Substitution mapping of the major quantitative trait loci controlling stigma exsertion rate from *Oryza glumaepatula*

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Abstract

Background: Stigma exertion rate (SER) is a key determinant for outcrossing ability of male sterility lines (MSLs) in hybrid rice seed production. Outcrossing ability in cultivated rice varieties has diminished during the process of domestication, while wild *Oryza* species keep strong outcrossing ability. Here, we detected the quantitative trait loci (QTLs) controlling SER using a set of single-segment substitution lines (SSSLs) derived from *O. glumaepatula*, a wild *Oryza* species.

Results: Seven QTLs for SER, *qSER-1a*, *qSER-1b*, *qSER-3a*, *qSER-3b*, *qSER-5*, *qSER-9* and *qSER-10*, were located on 5 chromosomes. *qSER-1a* and *qSER-1b* were located on chromosome 1. *qSER-3a* and *qSER-3b* were mapped on chromosome 3, and *qSER-3b* was further located at an interval of 931.0kb by secondary substitution mapping. *qSER-5*, *qSER-9* and *qSER-10* were identified on chromosomes 5, 9 and 10, respectively, and *qSER-9* was delimited to a region of 608.2kb by secondary substitution mapping. The additive effects of the 7 QTLs ranged from 10.6% to 14.8%, and the additive contribution variances explained by each of the QTLs were from 36.3% to 50.6%, which were higher than those of most loci for SER reported previously.

Conclusions: *qSER-1a* and *qSER-1b* were novel loci for SER on chromosome 1. All of the 7 QTLs had major effects on SER. The major QTLs of SER will help to develop MSLs with strong outcrossing ability.

Background

As one of the most important cereal crops, rice plays a key role in sustaining world food security and supplying more than 20% calories intake as a staple (Zuo and Li 2014). The success of hybrid rice commercialization results largely in improving yield of rice (Guo et al. 2017). However, cultivated rice is predominantly self-fertilizing with less than 1% natural cross-pollination (Virmani and Athwal 1973). Outcrossing ability in cultivated rice varieties has diminished along with changes in the morphology of rice flowers during the process of domestication. Previous studies have established that exerted stigma, as a main mating organ, could keep viable for several days after flowering (Long and Shu 2000; Zhou et al. 2017a), and then get more opportunities to catch pollens (Rahman et al. 2017; Zhang et al. 2018). Therefore, stigma exertion rate (SER) is a key determinant for outcrossing ability of male sterility lines (MSLs) in hybrid rice seed production.

Wild *Oryza* species have strong outcrossing ability. It is reported that perennial wild rice had higher outcrossing than annual types (Oka and Morishima 1967). Cultivated rice tends to have a shorter stigma than the annual wild species. The annual wild species have shorter stigma than their perennial progenitors. The wider variability for stigma, style, and their total lengths in wild species of rice might have developed simultaneously during evolution and domestication (Marathi et al. 2015). In the past two decades, many of QTLs controlling SER and related traits have been located on 12 chromosomes of rice genome from various genetic resources (Marathi and Jena 2015; Zhou et al. 2017a; Liu et al. 2019). However, only 2 wild *Oryza* species were used to identify QTLs of SER until now, and *O. rufipogon* was

most commonly used. Xiong et al. (1999) reported an QTL for extruding stigma on chromosome 6 using an F₂ population derived from a cross between *O. sativa*, Aijiao Nante, and *O. rufipogon*, P16. Li et al. (2001) found two QTLs of SER on chromosomes 5 and 8 by a backcross-F₁ population between *O. rufipogon* and Guichao 2, an elite *indica* rice. Uga et al. (2003a) identified two QTLs for rate of exerted stigma between the *indica* line Pei-kuh and W1944 of *O. rufipogon*. Huang et al. (2012) found 8 QTLs for SER using the 271 F₂ lines from the cross between *O. sativa*, Guangluai-4, and *O. rufipogon*. Bakti and Tanaka (2019) detected five QTLs for SER using an F₂ population generated from a cross between the *japonica* rice cultivar 'Akidawara' and 'W0120' of *O. rufipogon*. From another wild species, *O. longistaminata*, several QTLs of SER were identified from an introgression line of the wild species in the genetic background of Asian cultivated rice (Li et al. 2010).

