Parental polymorphic marker survey and genetic diversity studies among the popular maintainer lines of hybrid rice (Oryza sativa L.) for stigma exsertion trait

Abstract

Parental polymorphism survey is a pre-requisite for genotypic screening to identify the loci associated with trait of interest among mapping population. In the present study, eight popularly used rice maintainer lines viz.,APMS-6B (Improved for Bacterial leaf blight (Yugander Arra, et al.,2018), IR58025B, IR68897B, IR79156B, DRR-6B, DRR-9B, BF-16B and BF2096B were used to study stigma exsertion trait as a single, double and total stigma exsertion. The parental polymorphism survey has been carried out by using rice microsatellite (RM) simple sequence repeat (SSR) markers which predominantly employed in rice marker assisted selection. A total of 630RM markers were used to study parental polymorphism among eight maintainer lines and also to map their association with stigma exsertion trait. Among 630, 253RM markers showed polymorphism among the eight maintainers which are distributed across twelve chromosomes of rice. The overall parental survey revealed 40.18per cent of polymorphism among the maintainer lines. Number of alleles detected by 253 polymorphic markers was 635 with a maximum and minimum frequency of 5 and 2 alleles, respectively. The maximum and minimum polymorphic information content values were found to be 1.00 and 0.219 for the primers HRM25754, RM258 and RM10209, respectively. The genetic similarity coefficient for the most number of pairs ranged between of 0.2-0.9 with the average value of 0.60 for all possible combinations, indicating moderate genetic diversity among the chosen genotypes. Analysis of variance revealed the presence of considerable amount of variability among the maintainers. Based on cluster analysis, the maintainers were grouped into three clusters of which cluster II was the largest consisting of four maintainers with 0.57% of similarity. While, clusters I and III were the smallest with only two maintainers with 0.61% and 0.47% similarity~~ each other~~. The genotypes grouped according to their place of origin and represents genetic closeness between them. The identified RM polymorphic markers could be used to construct the linkage map and subsequently, to identify the stigma exsertion related QTLs from mapping population developed from different combinations of the rice maintainer lines.

Key words

Simple sequence repeats (SSR), Parental polymorphism, Diversity, Rice maintainer lines, Stigma exsertion, Marker assisted selection

Introduction

Rice (Oryza sativa L.) is the staple food for more than half of the world’s population USDA (2016) and it occupies 23 per cent of the total area in the world under cereal production. Rice is a model cereal crop due to its small genome size, around 430 Mb (2n = 24) which is one-tenth of the human genome (Kurata et al., 2002).

The development of hybrid rice breeding technology involves improvement and evaluation of parental lines, evaluation of the degree of heterosis for yield and techniques for seed production. Customarily to produce hybrids on a commercial scale, it is essential to change the function of male and female reproductive systems of rice plants.

The low yield of F_1seed production, and thus the availability of F_1 seed at reasonable prices, had been cited as a major constraint to the wide adoption of hybrid rice in countries outside china (Nguyen 2000, Tran 2002, Virmani 2003). The availability of affordable hybrid seeds to farmers is crucial to the success of hybrid rice commercialization since farmers have to use fresh hybrid seeds in each crop season. The low yield of hybrid seed production and high cost of hybrid ~~rice~~ seeds have been the main complaints raised by seed producers and farmers, and were listed as one of the major limitations in Asian countries for hybrid seed extension.

In self pollinated crops like rice, hybrid breeding appeared to be difficult, as the floral traits are unfavorable for out crossing. Use of male sterility system has immensely helped in hybrid breeding. ~~(~~Virmani. 1994~~)~~ reported that out crossing is influenced by many floral traits like size of pistil and stamen, stigma exsertion, angle of glume opening. Among them, stigma exsertion is emphasized as a major component in increasing pollination and seed set (Sheeba et al., 2006). Stigma exsertion is an important trait that contributes to the improvement of seed production in hybrid rice and is closely related to seed productivity in hybrid rice (Takano-Kai et al., 2011). Previous studies have demonstrated that the stigma exsertion rate of the male sterile line, the female parent in production of hybrid rice, is a key factor contributing to the efficient improvement of hybrid seed production, since exserted stigmas remain viable upto about 4 days and could continue to accept pollens (Li et al., 2004; Tian et al., 2004). A male sterile line with high stigma exsertion rate is expected to trap more pollen, thus improving the efficiency of hybrid seed production. With an increase in the efficiency of stigma exsertion in male sterile lines of hybrid rice, the seed- setting rate in hybrid seed production and the yield of hybrid seed also increased (Zetian and Yanrong., 2010).

Previous studies have shown that stigma exsertion is controlled by quantitative trait loci (QTL) and affected by environmental conditions. Several QTLs have been identified for stigma exsertion trait in different rice materials. The wild rice (*Oriza rufipogon*) often has large exserted stigmas, two QTLs were identified for rate of exserted stigma (qRES-5 and qRES-10) between the indica line Pei-kuh and the common wild rice accession W1944 (Uga et al., 2003). *O. longistaminata* is allogamous species, with a self-incompatibility system, and shows the extreme maximum values of stigma and anther length and number of pollen grains within the sativa species group (Oka and Morishima, 1967). This can be utilized as a genetic resource in breeding programme to introgress few of its allogamous floral traits in ~~to~~ *O.sativa*. There were ~~–~~three QTLs identified on chromosomes 2, 6, and 8~~,~~ for stigma exsertion rate (SER) in a cross between indica cultivar Guangluai-4 and the wild rice accession W1943 (Huang et al.,2012). Two QTLs were identified for percentage of exserted stigma (qPEST-5 and qPEST-8) in a cross between Dongxiang wild rice and the indica cultivar Guichao 2 (Li et al., 2001). In general, indica rice has longer and more exserted stigmas than japonica rice. Nine QTLs for frequency of stigma exsertion were detected in recombinant inbred lines (RILs) derived from a cross between japonica cultivar Asominori and indica cultivar IR24 and further identified a major QTL for exserted stigmas, qES3, in the same genomic region as the GS3 (Grain Size 3) gene on chromosome 3(Yamamoto et al., 2003 and Miyata et al., 2007). Recently, 11 QTLs identified for SER in a genome-wide association study (GWAS) of 217 indica CMS lines, and 23 genomic loci that significantly affected SER among diverse rice accessions (Guo et al., 2017 and Zhou et al., 2017). Transgenic experiments had shown the pleiotropic effect of GS3 on stigma length and exsertion (Takano-kai et al., 2011) detected that complementation of Asominori (with a gs3 allele from IR24) with the GS3 of Nipponbare reducing the length of the nonbrush- part of the stigma and the frequency of stigma exsertion. These results shows that the stigma exsertion trait is complex and controlled by many genes and that different rice material may carry different QTLs for stigma exsertion rate.

