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Molecular mapping of quantitative trait loci for stigma exsertion trait in rice (*Oryza sativa* L.)

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Abstract

Stigma exsertion is the most important floral trait of female parent/cytoplasmic male sterile (CMS) line for out crossing which increases hybrid seed production at farmer's field. The present study was aimed to map the genomic regions controlling the stigma exsertion in F_2 mapping population derived from IR68897B, a recipient maintainer line of IR68897A with low stigma exsertion (36.78 %) and BF-16B, a donor parent for high stigma exsertion (80.25%). Phenotyping and genotyping of stigma exsertion trait was done for 188 individuals of F_2 population developed from IR68897B X BF16B. Frequency distribution of F_2 population showed continuous variation which indicated polygenic nature of inheritance. Phenotypic evaluation revealed that significant variation for stigma exsertion trait and transgressive segregation was observed. A total of 15 QTLs were identified for total stigma exsertion rate (TSE) which includes single and dual stigma exsertion distributed on chrm 2, 3, 4, 5, 6, 8, 11 and 12 through QTL mapping. The phenotypic variance and LOD value explained by each QTL ranged from 1.19 to 5.83% and 2.54 to 9.29 respectively. In the present study, total 15 QTLs were minor effect QTLs (<10% phenotypic variance), indicating that stigma exsertion is controlled by many small effect QTLs. The QTL *qTSE11-1* on chrm 11 (9.29) and *qTSE3-1* on chrm 3 (6.83) were identified with higher LOD values and *qTSE3-2* on chrm 3 and *qTSE6-2* on chrm 6 explained 5.83 and 5.76 % of phenotypic variance, respectively

Keywords: QTL Mapping, stigma exsertion, maintainer line, phenotyping, grain yield

Introduction

Rice *(Oryza sativa* L.*)* is the second most important cereal crop and occupies a predominant position among major food grains. It is one of the crops responsible for the green revolution in the 1960s and 1970s. Global rice demand estimated to rise from 759.6 million tons in 2018 (FAO RMM, 2018) to 852 million tons in 2035 (Kush, 2013). This demand can be achieved by concentrating breeding efforts to improve rice yield potential with the shrinking natural resource base. With an ever-increasing population, crop scientists continuously work to meet the demands. Different strategies have been suggested to increase the yield potential of rice (Kush, 2005 and 2013). Among various strategies hybrid breeding is one of the strategies to improve rice productivity. Rice hybrids offer an yield advantage of 10-15 % over inbred varieties. Unfortunately, this technology was less adopted by the farming community. The high cost of hybrid seed is one of the major reasons affecting large scale adoption of the technology. This is mainly because of low hybrid seed yields i.e 1-2 t/ha. It is

difficult to reliably produce an acceptable quantity of hybrid rice seeds owing to its self-pollinating nature (Azzini and Rutger, 1982 and Kato and Nimai, 1987). The F_1 seed yield depends on outcrossing potential of female parent of a hybrid. Hybrid rice research should focus on to improve the F_1 seed yields by improving the out crossing potential of CMS lines and by managing seed production practices. This would maintain the hybrid seed cost. Therefore, improvement of hybrid seed production efficiency is an essential factor for large scale commercialization of hybrid rice (Tiwari *et al*., 2011).

In hybrid rice breeding programs, morphological characteristics of florets are of utmost importance in increasing the out crossing rate in hybrid rice seed production. The floral traits which can improve outcrossing potential of CMS lines include days to heading, pollen load, pollen longevity and morphological traits of floret, *viz.*, size of stigma and style, stigmatic receptivity, stigma exsertion, angle of floret opening and duration (Virmani, 1994). Among them, stigma exsertion is emphasized as a significant component in increasing pollination and seed set (Kato and Namai, 1987; Virmani, 1994; Sheeba *et al*., 2006). Stigma receptivity varies from 3-7 days, increasing the chance for pollination (Yan and Li, 1987; Xu and Shen, 1988; Li *et al*., 2004).Yang, (1997) reported that with 1 percent increase in stigma exsertion rate in male sterile lines, hybrid seed yields would increase by 47–68 kg /ha. Therefore stigma exsertion rate is extremely important floral trait for improving seed yields in hybrid seed production plots.