O. glumaepatula is one of the AA-genome wild *Oryza* species indigenous to Central and South America (Doi et al. 2008). Recently, Stein et al. (2018) elucidated that *O. glumaepatula* is the sister group only of African *O. barthii* and *O. glaberrima* based on the genome sequence alignment. Several hybrid pollen sterility genes between *O. sativa* and *O. glumaepatula* have been identified, such as *S12*, *S22*, *S23*, *S27* and *S28* (Sano 1994; Sobrizal et al. 2000a, b; Sobrizal and Yoshimura 2001, 2002; Sakata et al. 2014; Fang et al. 2019). It was found that the endosperm of *O. glumaepatula* had the high levels of total protein, albumin, and glutelin protein fractions and amino acids, showing the potential to increase the nutritional quality of rice storage protein (Santos et al. 2013). *O. glumaepatula* also showed the rapid internodal elongation potential under partial submergence (Sasayama et al. 2018), as well as its important yield and yield component traits (Zhang et al. 2015; Bhatia et al. 2017). It is important to note that *O. glumaepatula* had a longer stigma length, which is favorable for outcrossing (Marathi et al. 2015).

In order to detect QTLs for traits of agronomic importance in *O. glumaepatula*, 168 SSSLs were developed by using *O. glumaepatula* as donors and Huajingxian74 (HJX74), an *indica* variety of *O. sativa* as recipient. The total length of the substituted segments in the set of SSSLs was 3636.4 cM, which were distributed on 12 chromosomes (Zhao et al. 2019). In the present study, we used the set of SSSLs to identify QTLs for SER. Seven of the QTLs were located on 5 chromosomes. The QTLs had higher additive effects. These major QTLs of SER from *O. glumaepatula* will be helpful for developing of MSLs with strong outcrossing ability.

Results

SER in the SSSLs derived from *O. glumaepatula*

At the first, a set of 168 SSSLs derived from *O. glumaepatula* were investigated for SER and 9 of the SSSLs with higher SER were selected. The 9 SSSLs were then tested their SER for 5 cropping seasons. Compared with the HJX74 recipient, the 9 SSSLs were showed higher SER at the $P \leq 0.001$ level in every cropping season. Average SER of the 9 SSSLs in the 5 cropping seasons were from 48.1–60.8% with 18.5–31.2% higher than that of the control HJX74 (Fig. 1 and Additional file: Table S1).

The chromosomal substituted segments from *O. glumaepatula* in the 9 SSSLs were detected by molecular markers. The substituted segments in the 9 SSSLs were detected on chromosomes 1, 3, 5, 9 and 10, respectively. The estimated lengths of substituted segments were from 1467.0 kb to 16410.5 kb (Additional file: Table S2 and Table S3).

Eight of agronomic traits in the 9 SSSLs were investigated in two cropping seasons. Compared with HJX74, most of the traits in the SSSLs had no significant difference, except the plant height in SG25, the heading date in SG153, and so on (Additional file: Table S4).

Two QTLs for SER were mapped on chromosome 1

Four SSSLs with high SER, SG18, SG22, SG23 and SG25, carried substituted segments on chromosome 1. SG18 had a substituted segment at the interval from markers RM140 to RM5853 with the estimated length of 10741.8 kb (Fig. 1 and Additional file: Table S3). Therefore, the substituted segment of SG18 carried an QTL for SER, named *qSER-1a*.

Other three SSSLs, SG22, SG23, and SG25 carried substituted segments in the region of 29385.9-41082.0 kb and the substituted segments were overlapped. SG22 had the shortest substituted segment with the estimated length of 2611.3 kb from markers RM403 to RM6648. The estimated lengths of substituted segments were 5874.5 kb in SG23, and 11059.7 kb in SG25. These results indicated that the three SSSLs carried another QTL for SER, named *qSER-1b*, which located in the substituted segment of SG22 with the interval of 2611.3 kb (Fig. 2 and Additional file: Table S3).