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Material and Methods

In this study, eight hybrid rice maintainer lines APMS-6B (Improved for Bacterial leaf blight and Blast), IR58025B, IR68897B, IR79156B, DRR-6B, DRR-9B, BF-16B and BF2096B were used as parents. APMS6B: A maintainer line of APMS6A ,which is a female parent of popular medium slender Indian rice hybrid DRRH3 with good general combining ability, medium slender grain type and medium (106 days) duration. This line has been bred from APRRI, Maruteru and improved for bacterial leaf blight and blast at ICAR-IIRR, Hyderabad. (Yugander et al., 2018~~(BLB)~~). IR68897B: A maintainer line of IR68897A, which is a female parent of early maturing rice hybrid DRRH2 suitable to develop early duration (90-95 days) long slender grain type hybrids. IR58025B: A maintainer line of IR58025B, which is a female parent for a number of popular hybrids KRH2 and DRRH1, Sahyadri, CORH2, with long slender grain type and IR79156B: A maintainer line of IR79156A, with long slender grain type and medium duration (101-104 days) good outcrossing ability. DRR-9B: A medium slender grain and early maturity with moderate stigma exsertion maintainer line of DRR-9A. DRR-6B: A medium slender grain and early maturity with moderate stigma exsertion maintainer line of DRR-6A. BF-16B: Improved maintainer line with good stigma exsertion with medium bold grain type. BF-2096B: Improved maintainer line with good stigma exsertion with medium bold grain type (Fig. 1)


APMS6B
IR68897B
IR58025B
IR79156B
DRR-9B
BF-16B
BF2096B

Fig1: Figure showing the panicle and grain type of various maintainer lines used in the study

Genomic DNA extraction

Genomic DNA from the fresh leaves of the eight genotypes was extracted by cetyl-trimethyl ammonium bromide method (CTAB) as described (Rajendrakumar et al., 2007).The quality and quantity of extracted DNA was estimated through agarose gel electrophoresis (Alpha Imager UV gel documentation system, M/s Alpha Innotech Corporation, USA) and NanoDrop(ND100 spectrophotometer, NanoDrop Technologies Inc., USA), respectively. DNA samples with 260/280 ratio between 1.8 -1.9 were used for PCR to study parental polymorphism.

Primers used in the study

For studying the parental polymorphism among eight maintainer lines viz., APMS-6B (Improved for Bacterial leaf blight), IR58025B, IR68897B, IR79156B, DRR-6B, DRR-9B, BF-16B and BF2096B, total 630 SSR markers were used. The information regarding chromosomal location and sequences of primers were obtained from www.gramene.org.

PCR analysis

The polymerase chain reaction (PCR) was carried out in thermal cycler (Applied Bio systems, USA) using 630 SSR markers. The PCR reaction mix includes the following: 20-50ng of genomic DNA, 1x Buffer (containing 1.5 mM $MgCl_2$), 125 µM of dNTPs, 0.2 µM of each (forward and reverse) primer and 0.5 unit of Taq DNA polymerase (Bangalore Genei, India). The PCR profile was included with initial denaturation at 94˚C for 5 min followed by 35 cycles (denaturation at 94˚C for 30 s + annealing at 55˚C for 30 s + extension at 72˚C for 1 min) and the final extension at 72˚C for 5 min. The PCR amplicons were resolved in a 3% agarose gel prestained with ethidium bromide in 1X TAE (40mM Tris-acetate and 2mM EDTA pH ~8.0) buffer. The electrophoresed products were visualized under UV light and documented using Alpha lmager Documentation System (M/s Alpha Innotech, USA).

Results and Discussion

The ratio of UV absorbance at OD260/OD280 ranged between 1.8-1.9, and hence DNA samples were rated as good and standard. The quantity of DNA in the isolated samples ranged from 1100 to 1500ng/µl. Parental polymorphism at the molecular level was determined by genotyping them with SSR markers. The parental polymorphism survey indicated that a clear polymorphism was observed among the parents. A total of 630 SSR markers mapped on all the 12 chromosomes (Fig. 3) including 8 reported markers (Table 2 & Fig 5) for stigma exsertion trait and highly variable rice microsatellite markers (HRM) were used for testing polymorphism among the parents. The 8 reported markers and their chromosome number and physical position on chromosome given in table. 2. Out of 630 markers 253 SSR primer pairs were exhibited polymorphism among the eight parents and remaining 377 primers were monomorphic. Percentage of polymorphism highest (Table 3) on chromosome 1 (57.14) and least on chromosome 7 (17). Out of 8 reported markers (Yan et al. 2009), 4 markers were showing minor polymorphism among eight maintainers, they are RM3642 on chromosome 1 for DSE, RM5 on chromosome 1for SSE & DSE, RM105 on chromosome 9 for SSE & TSE, RM25669 on chromosome 10 for SSE & TSE. The reported markers ~~form~~ from the earlier study (Yan et al. 2009) did not work well with the present set of genotypes. This might be because of novel regions contributing for the stigma exertion trait. The average per cent of polymorphism on all the chromosomes was 43.39. The lack of detectable polymorphism among the eight parents would be due to the fact that all the parents are indica lines. Lack of molecular marker polymorphism among the indica genotypes has been earlier noticed in studies by Xu et al., (2002) and Biradar et al., (2004).