Stigma exsertion includes single, dual stigmas and other stigma traits that play important roles in hybrid seed production and received consistent attention from rice researchers (Virmani and Athwal, 1973; Virmani, 1994; Yan and Li, 1987; Tian, 1993; Li *et al*., 2001; Uga *et al*., 2003; Xu 2003; Yamamota *et al*., 2003; Miyata *et al*., 2007; Sidharthan *et al*., 2007 Yan *et al*., 2009, Huang *et al*., 2012; Dang *et al*., 2016; Zhou *et al*., 2017; Guo *et al*., 2017; Li *et al*., 2014; Liu *et al*., 2015; Sruthi *et al*., 2016; Rahman *et al*., 2017; Zhang *et al*., 2018; Liu *et al*., 2019, Bakti and Tanaka, 2019; Xu *et al*., 2019). Earlier studies have suggested that stigma exsertion is partially dominant (Virmani and Athwal, 1974) or completely dominant (Li *et al*., 1997b), and is a qualitative character controlled by a major gene (Hassan *et al*., 1984). Many studies reported that stigma exsertion is a quantitative trait controlled by polygenes (Li *et al*., 1995; Virmani and Athwal, 1974; Li and Chen, 1985; Wang *et al*., 2008; Xu and Shen, 1987; Sruthi *et al*., 2014). The small size of rice spikelets and the large effect of the environment on flowering in rice add to the difficulty in traditional selection-based breeding. As a result, the development of DNA markers associated with desirable floral traits for breeding programs has received increased attention at the national and international levels. Recently many studies have reported QTLs for stigma exsertion traits. Tan *et al*., (2020) mapped 7 QTLs on 5 chrm. (1, 3, 5, 9 and 10) using single segment substitution lines (SSSLs) derived from *O. glumaepatula*. Xu *et al*., (2019), identified a major QTL for stigma exsertion rate *viz.*, qSER-3.1 on chrm 3 in a 3.9 Mb region. Zhang *et al*., (2018), mapped qSE7 between RM5436 and RM5499 markers, within a physical distance of 1000-kb, with the use of INDEL markers it was finally mapped to a region of 322.9-kb between InDel SER4-1 and RM5436. Rahman *et al*.,(2017) dissected a major QTL qSE11on chrm 11 to a region of 350.7-kb. Liu *et al*., (2019) fine mapped qSER7 on chrm 7 and narrowed it down to a region of 28.4-kb between the markers RM3859 and Indel4373. Rahman *et al*., (2016) identified 8 QTLs (qSES6, qSSE11, qDSE1a, qDSE1b, qDSE10, qDSE11, qTSE1 and qTSE11) for single, dual and total stigma exsertion. Bakti and Tanaka, (2019) mapped QTLs for stigma exsertion ratio on chrms 2, 3, 4, 8, and 11 from *Oryza rufipogon* accession 'W0120'. Although many QTLs have been identified for stigma exsertion, their expression may vary one genetic back ground to other. We have attempted to map QTLs in the back ground of popularly used maintainer lines in hybrid breeding programme in India. Hence, the present investigation was carried out with an objective to identify the QTLs for stigma exsertion traits.

Materials and Methods

Plant material

The F_2 mapping population consisting of 188 lines was developed from a cross between IR68897B as a recipient parent with low stigma exsertion and BF-16B as a donor parent with high stigma exsertion **(Figure 2).** IR68897B is an early duration maintainer line for wild abortive male-sterile cytoplasm with long slender grain type developed by IRRI, Philippines and BF-16B is also a maintainer line for WA-CMS improved for stigma exsertion trait by Barwale Company. Hybrid nature of F_1s was confirmed using reported markers (**Table 1**) which are polymorphic between parents and true F_1s were raised in the field to develop F_2 population for QTL mapping.

Field experiments

A total of 188 F_2 mapping population along with its parents was grown in field at Research Farm, Indian Council of Agriculture Research (ICAR)- Indian Institute of Rice Research (IIRR), Hyderabad during *kharif* 2014 for phenotypic evaluation of stigma exsertion trait.