Because the *qSER-1b* was linked to *Sd1*, SG25 carried the *Sd1* in the substituted segment from *O. glumaepatula* and showed tall in plant height with 160.3 cm in FCS and 135.7 cm in SCS. SG22 and SG23 carried shorter substituted segments and had no *Sd1* from *O. glumaepatula*. Although SG22 and SG23 were semi-dwarf in plant height, the SER had no significant difference with SG25 (Fig. 2 and Additional file: Table S4). These results indicated that the *qSER-1b* of SER was not influenced by plant height.

Two QTLs for SER were mapped on chromosome 3

Two SSSLs with high SER, SG59 and SG65, carried substituted segments on chromosome 3. SG59 had a substituted segment at the interval from markers RM282 to InDel3 with the estimated length of 4632.1 kb (Fig. 1 and Additional file: Table S3). Therefore, the substituted segment of SG59 carried an QTL for SER, named *qSER-3a*.

The SSSL SG65 carried a substituted segment at the interval of 26747.5-35843.1 kb on chromosome 3. The substituted segment was in a different region from that in SG59. Therefore, SG65 had another QTL for SER, *qSER-3b*, in its substituted segment (Fig. 3b, Additional file: Table S3).

To narrow the interval of *qSER-3b*, 5 secondary SSSLs were developed from an $F_{2:3}$ population derived from the cross of HJX74/SG65. The secondary SSSL, SG65-14, carried a substituted segment in the

region from markers RM15678 to ID03MC07 on the left. Two of the secondary SSSLs, SG65-48 and SG65-61, had substituted segments in the regions from PSM132 and RM186 to RM442 on the right, respectively. The three secondary SSSLs showed lower SER as HJX74. Other two secondary SSSLs, SG65-9 and SG65-55, carrying substituted segments covered the region from markers ID03MC07 to RM186 showed higher SER as SG65. These results indicated that the *qSER-3b* was narrowed to an interval of 931.0 kb between markers ID03MC07 and RM186 (Fig. 3).

Secondary substitution mapping of *qSER-9*

One SSSL, SG135, carried a substituted segment on chromosome 9. Therefore, the substituted segment had an QTL for SER, *qSER-9*. To further map *qSER-9*, 5 secondary SSSLs were developed from an $F_{2:3}$ population from the cross of HJX74/SG135. Two of the secondary SSSLs, SG135-26 and SG135-30, carried respectively substituted segments in the regions from ID09M06 to ID09M16 and RM105 on the left. The secondary SSSL, SG135-6, had a substituted segment in the region from ID09M23 to RM3600 on the right. The three secondary SSSLs showed lower SER as HJX74. Other two secondary SSSLs, SG135-44 and SG135-68, carried substituted segments from ID09M16 to ID09M23, and had higher SER as SG135. These results indicated that the *qSER-9* was delimited to an interval of 608.2 kb between ID09M16 and ID09M23 (Fig. 4).

Other QTLs for SER identified in the SSSLs

One SSSL, SG85, carried a substituted segment from markers RM7444 to RM291 on chromosome 5 with the estimated length of 16410.5 kb. Therefore, the SSSL had an QTL for SER, *qSER-5*, in the substituted segment (Fig. 1 and Additional file: Table S3).

Another SSSL, SG153, carried a substituted segment from markers RM484 to RM25886 on chromosome 10 with the estimated length of 1467.0 kb and had an QTL for SER, *qSER-10*, in the substituted segment (Fig. 1 and Additional file: Table S3).

Summarily, a total of 7 QTLs for SER was mapped on 5 chromosomes. Among of them, chromosomes 1 and 3 each carried two of the QTLs, and chromosomes 5, 9 and 10 each carried one of the QTLs (Fig. 5).