Parental line phenotyping for stigma exsertion traits

The 253 rice microsatellite (SSR) markers identified as polymorphic among the eight parents ~~(APMS 6B (Improved for Bacterial leaf blight), IR58025B, IR68897B, IR79156B, DRR 6B, DRR 9B, BF 16B and BF2096B)~~ will be useful as a pointer to the existence of different alleles at each of the loci. As the parents differ from each other with respect to stigma exertion traits (e.g., single stigma exsertion (SSE), double stigma exsertion (DSE), Total stigma exsertion (TSE) and no stigma exsertion (NSE)).Based on the mean values of different genotypes, the highest mean value of 80.25% of total stigma exsertion was performed by BF16B, ~~immediately~~ followed by BF2096B with 78.03%, and DRR6B with 61.46% for TSE. The minimum or lowest mean value was recorded for APMS6B with 14.97%, followed by IR68897B (36.78%) and DRR9B (42.46%) (Table.1 and Fig.2).

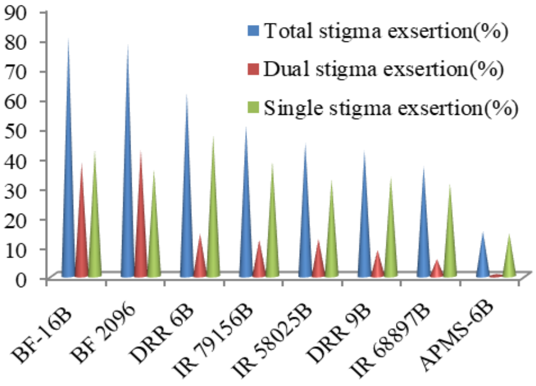

Fig 2: The chart depicting the total, dual and single stigma exsertion percentages for various maintainer lines

Comparison of these mean values using the LSD value (7%) indicated that BF16B and BF2096B did not differ significantly whereas the difference between BF16B and APMS6B was significant, with the highest and lowest mean values performance. APMS6B recorded the lowest mean value for DSE (0.67%) and for SSE (14.3%). BF2096B had shown maximum mean value for DSE (42.34%), for SSE (35.69%). BF16B was shown second highest mean value with 38.04% for DSE, where the highest mean value for SSE with 42.21%. DRR6B, IR79156B and IR58025B were performed the moderate mean values for TSE with 61.46%, 50.54%, and 44.86%, respectively. ~~Individual proportions of TSE, DSE, and SSE were shown in various studies.~~ The genotypes BF16B, BF2096B, DRR6B, and IR79156B had recorded more than 50% for TSE, where as all the other genotypes of mean values were below 50% for DSE and SSE. The genotypes DRR6B, IR79156B, IR58025B, DRR9B, IR68897B and APMS6B have exhibited less than 25% DSE and where as BF2096B and BF16B have exhibited more than 50%. Further, the association of identified polymorphic markers to stigma exsertion trait can be studied through QTL mapping.

SSR Polymorphism among maintainer lines of hybrid rice

All the 8 maintainer lines of hybrid rice were genotyped with 630 microsatellite markers (Fig 4); and were selected for their ability to produce amplified product at optimum concentration, polymorphism level among the maintainers and consistency of the pattern. Out of 630 microsatellite markers, 377 were found to be monomorphic revealing one allele at each locus in all the genotypes. Total 635 alleles were scored from these 253 polymorphic primer pairs, and 40.63 percent of the total number of markers was found to be polymorphic. The banding pattern of different polymorphic markers among 8 genotypes of maintainer lines is shown in Fig 4 & 5. The respective values for overall genetic variability for polymorphism information content, resolving power (RP), major allele frequency, percentage of polymorphism, number of alleles across all the 8 genotypes are given in Supplementary table 1. Highest PIC value (1) was observed for the primer HRM25754, RM258 and lowest PIC value (0.219) was recorded for the primer RM10209 (Supplementary table 1) with an average of 0.503. The percentage of polymorphism values ranged from 100 to 62.50 with an average of 98.17. The resolving power (RP) is a feature of marker that indicates the discriminatory potential of the primer. RP ranged from 1 to 0.250 with an average of 0.600 for polymorphic marker. In case of polymorphic markers the major allele frequency ranged from 0.156 to 0.781 with an average of 0.501 (Supplementary table 1). The allele number per locus varied from 2 to 5 with an average of 3 alleles per locus (Supplementary table 1).

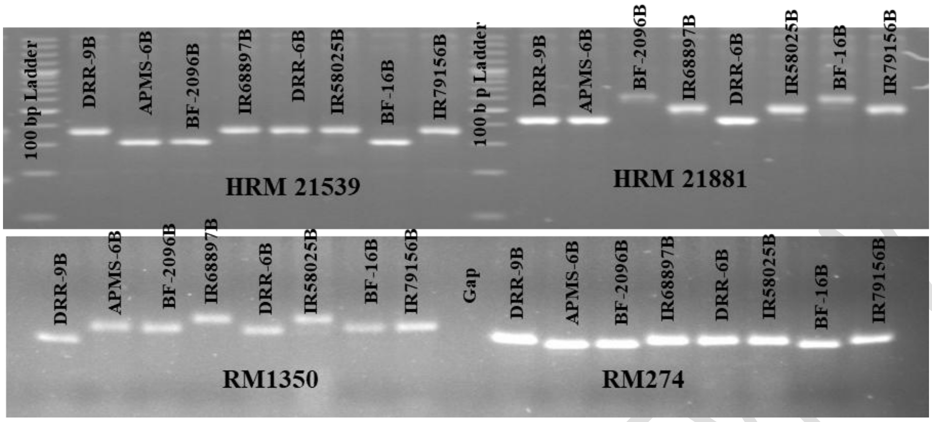

Fig. 4. Gel picture showing the polymorphic banding pattern of highly variable SSRs (HRM) and SSRs (RM) among eight maintainer lines