Phenotyping of stigma exsertion trait

For phenotypic evaluation of stigma exsertion trait, panicles were collected at post anthesis. Immediately after collecting panicles, panicles were wrapped in water soaked blotting paper and stored at -20°C. This is to avoid damage to stigma and to keep panicles afresh. For assessing the type of stigma exsertion in each spikelet of a panicle by the whole panicle method, all the individual spikelets in each panicle were separated and observed under illuminated magnifier lens to categorize them into dual (DStgE), single (SStgE) (**Figure 1**) or no stigma exsertion (NStgE) types. Then, these counts were converted to:

SStgE (%) = [SStgE / (SStgE + DStgE + NStgE)] x100

DStgE (%) = [DStgE/ (SStgE +DStgE +NStgE) x100

Total stigma exsertion [TStgE] (%) = SStgE (%) + DStgE (%) and NStgE (%) =100 - TStgE (%).

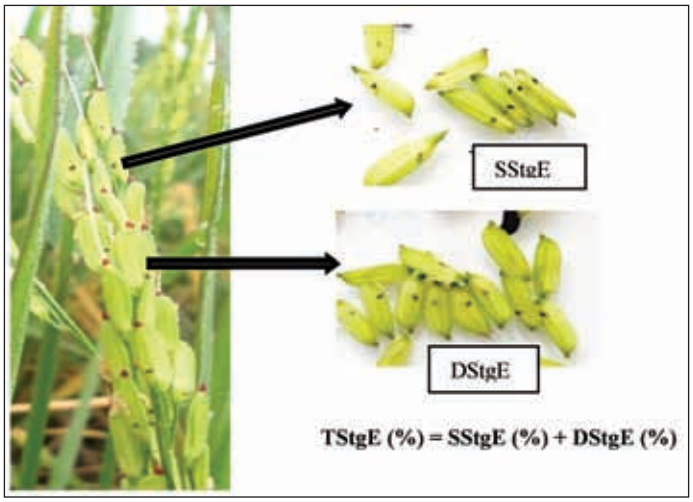

Figure 1: Figure showing the phenotyping of stigma exsertion trait

Genotyping

Genomic DNA was isolated from total 188 F_2 population and the parents using CTAB method of Zheng *et al.*, (1991) with minor modifications. Parental polymorphism survey was done using 513 SSR markers, which are distributed across 12 chrm. Out of 513 SSR markers only 124 markers showed (**Table 3**) polymorphism between parents. PCR amplification was carried out using thermal cycler (Veriti Thermo Cycler, Applied Bio systems). Master mix was prepared for 10 µl reaction volume containing 2 µl DNA template, 4.5 µl of nuclease free water, 1.0 µl 10X buffer, 1 µl dNTPs, 1 µl for both forward and reverse primers and 0.5 µl of 2U/ µl Taq DNA polymerase. PCR thermal profile is as followed: initial denaturation cycle of 95ºC for 5 min was followed by 35 cycles at 95ºC for 30 sec, 55°C for 30 sec , 1 min of extension at 72ºC with an additional step of 10 min at 72ºC. PCR products were resolved in 3% agarose gel in 0.5X TBE buffer stained with Ethidium Bromide and documented using gel documentation system (Bio-Rad, USA).

Linkage map construction and QTL mapping

The polymorphic 56 SSR (**Table 2**) markers were used to analyze the genomic DNA of the parents, IR68897B and BF-16B and F_2 population (188 plants). Each gel was scored for maternal (A), paternal (B) and heterozygous (H) banding pattern and later these converted into 2 (A), 0 (B) and 1 (H) score. QTL and

their effects were obtained using framework linkage map constructed for the genotypic data of 188 F_2 mapping population using 56 polymorphic markers and phenotypic data of 188 F_2 population using QTL ICI (Integrated Composite Interval Mapping) Mapping V4.2 (Meng *et al.*, 2015) employing Inclusive Composite Interval Mapping (ICIM) method. The LOD profiles were used to identify most of the likely position for a QTL in relation to the linkage map, which was the position where the highest LOD value was obtained. For QTL mapping, logarithm of the odds (LOD) threshold value was set to 2.5 using 1000 permutations at 0.05 level of significance.

