ISSN 0974-0775
NAAS Rating : 4.79

GREEN FARMING

International Journal of Applied Agricultural & Horticultural Sciences

Volume 7 ❖ Number 4 ❖ July-August 2016 ❖ Bimonthly

www.greenfarming.in

GREEN FARMING

(International Journal of Applied Agricultural & Horticultural Sciences)

(Abbr. - Green Farming Int. J.)

Editor	Editorial Asstt.	Chief Editor (Hony.)
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Publisher, Printer & Owned by : Dr. Anwar Hussain, and Published at White House, 78-A, Bank Colony Road, Near Lal Bangla, Raikabagh, **JODHPUR** - 342 006 (Rajasthan) INDIA. **Printed at :** Anmol Offset, Stadium Shopping Centre, Opp. Old Stadium Ground, Jodhpur - 342 002 (Raj.) INDIA, **Editor -** Dr. Anwar Hussain

Registration No. RAJENG/2010/32178 **NAAS Rating - 4.79 (2015)** **ISSN 0974-0775**

Citation : Abstracting & Indexing in Indian Science Abstracts (NISCAIR) & CABI International; Indexing in Agricultural & Biological Abstracts etc.

All Correspondence to : Editorial Office, GREEN FARMING, White House, 78-A, Bank Colony Road, Near Lal Bangla, Raikabagh, **JODHPUR** - 342 006 (Raj.)

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Volume 7 **Number 4** **July-August 2016** **Bimonthly**

Previous issue :
Vol. **7,** No. **3,** pp. 507-759

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Green Farming Vol. **7** (4) : 783-786 ; July-August, 2016 **Research Paper**

SSR marker aided parental polymorphism survey for stigma exsertion in maintainer lines of hybrid rice

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Received : 07 March 2015 ; Revised accepted : 26 May 2016

ABSTRACT

To begin marker assisted selection or marker assisted back cross breeding for introgression of a desirable trait into a variety from the donor genotype, study of parental polymorphism is a pre-requisite. Unless the parents are polymorphic for the traits of interest, the further selection of plants carrying the traits of interest is not possible in the progenies. The experiment was conducted in Directorate of Rice Research, Hyderabad. In the present study 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. Construction of a Linkage Map could be ensued procuring the generated genotyping data which could further avail QTL analysis and identification of markers linked to stigma exsertion trait.

Key words : *Hybrid rice, Parental polymorphism, Simple sequence repeats (SSR), Stigma exsertion.*

INTRODUCTION

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_1 seed production, and thus the availability of F_1 seeds 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 rice 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 rice extension.

In self pollinated crops like rice, hybrid breeding appeared to be difficult, as the floral traits are unfavourable for outcrossing. Use of male sterility system has immensely helped in hybrid breeding. Virmani (1994) reported that outcrossing 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 seed, is a key factor contributing to the efficient improvement of hybrid seed production, since exserted stigmas remain viable up to 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 frequency of stigma exsertion in male sterile lines of hybrid rice, the seed-setting rate in hybrid seed production

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and the yield of hybrid seed also increased (Zetian and Yanrong , 2010).

Microsatellites or simple sequence repeats (SSRs) are widely used in rice genetic studies. SSRs are highly polymorphic genetic markers and because of their widespread distribution in the genome, high allelic diversity and inherent potential have become a valuable source of genetic markers. Identification of markers that are associated with stigma exsertion and can be used in marker assisted breeding for development of new parental lines with higher outcrossing rate.

MATERIALS AND METHODS

In order to map QTLs for stigma exsertion trait, parents were first screened with DNA markers to establish parental polymorphism survey between them. Parents selected for polymorphism survey are with high stigma exsertion maintainer line BF-16B and low stigma exsertion maintainer line APMS-6B screened with the selected RM primers. In the present study, Rice Microsatellite (RM) markers were used to study the parental polymorphism between the selected parents. About 454 RM markers located on 12 rice chromosomes, based on the reported distribution.