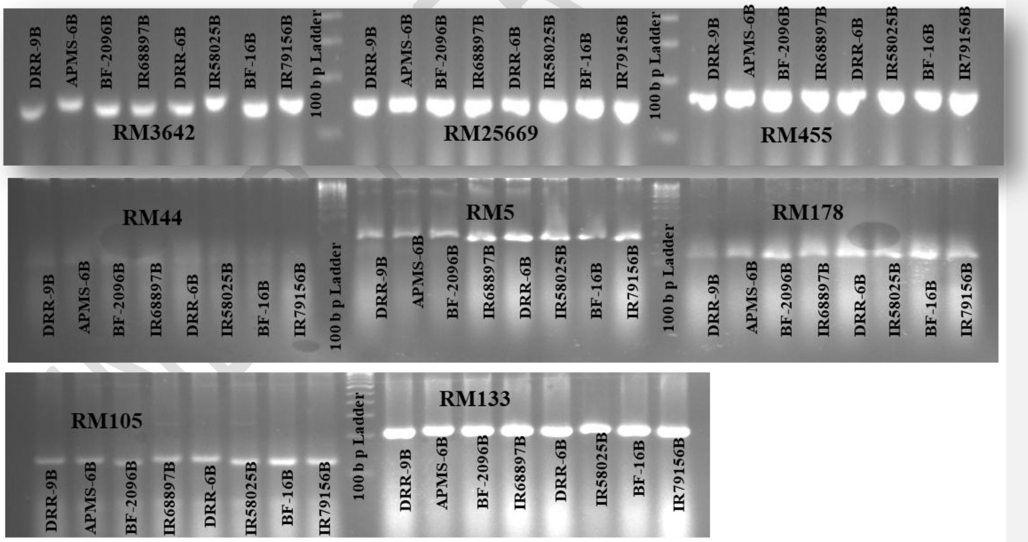

Fig. 5. Gel picture showing the banding pattern of reported markers (Yan et al., 2009) among 8 maintainer lines

Genetic relationship

To find out the genetic relationship between different maintainer rice genotypes, SSR data were used for analysis using NTSYSpc version 2.02e. The genetic similarity coefficients found in the genotype comparison matrix were relatively moderate. A dendrogram was constructed to understand the diversity among eight popularly used maintainer lines using genotypic data of 253 polymorphic markers. The cluster analysis was performed using UPGMA method on the basis of Jaccard's coefficients with one possible tie found between the closest pairs. The neighbour-joining tree based on all SSR fragments grouped eight germplasm accessions into three major clusters (Fig 6.)

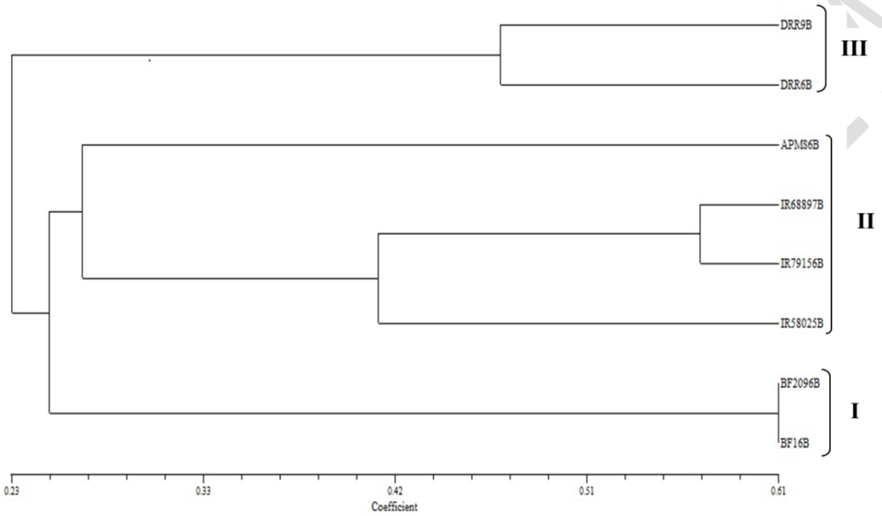

Fig 6: Dendrogram depicting the diversity among eight maintainer lines

The first cluster (P1) is formed between two accessions BF16B and BF2096B evincing 0.61% similarity with each others. Where these two B lines developed by Barwale foundation with higher stigma exsertion percentages. The other phenon (P2) is constructed between IR79156B and IR68897B showing mere 0.57% similarity with P1 and joined with IR58025B with a much higher distance. These three B lines developed at IRRI, Philippines and grouped under one cluster. The three B lines connected with other B line APMS6B where this line bred at APRRI, Maruteru with medium slender grain type. The third cluster (P3) is of two accessions of DRR6B and DRR9B at 0.47% similarity. These lines were developed at ICAR-IIRR, Hyderabad. The genotypes grouped according to their place of origin and represents genetic closeness between them.

