allele had a positive effect on PDES and PES. Xiao et al. (1996) observed that QTLs for correlated traits are often found in the same region. This trend was also observed in the two clusters above. To confirm whether these phenomena result from the pleiotropic effect of a single gene or from a tight linkage between two genes, fine mapping using near-isogenic lines is necessary. *qPDES-7* revealed in this study was close to associated marker reported by Yan et al. (2009).

Epistasis has been demonstrated as an important factor in the genetic basis for rice flowering time and heterosis (Yamamoto et al., 2000; Yu et al., 1997). In this research, epistatic interaction also played an important role in stigma exertion in rice (Table 4). Among the three traits surveyed, only PES had small effect of dominant-by- additive interaction. Epistatic QTLs explained a larger proportion of the phenotypic variation than major QTLs for PSES, and considerable proportion for PDES.

Utilizing QTLs of Stigma Exsertion

For the commercial use of hybrids in cultivated rice as an autogamous crop, maternal lines need to have exerted stigma for trapping more pollen dispersed from male parent, overcoming the barrier of pollination and increasing yield of hybrid seeds. QTLs with large effects have been improved as valuable resources in genetic improvement of quantitative traits (Jiang et al., 2004; Toojinda et al., 2005). In this study, the interval flanked by RM3575 and RM3351 on chromosome 5 had a main effect, but reverse function on PSES and PDES in the present research (Tables 2 and 3, Figure 2). If *qPSES-5* and *qPDES-5* were two tightly linked QTLs, recombination between these loci could improve both PSES and PDES. Otherwise PDES is more favored parameter than PSES due to exertion of dual stigmas doubles the probability of outcrossing for production of hybrid seed in comparison with the exertion of one out of two stigmas in rice. Therefore, the application of *qPDES-5* to improve PDES could be selected by MAS in rice breeding practice. Significant positive correlation was observed between PDES and PES. *qPDES-6* and *qPES-6* influencing PDES and PES was linked closely and functioned in the same way. So it is suitable for MAS to improve PDES and PES simultaneously in breeding program (Zhou et al., 2003).

Conclusions

With the development of hybrid rice, improvement of stigma exertion in CMS lines has become a major goal in many rice breeding programs. In our study, QTL analysis was conducted by using Huhan1B and K17B cross population to reveal the genetic basis of stigma exertion rate. Totally five main QTLs and nine pairs of epistatic QTLs influencing stigma exertion were detected. Utilizing these QTLs could improve stigma exertion rate in Huhan1B through marker-assisted selection strategies.

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