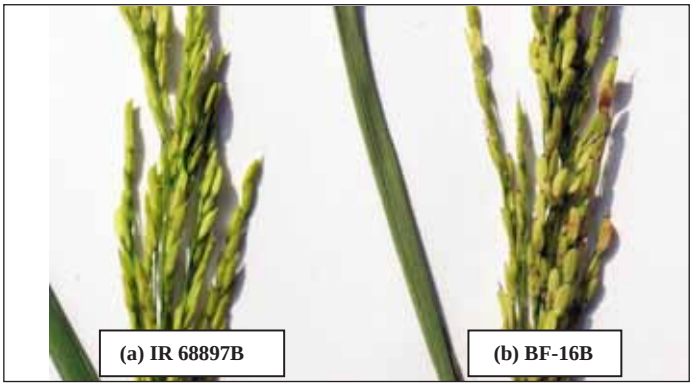

Figure 2: Figure showing the panicle type of parents;
(a) Recipient (IR 68897B) and (b) donor parent (BF-16)

Results and discussion

Phenotypic performance of stigma exsertion in the F_2 mapping population

The two parents used in the cross had contrasting stigma exsertion rates, *viz*., 36.78 and 80.25 %, while the F_1 was lower than better parent and higher than recipient parent *i.e*., intermediate with 60.44 % stigma exsertion rate.

Frequency distribution of stigma exsertion in F_2 population

The frequency distribution of the stigma exsertion rate in the F_2 population showed a continuous variation. The total phenotypic data were divided as per the scale of IRRI. The scale of stigma exsertion plotted against X-axis and number of plants on Y-axis. The graph showed a bell-shaped normal distribution curve and skewed towards low stigma exsertion. The frequency distribution of stigma exsertion (**Figure 3**) indicated that both parents have several chromosomal regions increasing the frequency of stigma exsertion and the trait is affected by the environment. In the present cross transgressive segregants were observed in the F_2 population.

Genotyping

Of the 513 SSR markers screened for parental polymorphism between IR 68897B and BF-16B, only 124 (24.17 %) showed clear polymorphism. A set of 56 polymorphic SSR markers, evenly distributed on rice genome covering all 12 chrm and showing clear polymorphism between stigma exsertion recipient and donor parents were used for genotyping of 188 F_2 population.

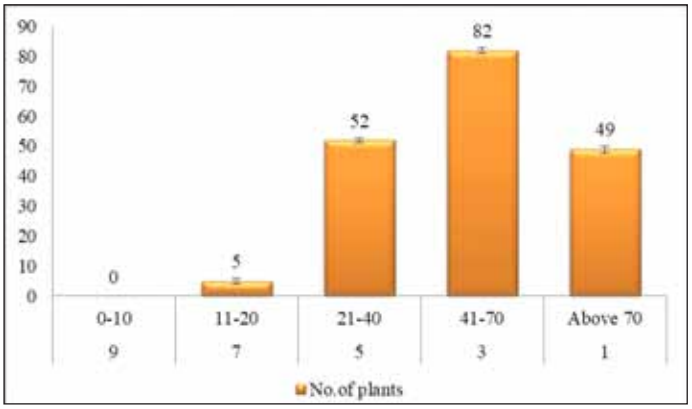

Figure 3: The graph dipicts the frequency distribution of stigma exsertion trait in 188 F_2 mapping population; 1, 3, 5, 7 and 9 the scale given by IRRI for phenotyping of stigma exsertion trait.

QTL mapping of stigma exsertion in F_2 mapping population

A total of 15 QTLs were identified for total stigma exsertion rate which included single, dual stigma exsertion using 56 polymorphic SSR markers with 188 F_2 mapping population and they were distributed on all 12 chrm except on chrm 1, 7, 9 and 10. The phenotypic variance and LOD value explained by each QTL ranged from 1.19 to 5.83% and 2.54 to 9.29, respectively. The majority of QTLs were contributed from the donor parent. On chrm 2, three QTLs were identified. The QTL, *qTSE2-1* was mapped at 24 cM and flanked by RM151 and RM12398 which accounts for 4.5% of the phenotypic variance with a LOD value of 3.75, the *qTSE2-2* was mapped at 129 cM, flanked

by markers RM13131 and RM208 and showed 4.94% of the phenotypic variance with a LOD value of 3.30 and the third QTL on chrm 2, *qTSE2-3* was flanked by RM208 and RM13238 at 218 cM map position and explained 4.98% of the phenotypic variance with 4.69 LOD value.