In the present study, 253 out of 630 markers were polymorphic and produced unique allelic profiles for the 8 maintainer rice genotypes. In total 635 alleles were detected among 8 rice genotypes with an average number of 3 alleles per locus and an average polymorphism information content (PIC) of 0.503, an average percentage of polymorphism 98.17, average resolution power (RP) of 0.600 and an average major allelic frequency of 0.501. The genetic diversity observed in the present study is similar to earlier studies (Babu BK, et al., 2014), they detected 4.8 alleles per locus and an average PIC value of 0.50. Three alleles per locus with an average PIC value of 0.41 among 88 Indian rice varieties collected from different agro-climatic regions of India were also reported (Yadav S, et al., 2013). Similarly, the average PIC values of 0.405, an average RP values of 1.01, the average values of major allelic frequencies of 0.74, an average number of 3 alleles per locus detected among the 141 basmati rice accessions were also reported (Salgotra RK,et al., 2015). Similarly, the average PIC value of 0.44 was observed among 43 Thai and 57 IRRI germplasm of rice (Chakhonkaen S, et al., 2012). In another study, an average PIC value of 0.45 was observed among the 183 Indonesian rice landraces on the Islands of Borneo (Michael JT, et al., 2009). A slightly lower genetic diversity was reported with an average of 2.75 alleles per locus and average PIC value of 0.38 among 40 rice accessions of Pakistan (Shah SM, et al., 2013). Similarly, a lower SSR diversity was also observed in a study with 36 polymorphic HvSSRs in which they detected 2.22 alleles per locus and an average PIC value of 0.25 in 375 Indian rice varieties collected from different regions of India (Singh N, et ai., 2013).

The dendrogram showed that all eight maintainer lines were grouped into three major clusters (Fig 6). The genotypes were well clustered based on their place of collection and geographical region. The genotypes from Barwale foundation BF-16B and BF-2096B were grouped in cluster I. Similarly, the genotypes from IRRI, IR79156B, IR68897B, IR58025B and APMS-6B from APRRI Maruteru were clustered in cluster II and genotypes from IIRR, DRR-6B and DRR-9B were grouped in cluster III. Thus, most of the IRRI maintainer lines were clustered in cluster II suggesting moderately less genetic diversity among these genotypes. It is because of similar breeding material were used for the development of these genotypes or in other words they have same ancestry. APMS-6B was distant in dendrogram.~~ This is ~~because of different types of material have been used for the breeding of this genotype.

Recently (Sruthi et al.,2016) Rice microsatellite (RM) markers were used to study the parental polymorphism between the selected two parents APMS-6B a popularly used maintainer line with low stigma exsertion (14.95%) and BF-16B, another maintainer line with high stigma exsertion

(80.25%). The two parents were screened for parental polymorphism using 454 SSR markers, of which 118 markers exhibited polymorphism. The overall polymorphism level for the surveyed SSR markers was 25.99% across the 12 chromosomes. (Miyata et al., 2007) identified the major QTLs for stigma exsertion rate in F2 mapping population using 269 polymorphic SSR markers by crossing Koshihikari / 98SQ1496 of japonica rice genetic background and the population size of 150 segregating plants. Similarly, (Li et ai., 2014b) mapping of minor QTLs for stigma exsertion rate in 225 NILs population using 171 SSR polymorphic markers derived from a cross between ZX and Cx29B. Similarly, (Shouling Xu et al., 2019) Identified a major QTL and its candidate gene for stigma exsertion trait on chromosome 3 in F_3 mapping population using 307 SNPs and 27 Indels by crossing ZS616 [*Oryza sativa* subsp. Xian (indica)], a male sterile line with a stigma exsertion rate (SER) as high as 94.5%, was crossed to DS552, a japonica line with almost no exserted stigmas.

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The 253 rice microsatellite (SSR) markers identified as polymorphic among the eight parents in this study and it will be useful as a pointer to the existence of different alleles at each of the 253 marker loci.

The screening of markers for parental polymorphism among the rice cultivars forms the basis for tagging of the desired gene, fine mapping of the gene in the rice chromosome and in the subsequent Marker assisted selection (MAS) programmes. The polymorphic rice markers can be used in the fine mapping of the stigma exsertion trait and to study the mapping populations of crosses obtained from these parents.

Conclusion

QTL analysis of various stigma exsertion related traits can be performed by utilizing these ready to use polymorphic markers along with mapping population developed from the crosses among the eight genotypes. These polymorphic markers can be used for background selection of these combinations during marker assisted breeding programmes. Moreover, these identified polymorphic markers can be used for diversity analysis and linkage analysis for various traits in rice.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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Table 1. Table showing the stigma exsertion percentage of eight maintainer lines used in the study

S.No.	Genotype	Total stigma exsertion(%)	Dual stigma exsertion(%)	Single stigma exsertion(%)
1	BF-16B	80.25	38.04	42.21
2	BF 2096	78.03	42.34	35.69
3	DRR 6B	61.46	14.2	47.27
4	IR 79156B	50.54	12.15	38.4
5	IR 58025B	44.86	12.44	32.42
6	DRR 9B	42.26	8.78	33.48
7	IR 68897B	36.78	5.72	31.06
8	APMS-6B	14.97	0.67	14.3

Table 2. List of reported markers (Yan et al., 2009) for stigma exsertion trait employed for polymorphism study

Sl.No.	Marker	Chromosome number	Position (cM)	Traits
1	RM5	1	98.5	SSE & DSE
2	RM3642	1	102.3	DSE
3	RM178	5	104.4	DSE & TSE
4	RM133	6	0.5	SSE
5	RM455	7	78.9	DSE & TSE

6	RM44	8	46.9	DSE
7	RM105	9	7.8	SSE & TSE
8	RM25669	10	55.2	SSE & TSE

Table 3.Chromosome wise percentage of primers showing polymorphism among the parental lines

Sl. No.	Chromosome	Total markers used on each chromosome	Polymorphic markers on each chromosome	Number of monomorphic primers on each chromosome	%of primers showing polymorphic on each chromosome
1	Chromosome-1	49	28	21	**57.14**
2	Chromosome-2	37	19	18	51.35
3	Chromosome-3	44	22	22	50.00
4	Chromosome-4	45	20	25	44.44
5	Chromosome-5	35	18	17	51.42
6	Chromosome-6	45	18	27	40.00
7	Chromosome-7	100	17	83	**17.00**
8	Chromosome-8	57	30	27	52.63
9	Chromosome-9	39	16	23	41.02
10	Chromosome-10	47	22	25	46.80
11	Chromosome-11	65	26	39	40.00
12	Chromosome-12	67	17	50	25.37
	Total	630	253	377	43.09