The additive effects of QTLs for SER identified in the SSSLs

The additive effects of the 7 QTLs for SER, *qSER-1a*, *qSER-1b*, *qSER-3a*, *qSER-3b*, *qSER-5*, *qSER-9* and *qSER-10*, ranged from 10.6–14.8% and the additive contribution variances explained by each of the QTLs for SER were from 36.3–50.6%. Among of them, *qSER-10* had the highest additive effect of 14.8% and the highest additive contribution variance of 50.6% (Table 1).

Table 1
Additive effects of QTLs for stigma exsertion rate detected in the SSSLs

QTL	Chr.	Interval (kb)	Estimated length (kb)	Pvalue	A (%)	ACV (%)
<i>qSER-1a</i>	1	12291.6-23033.4	10741.8	2.5E-08	11.9 ± 0.8	40.7 ± 3.7
<i>qSER-1b</i>	1	29608.2-32219.6	2611.3	3.6E-06	12.4 ± 1.8	42.5 ± 6.3
<i>qSER-3a</i>	3	11656.8-16288.9	4632.1	1.2E-07	11.1 ± 1.1	38.0 ± 4.4
<i>qSER-3b</i>	3	27677.1-28608.1	931.0	2.4E-07	10.7 ± 1.2	37.1 ± 5.5
<i>qSER-5</i>	5	3216.5-19626.9	16410.5	1.3E-09	13.5 ± 1.6	46.5 ± 6.6
<i>qSER-9</i>	9	14342.2-14950.4	608.2	3.1E-07	10.6 ± 1.0	36.3 ± 4.2
<i>qSER-10</i>	10	21132.3-22599.3	1467.0	1.2E-10	14.8 ± 2.0	50.6 ± 7.2
A additive effect, ACV additive contribution variance, A and ACV was represented as mean ± S.E. in five cropping seasons.						

Discussion

In the past two decades, many of QTLs that affect stigma exsertion were identified in rice genome. The QTLs were distributed across all 12 chromosomes (Marathi and Jena 2015; Zhou et al. 2017a; Liu et al. 2019). Among of them, a limited number of QTLs for SER were identified from the wild *Oryza* species, *O. rufipogon* (Xiong et al. 1999; Li et al. 2001; Uga et al. 2003a; Huang et al. 2012; Bakti and Tanaka 2019) and *O. longistaminata* (Li et al. 2010). In the present study, we detected 7 QTLs of SER on 5 chromosomes using a set of SSSLs derived from *O. glumaepatula*. On chromosome 1, *qSER-1a* and *qSER-1b* are novel loci, in which region no other QTLs for SER were reported previously. On chromosome 3, *qSER-3a* was located in the QTL cluster of *qES3* (Miyata et al. 2007), *PES-3* (Yue et al. 2009), *qSPE3* (Feng et al. 2010) and *qSSE3* (Li et al. 2014a). *qSER-3b* was mapped in the region locating of *qTSE-3b* (Li et al. 2014b) and *qSER-3.2* (Xu et al. 2019). On chromosome 5, the region of *qSER-5* covered those of *qPEST-5* (Li et al. 2001), *qPES-5* (Deng et al. 2010), *qTSE-5a* (Li et al. 2014b), *TSE* (Zhou et al. 2017a) and *qSER-5.1* (Xu et al. 2019). On chromosome 9, the *qSER-9* region was partly overlapped with the segment of *qPES-9* from T821B, an introgression line from *O. longistaminata* (Li et al. 2010). In addition, the *qSER-10* interval on chromosome 10 was overlapped with the region of *qDSE-10* (Li et al. 2014a). Although most of the loci identified in this study were located in the same regions as the previously reported loci, it was noted that the additive effects of the loci identified from *O. glumaepatula* were generally greater. The additive effects of the seven loci identified in this study were between 10.6% and 14.8%, while the additive effects of most of the previously reported loci were less than 8.0%. It indicated that the QTLs from *O. glumaepatula* had major effects on SER, which will be favorable for molecular breeding of MSLs with greater ability for outcrossing.