The QTL cluster *qTSE-3-1*, *qTSE-3-2* and *qTSE-3-3* on chrm 3 were flanked by RM1350-RM15466, RM1350-RM15466 and RM15466-RM7000 respectively and collectively explained 16.12% of the phenotypic variance with LOD value 6.83, 5.66 and 4.66 respectively.

A single QTL, *qTSE-4-1* was identified on chrm 4 at map position of 47 cM flanked by RM16338 and HRM17405 and explained 4.84 % of phenotypic variance with a LOD score of 3.5. On chrm 5, two QTLs, *qTSE-5-1 and qTSE-5-2* were identified at map positions of 117 and 184 cM flanked by the markers RM5592- HRM18222 and HRM18222-RM17950. Two QTLs *qTSE-5-1 and qTSE-5-2* on chrm 5 collectively accounted for 6.51 % of phenotypic variance with LOD scores of 2.54 and 2.59, respectively.

Two QTLs *qTSE-6-1 and qTSE-6-2* were identified on chrm 6 flanked by RM 20615-RM19569 with LOD value of 5.16 and 6.30 and explaining phenotypic variance of 1.19 and 5.76 %, respectively. Two QTLs *qTSE-8-1 and qTSE-8-2* on chrm 8 flanked by the markers RM22977-RM23643 and RM5493-RM6925 with LOD values of 3.88 and 3.77 and explaining 5 and 1.43 % of phenotypic variance, respectively. A single QTL *qTSE-11-1 was* detected on chrm 11 flanked by RM27183-RM26213 and explained 3.10 % phenotypic variance with LOD value of 9.29. A single QTL *qTSE-12-1* on chrm 12 flanked by RM7102-RM28157 and explained 4.28% phenotypic variance with LOD value of 2.72. The study detected contributing alleles for the trait were distributed in both i.e., donor and recipient parents. Eleven QTLs distributed on chrm 3, 4, 5, 6, 8, 11 and 12 from donor parent (BF-16B) and four QTLs present on chrm 2 and 8 were from recipient parent (IR 68897B). The QTL clusters identified on chrm 2(3 QTLs), 3(3 QTLs), 5(2 QTLs), 6(2 QTLs), 8(2 QTLs) collectively explained 14.42%, 16.12%, 6.51%, 6.95%, and 6.43%, respectively. The details of the putative QTLs for stigma exsertion were given in the **Table 4 and Figure 4.**

Table 4. Putative QTLs for total stigma exsertion detected in the F_2 population derived from IR 68897B and BF-16B

S.No.	QTL	Chr.	Position (cM)	Left Marker	Right Marker	LOD	PVE (%)	Add	Dom
1	qTSE-2-1	2	24	RM151	RM12398	3.75	4.50	0.87	21.17
2	qTSE-2-2	2	129	RM13131	RM208	3.30	4.94	1.31	21.46
3	qTSE-2-3	2	218	RM208	RM13238	4.69	4.98	4.23	-20.08
4	qTSE-3-1	3	28	RM1350	RM15466	6.83	5.19	-13.84	6.05
5	qTSE-3-2	3	94	RM1350	RM15466	5.66	5.83	-3.91	22.96
6	qTSE-3-3	3	153	RM15466	RM7000	4.66	5.10	-2.23	21.92
7	qTSE-4-1	4	47	RM16338	HRM17405	3.50	4.84	-3.86	-21.48
8	qTSE-5-1	5	117	RM5592	HRM18222	2.54	1.79	-7.98	-6.05
9	qTSE-5-2	5	184	HRM18222	RM17950	2.59	4.72	-0.35	21.37
10	qTSE-6-1	6	0	RM20615	RM19569	5.16	1.19	-1.02	-10.42
11	qTSE-6-2	6	58	RM20615	RM19569	6.30	5.76	-2.12	23.28
12	qTSE-8-1	8	27	RM22977	RM23643	3.88	5.00	-1.79	22.06
13	qTSE-8-2	8	155	RM5493	RM6925	3.77	1.43	0.16	12.45
14	qTSE-11-1	11	96	RM27183	RM26213	9.29	3.10	-10.42	3.97
15	qTSE-12-1	12	35	RM7102	RM28157	2.72	4.28	-1.10	20.05