Supplementary table-1. Marker analysis: Major allele frequency, Polymorphic information content, number of alleles, percentage of polymorphism and Resolving Power

Sl.No	Marker	Chro. No	Major allele frequency	PIC	No. of Alleles	Percentage of Polymorphism	Resolving Power(RP)
1	HRM11099	1	0.469	0.531	3	100.00	0.500
2	HRM10936	1	0.625	0.375	2	100.00	0.500
3	HRM11111	1	0.469	0.531	3	100.00	0.500
4	HRM10167	1	0.406	0.594	3	100.00	0.667
5	HRM11114	1	0.313	0.688	4	100.00	0.500
6	RM151	1	0.625	0.375	2	100.00	0.500
7	RM562	1	0.375	0.625	3	100.00	0.667
8	RM580	1	0.594	0.406	3	100.00	0.333
9	RM 18	1	0.625	0.375	2	100.00	0.500
10	RM10843	1	0.500	0.500	2	100.00	1.000
11	RM10720	1	0.625	0.375	2	100.00	0.500
12	RM580	1	0.625	0.375	2	100.00	0.500
13	RM8068	1	0.469	0.531	3	100.00	0.500
14	RM579	1	0.500	0.500	2	100.00	1.000
15	RM10649	1	0.406	0.594	3	100.00	0.667
16	RM10018	1	0.531	0.469	2	100.00	0.750
17	RM1247	1	0.297	0.703	3	87.50	0.583
18	RM10209	1	0.781	0.219	2	100.00	0.250
19	RM6289	1	0.500	0.500	2	100.00	1.000
20	RM600	1	0.344	0.656	3	100.00	0.667
21	RM595	1	0.625	0.375	2	100.00	0.500
22	RM129	1	0.625	0.375	2	100.00	0.500
23	RM11307	1	0.281	0.719	4	100.00	0.500
24	RM246	1	0.469	0.531	3	100.00	0.500
25	RM5536	1	0.531	0.469	2	100.00	0.750
26	RM6840	1	0.781	0.219	2	100.00	0.250
27	RM5	1	0.406	0.594	3	100.00	0.667
28	RM3642	1	0.625	0.375	2	100.00	0.500
29	HRM13155	2	0.500	0.500	2	100.00	1.000
30	HRM13238	2	0.406	0.594	3	100.00	0.667
31	HRM12690	2	0.625	0.375	2	100.00	0.500
32	HRM13867	2	0.266	0.734	3	87.50	0.583
33	HRM12983	2	0.500	0.500	2	100.00	1.000
34	HRM13659	2	0.203	0.797	2	62.50	0.625
35	HRM13154	2	0.531	0.469	2	100.00	0.750
36	HRM12469	2	0.469	0.531	3	100.00	0.500
37	RM263	2	0.531	0.469	2	100.00	0.750

38	RM208	2	0.500	0.500	2	100.00	1.000
39	RM6843	2	0.469	0.531	3	100.00	0.500
40	RM12368	2	0.531	0.469	2	100.00	0.750
41	RM279	2	0.344	0.656	3	100.00	0.667
42	RM6375	2	0.469	0.531	3	100.00	0.500
43	RM6509	2	0.500	0.500	2	100.00	1.000
44	RM573	2	0.531	0.469	2	100.00	0.750
45	RM6	2	0.531	0.469	2	100.00	0.750
46	RM5643	2	0.781	0.219	2	100.00	0.250
47	RM7485	2	0.375	0.625	3	100.00	0.667
48	HRM15679	3	0.469	0.531	3	100.00	0.500
49	HRM15337	3	0.625	0.375	2	100.00	0.500
50	HRM15831	3	0.281	0.719	4	100.00	0.500
51	HRM15580	3	0.469	0.531	3	100.00	0.500
52	HRM15630	3	0.375	0.625	3	100.00	0.667
53	HRM16006	3	0.531	0.469	2	100.00	0.750
54	HRM14250	3	0.625	0.375	2	100.00	0.500
55	HRM15626	3	0.625	0.375	2	100.00	0.500
56	HRM15855	3	0.625	0.375	2	100.00	0.500
57	RM1350	3	0.469	0.531	3	100.00	0.500
58	RM14725	3	0.531	0.469	2	100.00	0.750
59	RM15580	3	0.406	0.594	3	100.00	0.667
60	RM85	3	0.781	0.219	2	100.00	0.250
61	RM14303	3	0.531	0.469	2	100.00	0.750
62	RM3392	3	0.422	0.578	3	87.50	0.417
63	RM7576	3	0.234	0.766	4	87.50	0.438
64	RM7	3	0.531	0.469	2	100.00	0.750
65	RM15064	3	0.531	0.469	2	100.00	0.750
66	RM15741	3	0.625	0.375	2	100.00	0.500
67	RM7000	3	0.344	0.656	3	100.00	0.667
68	RM6832	3	0.781	0.219	2	100.00	0.250
69	RM15189	3	0.625	0.375	2	100.00	0.500
70	HRM17201	4	0.625	0.375	2	100.00	0.500
71	HRM16913	4	0.469	0.531	3	100.00	0.500
72	HRM16801	4	0.406	0.594	3	100.00	0.667
73	HRM17405	4	0.375	0.625	3	100.00	0.667
74	RM17162	4	0.781	0.219	2	100.00	0.250
75	RM16553	4	0.781	0.219	2	100.00	0.250
76	RM16447	4	0.281	0.719	4	100.00	0.500
77	RM16396	4	0.469	0.531	3	100.00	0.500
78	RM16458	4	0.625	0.375	2	100.00	0.500
79	RM16649	4	0.344	0.656	4	100.00	0.500
80	RM16601	4	0.375	0.625	3	100.00	0.667
81	RM16649	4	0.500	0.500	2	100.00	1.000
82	RM551	4	0.375	0.625	3	100.00	0.667