During the process of domestication, cultivated rice has already lost some traits of natural outcrossing by varying from a vegetative to sexual manner of reproduction (Parmar et al. 1979). Wild *Oryza* species have a strong outcrossing ability due to their larger stigma, longer style, greater exertion of the stigma, and longer periods of spikelet opening (Marathi et al. 2015; Marathi and Jena 2015). Previous studies revealed dominant differences for SER and floral traits between cultivated rice and wild rice (Virmani and Athwal 1973; Uga et al. 2003b). It was found that *O. glumaepatula* had longer of stigma length than cultivated rice (Marathi et al. 2015). In the present study, we identified 7 QTLs for SER with major effects from the SSSLs derived from *O. glumaepatula*. It indicated that the favorable alleles for outcrossing traits of larger stigma, longer style, greater exertion of the stigma, and longer periods of spikelet opening, and so on, which were lost in cultivated rice, could be mined in wild *Oryza* species.

In the past two decades, we have constructed an SSSL library by the using of HJX74 as the recipient and 43 accessions from 7 species, including 5 wild *Oryza* species of AA genome as donors (Zhang et al. 2004; Xi et al. 2006; Zhang 2019). The SSSLs have been used to map QTLs (Zhang et al. 2012; Yang et al. 2016; Zhou et al. 2017b), to clone genes (Wang et al. 2012; Sui et al. 2019), to analyze allelic variation (Teng et al. 2012; Cai et al. 2013) and to conduct molecular breeding by design (Dai et al. 2015, 2016; Luan et al. 2019). In the present study, the SSSLs derived from *O. glumaepatula* were used to identified the QTLs for SER. Seven QTLs of SER with major effects were located on 5 chromosomes. These results indicated that the HJX74-SSSL library is a powerful tool for mapping of QTLs of agronomic importance and identifying of naturally occurring alleles in germplasm, particularly for mining of the favorable alleles lost in cultivated rice from wild species.

Conclusion

Using the SSSLs derived from *O. glumaepatula*, seven major QTLs controlling SER, *qSER-1a*, *qSER-1b*, *qSER-3a*, *qSER-3b*, *qSER-5*, *qSER-9* and *qSER-10*, were located on 5 chromosomes by substitution mapping. *qSER-1a* and *qSER-1b* were novel loci for SER, in which regions no other QTLs of SER were reported previously. Compared with the previously reported loci, the 7 QTLs have major additive effects and additive contribution variances. The major QTLs will be favorable for molecular breeding of MSLs with strong outcrossing ability. The favorable alleles for outcrossing traits which were lost in cultivated rice could be mined in the wild *Oryza* species.

Methods

Materials and field experiment

The set of SSSLs derived from *O. glumaepatula* (Zhao et al. 2019) and their parents HJX74 and *O. glumaepatula* were grown in the paddy fields at the experimental farm of South China Agricultural University in Guangzhou (23°07'N, 113°15'E), China. The materials were grown in 5 cropping seasons from 2016 to 2018, two cropping seasons per year, the first cropping season (FCS) from late February to middle July and the second cropping season (SCS) from late July to middle November. Single-seedling

transplanting was applied in field experiment. Each plot had four rows with ten single plants per row. Standard cultivation practices and controlling of diseases and insect pests were followed the typical methods in South China.

Molecular markers and PCR protocol

SSR markers labeled “RM” were selected from online resources (<https://archive.gramene.org/markers/>). The “PSM” and “Indel” markers were designed using software of Primer Premier 5.0 (Lalitha 2000). DNA was extracted from the fresh leaves of each plant referencing a reported method (Murray and Thompson 1980). The PCR products were amplified and analyzed on 6% denatured polyacrylamide gel, and detected using the silver staining using the methods described by Fang et al. (2019).

Phenotyping of traits and statistical analysis

The stigma exertion (SE) was subdivided into single stigma exertion (SSE) and dual stigma exertion (DSE). The SER refers to total stigma exertion rate, including single stigma exertion rate and dual stigma exertion rate. For investigating SER, 8–10 main panicles were sampled from plants in 1–2 days after flourishing florescence of florets. The SER per panicle was calculated based on visual aspect of exerted stigmas (Xu et al. 2019). Agronomic traits were measured by using the YTS rice phenotypic facility (Yang et al. 2014). Differences of traits were determined using one-way ANOVA. SPSS 23.0 and Origin 9.0 plus (<https://www.originlab.com>) were applied to analyze experimental data and draw figures in this paper.