PVE- Phenotypic variance explained by each QTL, LOD-Logarithm of odds ratio, qTSE-QTL for total stigma exsertion. Add-the additive effect of each QTL and dominant effect of each QTL

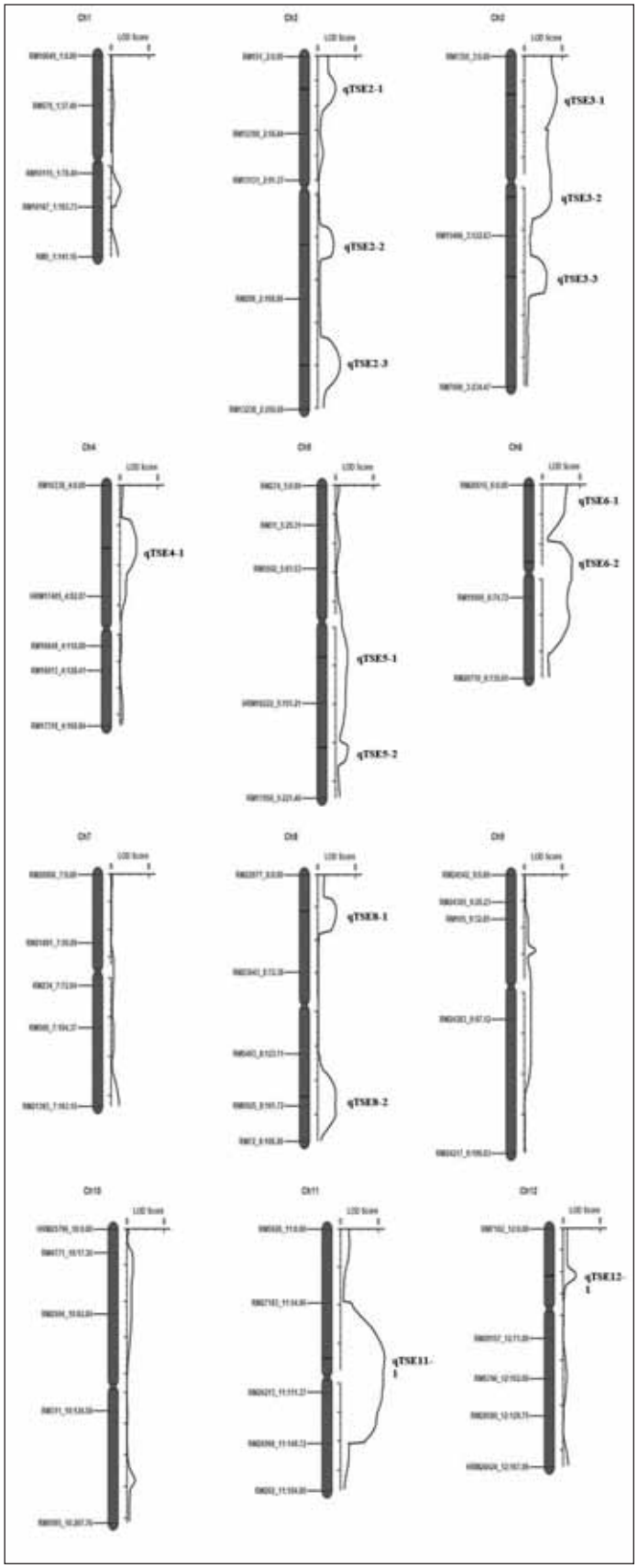

Figure 4: Distribution of QTLs for stigma exsertion trait on the linkage map

Breeding for hybrid rice is one of the pragmatic solutions for addressing the problem of food shortage, caused by marked increase in - global population with decreasing trend in available resources, land and water for agriculture. Breeding approaches may solve this problem by increasing yield per unit area through hybrid system of breeding. But the large scale adoption of hybrid rice has been hampered by low F_1 seed set and the consequent high price of hybrid seed. To overcome the extremely low seed set percentage of CMS line, there is a need to improve the parental lines for flowering behavior and floral traits and modifications in seed production practices are useful for pushing seed yields beyond 2.5 tonnes per hectare and also bring down the F_1 seed cost.

