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Identification of Restorer Lines through the Rf_3 and Rf_4 Genes Using SSR Markers in Rice Genotypes (*Oryza sativa* L.)

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ABSTRACT

Identification of parental lines is crucial for developing specific hybrids with more fertility restoration leads to higher yields. Conventionally, the process of screening for the trait of fertility restoration is by tedious test cross progeny evaluation. We screened total of 73 different ecology-specific Indian rice varieties for the presence offertility restorer genes, by earlier reported SSR markersRM10313 and RM6100tightly linked to Rf3 and Rf4, respectively. Among these genotypes, 53.42% carried Rf3Rf3/Rf4Rf4, 57.5% carried Rf3Rf3/rf4rf4 andremaining 69.8% carried Rf4Rf4/rf3rf3 allelic combinations. In this study observed that among 73 genotypes 39 lines were reported as high fertility restoring ability. So,these 39 lines are used for crossing programme to develop the high yielding rice hybrids suitable for aerobic cultivation.

Key words: *Hybrid rice, Molecular markers, Fertility restoration, Rf3, Rf4markers.*

INTRODUCTION

The tropical region of Asia or Monsoon Asia is the largest rice-producing area. The countries of this region together produce 90 per cent of the global output of rice. Globally rice is cultivating around 154 million ha. With 600 metric tonnes production and 3.9 tonnes/ha. productivity. In the present scenario of population explosion, global food production has to be increased by over 40% from the present. In India, the total area under irrigated rice is about 22.00 million hectares, which accounts about 49.5per cent of the total area under rice crop in the country and total area under upland rain fed rice in the country is about 6.00 million hectares, which accounts13.5per cent of the total area under rice crop in the country. Production is variable because of the lack of technology used in rice production. Production declined during 2009-10 due to severe drought, but it reached to 95.98 m tonnes in 2010-11 and further the highest record of 105.31 million tonnes in 2011-12.The target becomes even steeper taking intoaccount the fact that the challenge has to be met under ever-decreasing resources and also without disturbing the environ-mental balance.

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Genetic enhancement of the food crops targeted for better yield seems to be the most viable option. Indian rice production largely depends on monsoon rains and only 59 per cent rice area has assured irrigation. Hybrid rice technology is one of such options for increasing rice production in order to achieve global food security. It has been reported that hybrid rice yields 15 –20% higher than high-yielding varieties in similar growth condition[13]. India, hybrid rice is primarily developed using a three-line system. Male sterility is restored by the nuclear gene(s) known as restorer of fertility (*Rf*), which have the ability to suppress/modify the male sterilizing effect leading to production of fertile pollen. The molecular mechanism of fertility restoration of WA cytoplasm was experimentally proved recently for the *Rf4* gene. Identification of new fertility restorer genes in rice genotypes will help in development of superior restorer lines. Moreover, searching for restorer genes is desirable as phenotyping is verytime-consuming and requires determination of spikelet sterility intestcross progeny (Yao *et al.* 1997). Additionally, partial restorer lines that have good agronomic background canbe improved by transfer of fertility restorer genes without involving a sterile cytoplasm or extensive testcrossing with cyto-plasmic male sterile (CMS) lines. Revathi *et al.* haveevaluated the efficiency of tightly linked markers of *Rf3* and *Rf4*genes for fertility restoration in which 85–92% efficiency was identified[10]. However most of the genetic studies of fertility restoration for the WA CMS system have suggested that fertility restoration is governed by two genes namely *Rf4* and *Rf3* have been mapped to chromosomes 10 and 1 respectively[1,13].

MATERIAL AND METHODS

The present study conducted during *Kharif*-2014-15 at Indian Institute of Rice Research, Rajendranagar, Hyderabad. The leaf samples of seventy three breeding lines were collected from 15-20 days old seedlings during early hours (8am to 9am) and stored at -20^{0}C for DNA isolation.