83	RM6770	4	0.625	0.375	2	100.00	0.500
84	RM5633	4	0.313	0.688	2	75.00	0.750
85	RM16720	4	0.531	0.469	2	100.00	0.750
86	RM185	4	0.500	0.500	2	100.00	1.000
87	RM5979	4	0.781	0.219	2	100.00	0.250
88	RM273	4	0.281	0.719	3	75.00	0.500
89	RM17604	4	0.531	0.469	2	100.00	0.750
90	HRM17950	5	0.500	0.500	2	100.00	1.000
91	HRM18222	5	0.313	0.688	4	100.00	0.500
92	HRM18270	5	0.594	0.406	3	100.00	0.333
93	HRM18704	5	0.156	0.844	4	75.00	0.375
94	HRM18770	5	0.625	0.375	2	100.00	0.500
95	HRM18799	5	0.625	0.375	2	100.00	0.500
96	HRM18857	5	0.500	0.500	2	100.00	1.000
97	RM274	5	0.531	0.469	2	100.00	0.750
98	RM31	5	0.625	0.375	2	100.00	0.500
99	RM32	5	0.469	0.531	3	100.00	0.500
100	RM440	5	0.781	0.219	2	100.00	0.250
101	RM17959	5	0.625	0.375	2	100.00	0.500
102	RM169	5	0.313	0.688	4	100.00	0.500
103	RM5140	5	0.313	0.688	4	100.00	0.500
104	RM188	5	0.328	0.672	3	87.50	0.583
105	RM538	5	0.219	0.781	3	75.00	0.500
106	RM19218	5	0.578	0.422	2	87.50	0.375
107	RM178	5	0.500	0.500	2	100.00	1.000
108	HRM20196	6	0.781	0.219	2	100.00	0.250
109	HRM20710	6	0.375	0.625	3	100.00	0.667
110	HRM20060	6	0.406	0.594	3	100.00	0.667
111	HRM19697	6	0.438	0.563	4	100.00	0.375
112	HRM20615	6	0.375	0.625	3	100.00	0.667
113	RM510	6	0.328	0.672	3	87.50	0.583
114	RM20229	6	0.500	0.500	2	100.00	1.000
115	RM19670	6	0.500	0.500	2	100.00	1.000
116	RM20378	6	0.781	0.219	2	100.00	0.250
117	RM190	6	0.781	0.219	2	100.00	0.250
118	RM19715	6	0.375	0.625	3	100.00	0.667
119	RM510	6	0.469	0.531	3	100.00	0.500
120	RM19620	6	0.625	0.375	2	100.00	0.500
121	RM402	6	0.375	0.625	3	100.00	0.667
122	RM1370	6	0.406	0.594	3	100.00	0.667
123	RM3343	6	0.531	0.469	2	100.00	0.750
124	RM5463	6	0.406	0.594	3	100.00	0.667
125	RM133	6	0.781	0.219	2	100.00	0.250
126	HRM20866	7	0.219	0.781	5	100.00	0.400
127	HRM20818	7	0.500	0.500	2	100.00	1.000

128	HRM20948	7	0.344	0.656	3	100.00	0.667
129	HRM21539	7	0.531	0.469	2	100.00	0.750
130	HRM21881	7	0.344	0.656	3	100.00	0.667
131	HRM21258	7	0.531	0.469	2	100.00	0.750
132	RM234	7	0.344	0.656	3	100.00	0.667
133	RM21260	7	0.406	0.594	3	100.00	0.667
134	RM505	7	0.531	0.469	2	100.00	0.750
135	RM500	7	0.625	0.375	2	100.00	0.500
136	RM21925	7	0.625	0.375	2	100.00	0.500
137	RM295	7	0.391	0.609	2	87.50	0.875
138	RM20897	7	0.625	0.375	2	100.00	0.500
139	RM3859	7	0.469	0.531	3	100.00	0.500
140	RM500	7	0.625	0.375	2	100.00	0.500
141	RM234	7	0.531	0.469	2	100.00	0.750
142	RM118	7	0.625	0.375	2	100.00	0.500
143	HRM22622	8	0.250	0.750	5	100.00	0.400
144	HRM22732	8	0.625	0.375	2	100.00	0.500
145	HRM22977	8	0.500	0.500	2	100.00	1.000
146	HRM23578	8	0.500	0.500	2	100.00	1.000
147	HRM23146	8	0.531	0.469	2	100.00	0.750
148	HRM23578	8	0.500	0.500	2	100.00	1.000
149	HRM23237	8	0.500	0.500	2	100.00	1.000
150	RM23386	8	0.297	0.703	3	87.50	0.583
151	RM22612	8	0.375	0.625	3	100.00	0.667
152	RM22971	8	0.531	0.469	2	100.00	0.750
153	RM22416	8	0.500	0.500	2	100.00	1.000
154	RM5493	8	0.781	0.219	2	100.00	0.250
155	RM7285	8	0.375	0.625	3	100.00	0.667
156	RM22675	8	0.625	0.375	2	100.00	0.500
157	RM22772	8	0.781	0.219	2	100.00	0.250
158	RM531	8	0.625	0.375	2	100.00	0.500
159	RM23648	8	0.500	0.500	2	100.00	1.000
160	RM458	8	0.625	0.375	2	100.00	0.500
161	RM6925	8	0.594	0.406	3	100.00	0.333
162	RM72	8	0.266	0.734	3	87.50	0.583
163	RM22585	8	0.406	0.594	3	100.00	0.667
164	RM22675	8	0.781	0.219	2	100.00	0.250
165	RM2687	8	0.531	0.469	2	100.00	0.750
166	RM5647	8	0.469	0.531	3	100.00	0.500
167	RM22692	8	0.531	0.469	2	100.00	0.750
168	RM5428	8	0.531	0.469	2	100.00	0.750
169	RM22696	8	0.625	0.375	2	100.00	0.500
170	RM210	8	0.344	0.656	4	100.00	0.500
171	RM3845	8	0.406	0.594	3	100.00	0.667
172	RM3480	8	0.250	0.750	5	100.00	0.400