In our study, transgressive segregants were observed in both the directions signifying complementary action of the genes with additive effect that were dispersed in both the parents and a normal distribution for the phenotype was observed. According to Singh and Narayanan (1993) transgressive segregations are only possible from the crosses between two parents with mean values for a quantitative trait. Such segregants are not possible in case of qualitative traits. De Vicente and Tanksley, (1993) studied that the transgressive segregation is commonly observed in the population segregating for quantitative trait. Whereas Ricks and Smith (1953), mentioned that transgressive segregation in certain progeny is because of accumulation of complementary alleles at multiple loci inherited from the two parents.

These results were in conformity with the results of Virmani and Athwal, (1974), Li and Chen, (1985), Wang *et al*., (2008), Ling *et al.,* (1989), Miyata *et al*., (2007), Lou *et al*., (2014), Li *et al*., (2014) and Sruthi *et al*., (2014) who observed wide continuous variation with normal distribution and concluded that the stigma exsertion trait of rice is a quantitative trait, controlled by polygenes. Similarly, in tomato, stigma exsertion has been reported to be a quantitative trait and controlled by a few genes (Rick and Dempsey, 1969; Scott and George, 1978; Levin *et al*., 1994). However, earlier reports on the genetics of stigma exsertion in rice showed contradiction to the present finding. For instance, Hassan and Siddiq, (1984)

reported that fully exserted stigma to be monogenic and dominant over partially exserted stigma. Xu and Shen, (1987) also studied the inheritance of the stigma exsertion trait and reported that it was a dominant trait and in their study F_2 population showed continuous variation for stigma exsertion, this may be due to some minor genes supplementing the major gene in the expression of the trait. Experimental evidence suggested that environmental effects would also produce a continuous variation even if the number of genes governing a character was very small or even one.

We constructed 188 lines of the F_2 mapping population using IR68897B x BF-16B cross and genotyped these 188 lines with 56 polymorphic molecular markers to identify the QTLs for the trait of interest (stigma exsertion). Results showed that 15 QTLs were associated with stigma exsertion traits and were located on chrm 2, 3, 4, 5, 6, 8, 11 and 12. The phenotypic variance and LOD value explained by each QTL ranged from 1.19 to 5.83% and 2.54 to 9.29 respectively. The present QTL analysis results for stigma exsertion were in accordance with the result of Tan *et al*., (2020). They mapped the major QTLs for stigma exsertion rate from *Oryza glumaepatula* on chrm 1 and 3. Xu *et al.*, (2019) identified a major QTL on chrm 3 using double haploid population. Lou *et al*., (2014) identified a total of 5 QTLs and 9 epistatic QTL pairs were found to associate with the stigma trait in F_2 population of Huhan 1b and K17B. Li *et al.*, (2001) reported QTL on Chrm 5 and 8 using back cross-population of Dongxiang and Guichao 2. Li *et al.*, (2001) identified QTLs on chrm 2 and 3 using DH population of Zaiyeqing 8 and Jingxi 17. Uga *et al.*, (2003) identified QTL for the rate of stigma exsertion on chrm 5 and 10 using RILs of Pei-Kuh and a wild rice W1944 and Deng *et al*., (2011) observed on chrm 1, 2, 3 and 5 using F_2 population of (CMS) maintainer line II-32B and G46B. Miyata *et al*., (2007) reported QTLs for stigma exsertion on chrm 3 using F_2 population of Koshihikari and IR24 and Yue *et al*., (2009) demonstrated on chrm 3, 4, 7 and 9 using F_2 population of CMS maintainer line Huhan 1B and II-32B. Wang *et al*., (2008) studied high SE in chrm 2, 5 and 8 by introducing the genes of high stigma exsertion rate from *indica* into *japonica*.