Molecular analysis

DNA was isolated from young leaves by CTAB method reported by Dellaporta*et al*. With respect to the SSR markers, polymerase chain reaction was carried out using 15–20 ng of template DNA, 250 µM of dNTPs (Eppendorf, USA), 5 pmoles of each F and R primer, 1 unit of Taq DNA polymerase (Bangalore Genei, India), 1X PCR reaction buffer (Bangalore Genie, India) in a total volume of 10 µl. The cycling conditions were an initial denaturation at 940C for 5 min followed by 35 cycles of PCR amplification under the following parameters: 30 s at 940C, 30 s at 550C, and 1 min at 720C, followed by a final extension at 720C for 7 min. The sequences for the SSR primers are presented in (Table 1). Amplified PCR products were resolved in 3% agarose gel, stained with ethidium bromide and visualized under UV light using the Alpha Imager® 1220 gel documentation system (Alpha Innotech Corporation San Leandro, CA, USA).

Table 1: Primer sequence

Name of the Primer	Gene tagged	Primer Sequence(5’ - 3’)	AT(º C)
RM 6100	*Rf4*	F:TTCCCTGCAAGATTCTAGCTACACC R:TGTTCGTCGACCAAGAACTCAGG	55
RM 10313	*Rf3*	F: ACTTACACAAGGCCGGGAAAGG R: TGGTAGTGGTAACTCTACCGATGG	55

RESULTS AND DISCUSSION

The use of molecular markers linked to *Rf* genes can enhance the selection efficiency, save timeand avoid the complications associated with phenotype-based screening. The genetic linkage analysis indicated that the SSR markers RM6100 reported by Singh *et al.*[11], on the long arm of chromosome 10, linked with the *Rf4* gene at distance of 1.2cM and RM10313 reported by Neeraja[9], on the short arm chromosome 1, linked with *Rf3* gene at a distance of 4.2 cM have been utilized to screen 73 genotypes for the identification of restorers.

A total 73 germplasm lines were screened with two SSR primers namely RM6100 linked to fertility restorer gene*Rf4* located on chromosome 10 and RM 10313 linked to fertility restorer gene*Rf3* located on chromosome 1. The lines were scored as restorers based on the presence of restorer specific allele band. Out of 73 lines, 51 lines showed the presence of *Rf4* by amplifying 175- bp size fragment and 22 lines showed the absence of *Rf4* by amplifying 165-bpsize. In same way germplasm lines were screened with the help of SSR marker RM10313 linked to *Rf3* gene out of 73 lines screened, 42 lines showed the presence of *Rf3* by amplifying 215- bp size fragment and 28 showed the absence by amplifying 200- bp product size.

Based on molecular screening results we can assume that out of 73 germplasm lines, 51 lines had *Rf4*, 42 lines had *Rf3* and 39 lines showed the presence of both*Rf3*and *Rf4*. The lines which had both *Rf3*and *Rf4* were confirmed as restorers and the identified restorer lines could be effectively utilized in hybrid rice breeding program Table 1.These results are in agreement with the reports of Akthar *et al.*[2], Ahmadikhah *et al.*[1], Balaji Suresh *et al.*[5], Arun Kumar *et al.*[3], and Namaky, *et al*[8].

To confirm the fertility restoration the 39 lines which are identified as restorer lines, with*Rf4*and *Rf3* were used for further study.

Table 2: Screening of parental lines for fertility restoration through *Rf4* & *Rf3* markers

S.No.	Genotypes	*Rf4*	*Rf3*	*Rf4 & Rf3*
1	AR-9-21	*Rf4*	*Rf3*	*Rf4/Rf3*
2	AR-19-18	*Rf4*	*Rf3*	*Rf4/Rf3*
3	AR-7-75	*Rf4*	*Rf3*	*Rf4/Rf3*
4	NH-12-114	*Rf4*	*Rf3*	*Rf4/Rf3*
5	NH-12-103	*Rf4*	*Rf3*	*Rf4/Rf3*
6	ABU-11-37	*Rf4*	No	*Rf4*
7	L2-182	*Rf4*	*Rf3*	*Rf4/Rf3*
8	AR-19-42	*Rf4*	*Rf3*	*Rf4/Rf3*
9	SV-315-080	*Rf4*	No	*Rf4*
10	AR-7-65	*Rf4*	*Rf3*	*Rf4/Rf3*
11	BK-49-43	*Rf4*	*Rf3*	*Rf4/Rf3*
12	BK-49-80	No	*Rf3*	*Rf3*
13	TCP-650	*Rf4*	*Rf3*	*Rf4/Rf3*
14	AKSHAYADHAN	No	No	No
15	VARDAN	*Rf4*	No	*Rf4*
16	IR-40750	No	No	No
17	TCP-657	*Rf4*	*Rf3*	*Rf4/Rf3*
18	TCP-661	*Rf4*	*Rf3*	*Rf4/Rf3*
19	TCP-585	*Rf4*	*Rf3*	*Rf4/Rf3*
20	RPHR-1096	*Rf4*	No	*Rf4*