DNA Isolation

Total genomic DNA was isolated from the parental lines using the method of Zheng e*t al.* (1991) with modifications. Healthy leaf pieces (2-3 cm) from the youngest leaves of a 20-25 day old rice seedlings were taken into the well of a spot test plate (M/s Thomas Scientific, USA). 400 ml of extraction buffer (50 mM Tris HCL, pH 8.0, 25 mM EDTA, 300 mM NaCl and 1 % SLS) was added to the well. The leaf tissues were macerated for about 15-20 seconds till it was completely homogenized. Another 400 µl of extraction buffer was added to the well containing the leaf sample. Using a micropipette of 1 ml capacity, the entire content from the well was transferred to a 1.5 ml capacity micro centrifuge tube. About 400-500 µl Phenol (pH 8.0) : Chloroform : Isoamyl alcohol (25:24:1) was then added to the micro centrifuge tube. The contents were mixed well by inversion for about 10 minutes and centrifuged at 12000 rpm for about 10 minutes at room temperature. After the centrifugation, the supernatant was aliquoted from the micro-centrifuge tube without disturbing the intermediate layer into a fresh 1.5 ml micro-centrifuge tube. To the clear supernatant, 5-10 µl of RNAase (10 mg per ml) was added and incubated for 45-60 minutes at room temperature (~ 37 $^{\circ}$C). After completion of the incubation period, about 500 µl of Chloroform: Isoamyl alcohol (24:1) was added to the micro-centrifuge tube and mixed well by inversion for 10 minutes. The content was centrifuged at 12000 rpm for 10 minutes and the supernatant was collected into another fresh 1.5 ml micro-centrifuge tube without disturbing the intermediate layer. To the clear supernatant equal volume (~500-600 ml) of chilled isopropanol was added. The contents were mixed gently and centrifuged at 12000 rpm for 10 minutes at room temperature. The supernatant was drained gently without disturbing the DNA pellet. About 150-200 µl of 70 % ethanol was added to the pellet at the bottom of the micro-centrifuge tube. It was ensured that the pellet was completely immersed in 70% alcohol. Then the content was centrifuged at 5000 rpm for 5 minutes at room temperature. After centrifugation, the supernatant was drained and 70% alcohol wash was repeated once again. Finally, after centrifugation and draining out the supernatant, the pellet was left overnight air drying at room temperature. After complete drying of the pellet, depending upon the size of the pellet, about 50-100 µl of sterile distilled water was added for dissolving the pellet. About 3 µl of the DNA sample was taken and loaded in 0.8 % ethidium bromide stained agarose-TBE (concentration of TBE) gel, electrophoresed for about an hour and the bands of genomic DNA were visualized and documented in a UV gel documentation system (M/s Alpha Innotech Corporation, USA).

Polymerase chain reaction

The genomic DNA of parental lines subjected to PCR amplification as per the procedure described by Chen *et al.* (1997). The total reaction was scaled down to 10 MI.

PCR amplification was carried out in a thermocycler using the temperature profile, followed by rapid cooling to 4°C prior to analysis. For electrophoretic studies, The SSR-PCR products were resolved in a 3% Agarose gel prepared in 0.5 X TBE buffer stained with Ethidium Bromide. The gel was run at a constant voltage of 90 V for 1 h and visualized under UV light and documented using gel documentation system. The temperature specifications for the denaturation of DNA strands, Annealing of primers and extension steps are as following **Table 1**.

Table 1. Thermal profile for PCR reaction

Sr.No.	Steps	Temperature	Time
1	Initial denaturation	94°C	5 min
2	Denaturation	94°C	30 s
3	Annealing	57°C	1 min
4	Extension	72°C	1 min
5	Final extension	72°C	7 min
6	Cooling	4°C	A

RESULTS AND DISCUSSION

The parental polymorphism survey indicated that a clear polymorphism was observed between the parents. A total of 454 SSR primer pairs mapped on all the 12 chromosomes were used for testing polymorphism between two parents.

Table 2. Microsatellite markers exhibiting polymorphism between APMS-6B and BF-16B

1	RM10018F	40	RM273	79	RM3845
2	RM1247F	41	RM17604	80	RM3480
3	RM10649	42	RM6770	81	RM210
4	RM10843	43	RM5633	82	RM22696
5	RM562	44	RM16720	83	RM5428
6	HRM11099	45	RM31	84	HRM22622
7	HRM11111	46	RM440	85	HRM22732
8	HRM11114	47	RM5140	86	HRM22977
9	RM5	48	RM169	87	RM23900
10	RM3642	49	RM188	88	RM23916
11	HRM10167	50	RM538	89	HRM24217
12	RM5536	51	HRM18222	90	RM105
13	RM208	52	HRM18270	91	HRM24654
14	RM279	53	HRM17950	92	RM4771
15	RM7485	54	RM20229	93	RM311
16	RM6509	55	RM19670	94	RM2504
17	HRM13238	56	RM20378	95	RM5095
18	HRM12469	57	RM510	96	RM216
19	RM15189	58	RM402	97	RM6673
20	RM15580	59	RM5463	98	HRM24941
21	RM85	60	RM1370	99	HRM25796
22	RM14303	61	RM133	100	RM229
23	RM7	62	HRM20060	101	RM21
24	RM15064	63	HRM20615	102	RM26669
25	RM7000	64	HRM20710	103	RM202
26	RM15741	65	RM21260	104	RM209
27	HRM14250	66	RM500	105	RM6327
28	HRM15337	67	RM3859	106	RM332
29	HRM15626	68	RM20897	107	RM26652
30	HRM15630	69	HRM20818	108	HRM25970
31	HRM15679	70	HRM21881	109	HRM26213
32	HRM15855	71	RM23386	110	HRM26829
33	RM16447	72	RM22612	111	RM155
34	RM16458	73	RM22971	112	RM179
35	RM16601	74	RM22416	113	RM7102
36	RM22554	75	RM7285	114	RM5746
37	RM16649	76	RM23643	115	RM28404
38	RM551	77	RM22837	116	RM27542
39	RM5979	78	RM5647	117	HRM28580
				118	HRM28157