Mapping of QTLs

The lengths of substituted segments of SSSLs were calculated by the previous method (Xi et al. 2006; He et al. 2017; Zhao et al. 2019). The minimum length ($L_{\min}$) of a substituted segment refers to the length between two markers of donor genotype at the end of the substitution segment. The maximum length ($L_{\max}$) refers to the length between two markers of background genotype flanking the substitution segment. The estimated length ($L_{\text{est}} = (L_{\min} + L_{\max})/2$). The QTLs for SER were mapped by the substitution mapping method (Eshed and Zamir 1995; Wissuwa et al. 2002). Linkage map of QTLs was drawn by using MapChart2.2 (<https://www.wur.nl/en/show/Mapchart.htm>). QTLs were named by the method of McCouch et al. (1997).

Abbreviations

SER: Stigma exertion rate; QTL: Quantitative trait locus; SSSL: Single-segment substitution line; MAS: Marker-assisted selection; MSL: Male sterility line; FSC: First cropping season; SCS: Second cropping season

Declarations

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Availability of data and materials

All data generated or analysed during this study are included in this published article and its additional information files.

Authors' contributions

GZ and XF conceived and designed the experiments and supervised the research works. QT performed most of the experiments and prepared the data and the draft of manuscript. TZ, YN, MZ, XL, WY and ZY performed a part of experiments. HZ, RZ, GL, SW and GZ participated in material development. GZ analyzed the data and wrote the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Figures

Figure 1

Stigma exsertion rate in the 9 SSSLs and HJX74. SSSL: single-segment substitution line. SER: stigma exsertion rate. Data were presented as mean $\pm$ S.E. in five cropping seasons, one-way ANOVA, LSD, two-tailed, *** p-value $\leq$ 0.001. The percentages were converted to arcsine values.

Figure 2

Substitution mapping of *qSER-1b*. a, Morphology of HJX74 and SG22. Scale bar, 15 cm. b, The region of substituted segments of three SSSLs, SG22, SG23 and SG25, on chromosome 1. c, Substitution mapping of *qSER-1b*. Stigma exertion rate (SER) (%) is a mean $\pm$ S.E. in five cropping seasons. White and black blocks on chromosomes represent genotypes of HJX74 and donor, respectively. Threshold, $\alpha = 0.001$, arcsine value, one-way ANOVA, Duncan, two-tailed. The triangle represents the locus of *Sd1* (Sasaki et al. 2002).

Figure 3

Secondary substitution mapping of *qSER-3b*. a, Morphology of HJX74 and SG65. Scale bar, 15 cm. b, The substituted segment of SG65 on chromosome 3. c, Secondary substituted mapping of *qSER-3b* in the substituted segment of SG65. Stigma exsertion rate (SER) (%) is a mean $\pm$ S.E.. White and black blocks on chromosomes represent genotypes of HJX74 and donor, respectively. Threshold, $\alpha = 0.001$, arcsine value, one-way ANOVA, Duncan, two-tailed.

Figure 4

Secondary substitution mapping of *qSER-9*. **a**, Morphology of HJX74 and SG135. Scale bar, 15 cm. **b**, Substituted segment of SG135 on chromosome 9. **c**, Secondary substituted mapping of *qSER-9* in the substituted segment of SG135. Stigma exsertion rate (SER) (%) of secondary SSSLs is a mean $\pm$ S.E.. White and black blocks on chromosomes represent genotypes of HJX74 and donor, respectively. Threshold, $\alpha = 0.001$, arcsine value, one-way ANOVA, Duncan, two-tailed.

Figure 5

Chromosomal locations of the seven QTLs for SER in the SSSLs. Black bars on the right of each chromosome are the intervals of QTLs with their names on the right. Chr. chromosome, Mb megabase.

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