21	RPHR-1004	No	*Rf3*	*Rf3*
22	50-7	*Rf4*	No	*Rf4*
23	C-80	*Rf4*	No	*Rf4*
24	GQ-70	*Rf4*	*Rf3*	*Rf4/Rf3*
25	GQ-86	*Rf4*	*Rf3*	*Rf4/Rf3*
26	VG-13	No	No	No
27	VG-58	*Rf4*	No	*Rf4*
28	TCP-643	*Rf4*	*Rf3*	*Rf4/Rf3*
29	MTU-9992	No	*Rf3*	*Rf3*
30	E-20	No	No	No
31	BR-827-35	No	No	No
32	IR-66	*Rf4*	No	*Rf4/Rf3*
33	AJAY	*Rf4*	*Rf3*	*Rf4/Rf3*
34	50-13	*Rf4*	No	*Rf4*
35	363-5	*Rf4*	*Rf3*	*Rf4/Rf3*
36	IB2-517	*Rf4*	*Rf3*	*Rf4/Rf3*
37	RPHR-517	*Rf4*	*Rf3*	*Rf4/Rf3*
38	RPHR-518	*Rf4*	*Rf3*	*Rf4/Rf3*
39	RPHR-124	*Rf4*	*Rf3*	*Rf4/Rf3*
40	NRI-3158	*Rf4*	*Rf3*	*Rf4/Rf3*
41	PNR-3158	No	No	No
42	RPHR-255	*Rf4*	*Rf3*	*Rf4/Rf3*
43	SG-17-118-3	*Rf4*	*Rf3*	*Rf4/Rf3*
44	GQ-25	*Rf4*	*Rf3*	*Rf4/Rf3*
45	SG-27-72	*Rf4*	*Rf3*	*Rf4/Rf3*
46	SG-26-120	*Rf4*	*Rf3*	*Rf4/Rf3*
47	SG-27-31	*Rf4*	*Rf3*	*Rf4/Rf3*
48	SG-998	*Rf4*	No	*Rf4*
49	ICRD-16-1-421	*Rf4*	No	*Rf4*
50	SG-27-177	*Rf4*	*Rf3*	*Rf4/Rf3*
51	IBL-52-1	No	*Rf3*	*Rf3*
52	ICRD-16-9-21	No	No	No
53	GQ-54	*Rf4*	*Rf3*	*Rf4/Rf3*
54	SG-22-289-3	*Rf4*	*Rf3*	*Rf4/Rf3*
55	SG-27-105	*Rf4*	*Rf3*	*Rf4/Rf3*
56	SG-27-175	*Rf4*	*Rf3*	*Rf4/Rf3*
57	NDR-3026	*Rf4*	*Rf3*	*Rf4/Rf3*
58	VG-269	No	No	No
59	TCP-10274	*Rf4*	*Rf3*	*Rf4/Rf3*
60	TCP-3005	*Rf4*	*Rf3*	*Rf4/Rf3*
61	TCP-432	*Rf4*	*Rf3*	*Rf4/Rf3*
62	APMS-6B	No	No	No
63	IR-58025B	No	No	No
64	IR-79156B	No	No	No
65	IR-68897B	No	No	No
66	PUSA-5B	No	No	No
67	IR-68888B	No	No	No
68	IR-80561B	No	No	No
69	IR-80555B	No	No	No
70	CSR-36	No	No	No
71	BPT-5204	No	No	No
72	CST-7-1	*Rf4*	No	*Rf4*
73	FL-478	*Rf4*	No	*Rf4*
No. of confirmed lines		**51**	**42**	**39**

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