173	HRM24199	9	0.469	0.531	3	100.00	0.500
174	HRM23901	9	0.406	0.594	3	100.00	0.667
175	HRM24217	9	0.531	0.469	2	100.00	0.750
176	HRM24481	9	0.344	0.656	4	100.00	0.500
177	HRM24542	9	0.625	0.375	2	100.00	0.500
178	HRM24654	9	0.781	0.219	2	100.00	0.250
179	HRM24842	9	0.266	0.734	2	62.50	0.625
180	RM23948	9	0.469	0.531	3	100.00	0.500
181	RM16883	9	0.500	0.500	2	100.00	1.000
182	RM23966	9	0.531	0.469	2	100.00	0.750
183	RM105	9	0.531	0.469	2	100.00	0.750
184	RM23900	9	0.469	0.531	3	100.00	0.500
185	RM23742	9	0.625	0.375	2	100.00	0.500
186	RM5799	9	0.453	0.547	2	87.50	0.625
187	RM23916	9	0.281	0.719	4	100.00	0.500
188	RM105	9	0.625	0.375	2	100.00	0.500
189	HRM25754	10	0.450	1.000	3	100.00	0.500
190	HRM25796	10	0.453	0.547	2	87.50	0.625
191	HRM24941	10	0.406	0.594	2	75.00	0.500
192	RM5095	10	0.531	0.469	2	100.00	0.750
193	RM4771	10	0.625	0.375	2	100.00	0.500
194	RM311	10	0.625	0.375	2	100.00	0.500
195	RM3510	10	0.531	0.469	2	100.00	0.750
196	RM25149	10	0.625	0.375	2	100.00	0.500
197	RM474	10	0.625	0.375	2	100.00	0.500
198	RM2504	10	0.625	0.375	2	100.00	0.500
199	RM228	10	0.469	0.531	3	100.00	0.500
200	RM25919	10	0.531	0.469	2	100.00	0.750
201	RM24989	10	0.391	0.609	2	87.50	0.875
202	RM258	10	0.630	1.000	2	100.00	0.500
203	RM10852	10	0.625	0.375	2	100.00	0.500
204	RM24989	10	0.531	0.469	2	100.00	0.750
205	RM258	10	0.781	0.219	2	100.00	0.250
206	RM5095	10	0.469	0.531	3	100.00	0.500
207	RM24952	10	0.469	0.531	3	100.00	0.500
208	RM216	10	0.406	0.594	3	100.00	0.667
209	RM3834	10	0.453	0.547	2	87.50	0.625
210	RM6673	10	0.328	0.672	3	87.50	0.583
211	HRM26829	11	0.344	0.656	3	100.00	0.667
212	HRM26369	11	0.406	0.594	3	100.00	0.667
213	HRM26213	11	0.281	0.719	4	100.00	0.500
214	HRM27323	11	0.531	0.469	2	100.00	0.750
215	HRM27322	11	0.625	0.375	2	100.00	0.500
216	HRM27183	11	0.281	0.719	4	100.00	0.500
217	HRM27034	11	0.469	0.531	3	100.00	0.500

218	RM5590	11	0.531	0.469	2	100.00	0.750
219	RM206	11	0.188	0.813	3	75.00	0.500
220	RM26643	11	0.625	0.375	2	100.00	0.500
221	RM27172	11	0.266	0.734	2	62.50	0.625
222	RM5926	11	0.531	0.469	2	100.00	0.750
223	RM202	11	0.625	0.375	2	100.00	0.500
224	RM27318	11	0.391	0.609	2	87.50	0.875
225	RM209	11	0.406	0.594	3	100.00	0.667
226	RM2459	11	0.406	0.594	3	100.00	0.667
227	RM26998	11	0.594	0.406	3	100.00	0.333
228	RM229	11	0.500	0.500	2	100.00	1.000
229	RM21	11	0.344	0.656	4	100.00	0.500
230	RM26669	11	0.500	0.500	2	100.00	1.000
231	RM6327	11	0.594	0.406	3	100.00	0.333
232	RM332	11	0.469	0.531	3	100.00	0.500
233	RM202	11	0.469	0.531	3	100.00	0.500
234	RM26652	11	0.469	0.531	3	100.00	0.500
235	RM26999	11	0.625	0.375	2	100.00	0.500
236	RM27154	11	0.625	0.375	2	100.00	0.500
237	HRM27814	12	0.406	0.594	3	100.00	0.667
238	HRM27406	12	0.438	0.563	4	100.00	0.375
239	HRM28424	12	0.344	0.656	3	100.00	0.667
240	HRM28157	12	0.469	0.531	3	100.00	0.500
241	RM5746	12	0.531	0.469	2	100.00	0.750
242	RM28404	12	0.344	0.656	4	100.00	0.500
243	RM27406	12	0.531	0.469	2	100.00	0.750
244	RM5364	12	0.531	0.469	2	100.00	0.750
245	RM155	12	0.500	0.500	2	100.00	1.000
246	RM179	12	0.625	0.375	2	100.00	0.500
247	RM7102	12	0.531	0.469	2	100.00	0.750
248	RM5568	12	0.781	0.219	2	100.00	0.250
249	RM7315	12	0.625	0.375	2	100.00	0.500
250	RM27542	12	0.531	0.469	2	100.00	0.750
251	RM5746	12	0.781	0.219	2	100.00	0.250
252	RM28130	12	0.531	0.469	2	100.00	0.750
253	RM463	12	0.531	0.469	2	100.00	0.750

Fig. 3. Physical map of the 12 rice chromosomes showing location of 630 HRM and RM markers using Graphical genotyping (GGT)