118 SSR primer pairs exhibited polymorphism between recipient parent APMS-6B and donor parent BF-16B and remaining 336 were monomorphic. Percentage of polymorphism highest (**Table 3**) on chromosome 3 (37.84) and least on chromosome 9 (17.86). The average per cent polymorphism on all the chromosomes was 25.99. The lack of detectable polymorphism between two parents could be due to the fact that both parents, APMS-6B and BF-16B, are indica lines. Lack of molecular marker polymorphism between indica genotypes has been earlier noticed in studies by Xu *et al*. (2002) and Biradar *et al.* (2004).

Polymerase Chain Reaction was done to check each

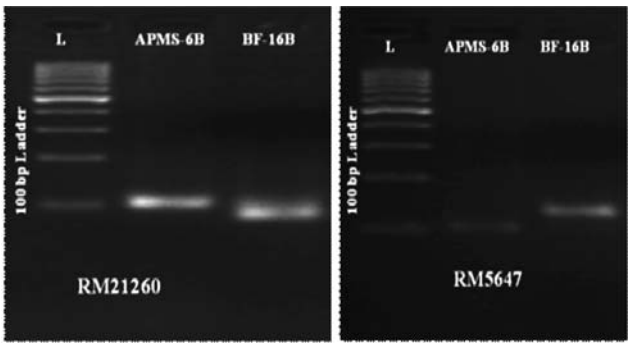

Fig. 1. Polymorphism between recipient parent APMS-6B and donor parent BF-16B using RM21260 and RM5647 SSR primers

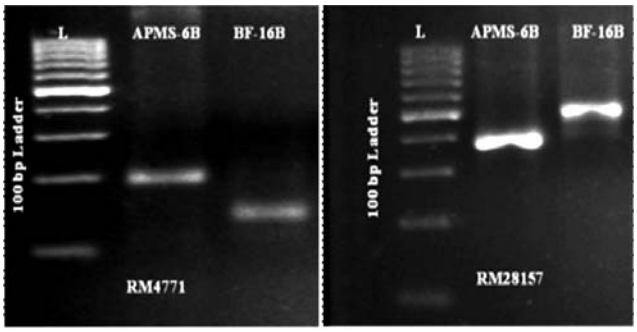

Fig. 2. Polymorphism between recipient parent APMS-6B and donor parent BF-16B using RM4771 and RM28157 SSR primers

sample for parental polymorphism using specific Rice Microsatellite (RM) primers as shown in **Fig. 1 and 2**. Out of 454 primers used the parents showed polymorphic only to 118 RM primers listed in **Table 2**.

The percentage of polymorphism was calculated using the formula :

$$= \frac{\text{RM Primers Showing Polymorphism}}{\text{The total number of RM primers used}} \times 100$$

The usage of molecular marker technology in breeding programs has greatly increased its efficiency and fastens the transfer of desirable genes among varieties and to introgress novel genes from related wild species. Polygenic markers which were previously difficult to analyze using traditional breeding methods, would now be easily tagged using molecular markers. 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 RM 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.

Table 3. Chromosome wise percentage of primers showing polymorphism between parents, APMS-6B and BF-16B

Chrom-osome No.	Total no. of primers screened on each chromosome	No.of polymorphic primers on each chromosome	No.of monomorphic primers on each chromosome	Percentage of primers showing polymorphism on each chromosome
1	40	12	28	30.00
2	32	6	26	18.00
3	37	14	23	37.84
4	39	12	27	30.77
5	30	9	21	30.00
6	39	11	28	28.21
7	32	6	26	18.75
8	55	16	39	29.09
9	28	5	23	17.86
10	42	8	34	19.05
11	44	11	33	25.00
12	36	8	28	22.22

CONCLUSION

The result of the present work represent the main approach in understanding the outcrossing rate of CMS lines which is associated with stigma exsertion rate in rice and it is expected that in future DNA markers will be available for screening stigma exsertion availability in a less expensive and less time consuming procedure in rice.

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