The National plant biology symposium proceeding in China (2011) confirmed the QTLs for PES (percentage of exerted stigma) to be present on all chrm except on chrm 7. The studies on stigma exsertion by Li *et al*., (2014) reported the presence of the trait in chrm 3, 6, 7, 9, 10 and 12 using RIL population of ZX and CX29B. Lou *et al*., (2014) also reported the presence of PSE in chrm 5 and 6, 7 using F_2 population of CMS lines, Huhan1B and K17B, which are similar to the results of the present study. Li *et al*., (2014) resolved exserted stigma determined by a few main and epistatic pairs QTLs, indicating mainly influenced by minor QTLs in three-way cross/backcross population of Zhenshan 97B and 9311. In Rahman *et al*., (2016) studies showed a total of two QTLs for TSE located on chrm 1 and 11 in two environments and QTL on chrm 11 is novel to date for exserted stigma in our result also.

In addition, results detected contributing alleles for trait were distributed in both i.e., donor and recipient parents. Eleven QTLs distributed on chrm 3, 4, 5, 6, 8, 11 and 12 from BF-16B and four are on chrm 2 and 8 from IR 68897B. Previous studies (Rahman *et a.*, 2016; Li *et al*., 2014; Yamamoto *et al.*, 2003; Yu *et al*., 2006) reported that favorable alleles present and contribute to trait expression by both the parents. Hence, for improvement of exserted stigma trait it is very much essential to select suitable breeding program for pyramiding of traits in parental lines from different sources.

In the present study one QTL cluster has been identified on chrm 3 flanked between RM 1350 and RM 7000 for TSE, it collectively explaining 16.12 % phenotypic variance. Earlier also reported by Lou *et al*., (2014) for PDES on chrm 5 and by Yu *et al*., (2006), mapped the region by Li *et al*., (2001) and Uga *et al*., (2003b). Li *et al*., (2014) also detected two QTLs clusters on chrm 1 and 6 for extruded stigma.

Many QTL identified by different researchers for stigma exsertion rate (SER), Percentage of exserted stigma (PES), Percentage of single exserted stigma (PSES) and Percentage of double exserted stigma (PDES) in rice. In the present investigation, 15 QTLs were identified, of which 11 were contributed from

the donor parent (BF-16B) and 4 from the recipient parent (IR 68897B) for stigma exsertion to the progeny. Both the parents contributed towards the stigma exsertion. However, more number of QTLs was contributed by the donor parent to the progeny for total stigma exsertion (TSE) in *indica* rice. All the15 QTLs identified for total stigma exsertion in this study were minor affect QTLs accounting for less than 10 % of the phenotypic variance. This clearly indicates the polygenic nature of the trait controlled by several genomic regions with small effects. Stigma exsertion is highly influenced by environment which might be affecting the QTL expression. In general, QTLs with large effects are more stable across environments than QTLs with smaller effects. stigma exsertion is controlled by many genes and has low heritability. Hence, it is advisable to phenotype the trait in multiple environments in order to identify the stable QTLs. In the present study, the markers flanked by the QTLs can be utilized to screen the uncharacterized germplasm for stigma exsertion trait to identify new donors and can be used in marker assisted breeding programmes to improve maintainer lines. These maintainers can be converted into new CMS lines with improved stigma exsertion rate to increase hybrid seed production efficiency. The QTLs identified in the present study need validation before they can be utilized in Marker-Assisted breeding programs for improvement of the maternal parent in hybrid rice technology. This may exsertion help in overcoming the pollination barriers like time (asynchronous flowering) and will allow accumulation of more pollen on stigmatic surface of female parent improving the hybrid seed production efficiency.

Authors Contribution

Conceptualization of research (KKB, SMM & ASH): Designing of the experiments (KKB, SMM & ASH): Contribution of experimental material (Barwale Foundation): Execution of field /lab experiments and data collection (GS & JK): Analysis of data and interpretation (GS, JK, KKB, SMM & SK): Preparation of the manuscript and correction (GS, JK, KKB, SMM, SK, VS, SJ, AKS, PK, LS, SP, GC, AMS, RMS & ASH).

Compliance with Ethical Standards: The authors declare the research work is in Compliance with Ethical Standards

Conflict of Interest: The authors declare they have no conflicts of interest.

Ethical approval: This article does not contain any studies with human participants or animals performed by any of the authors.

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