IDENTIFICATION OF BEST RESTORER LINES SUITABLE TO AEROBIC CULTIVATION THROUGH SSR MARKERS IN RICE (*Oryza sativa* L.)

[1]MADHUKAR, P.; [2]SENGUTTUVEL, P.; [3]RAJU CH. S. AND [4]VANISREE, S.

DEPARTMENT OF GENETICS AND PLANT BREEDING
COLLEGE OF AGRICULTURE
PROF. JAYASHANKAR TELANGANA STATE AGRICULTURAL UNIVERSITY
HYDERABAD – 500 030, TELANGANA, INDIA

EMAIL: madhu0743@gmail.com

[1]Department of Genetics and Plant Breeding, College of agriculture, PJTSAU, Hyderabad – 500 030
[2]Crop Improvement Section, ICAR-Indian Institute of Rice Research, Rajendranagar, Hyderabad – 500 030
[3]Rice Research Station, Agriculture Research Institute, PJTSAU, Rajendranagar, Hyderabad – 500 030
[4]Institute of Biotechnology, College of Agriculture, PJTSAU, Rajendranagar, Hyderabad – 500 030

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 fertility restoration through test cross progeny evaluation is a tedious job. Total of 125 different ecology-specific Indian rice varieties were screened for the presence of fertility restorer genes, by earlier reported SSR markers RM10313 and RM6100 tightly linked to Rf3 and Rf4, respectively. Out of one hundred twenty five genotypes screened using SSR RM6100, fifty three lines/genotypes showed the presence of Rf4 by amplifying 175-bp size fragment and seventy two lines showed the absence of Rf4 by amplifying 165-bp size fragment compared with KMR3 restorer. Based on these results, it was confirmed that out of one hundred twenty five genotypes, fifty three are restorers, seventy two are non- restorers. Similarly, Out of 125 genotypes were also screened along with known restorer KMR3 using RM 10313 marker and in that, seventy six lines showed the presence of Rf3 by amplifying 215- bp size fragment, forty two showed the absence by amplifying 200-bp product size and seven showed the heterozygous amplification pattern. Based on molecular screening results, it could be know that out of one hundred twenty five lines screened, seventy six are restorers, forty two are non- restorers and seven lines may be partial restorers by comparing with known restorer KMR3R. Overall, it was observed that among 125 genotypes 38 lines were reported as high fertility restoring ability. So,these 38 lines are used for crossing programme to develop the high yielding rice hybrids suitable for aerobic cultivation.

KEY WORDS: Fertility restoration, Molecular markers, Rice, Rf3, Rf4, RM10313, RM6100

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 per cent from the present. In India, the total area under

irrigated rice is about 22.00 million hectares, which accounts about 49.5 per cent of the total area under rice crop in the country and total area under upland rainfed rice in the country is about 6.00 million hectares, which accounts13.5 per cent of the total area under rice crop in the country. In India, low land rice area is about 14.4 million hectares, which accounts 32.4 per 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 environmental balance. 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 productionin order to achieve global food security. It has been reported that hybrid rice yields 15-20 per cent higher than high yielding varieties in similar growth condition (Virmani *et al*., 2003).

In 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 (Kazama and Toriyama, 2014; Tang *et al*., 2014). 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 very time consuming and requires determination of spikelet sterility in test cross progeny (Yao *et al.,* 1997; Komori *et al.,* 2003, Ahmadikhah and Karlov, 2006; Ahmadikhah et al., 2007). Additionally, partial restorer lines that have good agronomic background can be improved by transfer of fertility restorer genes without involving a sterile cytoplasm or extensive test crossing with cytoplasmic male sterile (CMS) lines. Revathi *et al.* (2013) have evaluated the efficiency of tightly linked markers of *Rf3* and *Rf4*genes for fertility restoration in which 85-92 per cent efficiency was identified. 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 (Yao *et al*., 1997; Zhang *et al.,* 1997; Ahmadikhah and Karlov, 2006; Ahmadikhah and Alavi, 2009).

MATERIAL AND METHODS

During *kharif* 2015, the 125 lines were grown in the crossing block under irrigated conditions and to collect leaf samples for molecular study at Rice Research Centre, Rajendranagar, Hyderabad. The leaf samples were collected from 15-20 days old seedlings during early hours (8.00 am to 9.00 am) from 125 lines available in the crossing blocks and stored at -20^0 C-DNA isolation. The entire laboratory work was accomplished in Hybrid Rice wing, Crop Improvement Section, Indian Institute of Rice Research, Hyderabad.

Molecular analysis

DNA was isolated from young leaves by CTAB method reported by Dellaporta *et al*. (1983). 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 94^0C for 5 min followed by 35 cycles of PCR amplification under the following parameters: 30 s at 94^0C, 30 s at 55^0C, and 1 min at 72^0C, followed by a final extension at 72^0C for 7 min. The sequences for the SSR primers are presented in (Table 1). Amplified PCR products were resolved in 3 per cent 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).

RESULTS AND DISCUSSION

The use of molecular markers linked to *Rf* genes can enhance the selection efficiency, save time and avoid the complications associated with phenotype-based screening. SSR marker RM6100 reported by Singh *et al.* (2005), on the long arm of chromosome 10, linked with the *Rf4* gene at distance of 1.2cM and RM10313 reported by Neeraja (2008), on the short arm chromosome 1, linked with *Rf3* gene at a distance of 4.2 cM have been utilized to screen 125 genotypes for the identification of best restorers suitable under aerobic cultivation.

The one hundred twenty five genotypes and one known restorer KMR3R have been screened for the presence of fertility restorer gene *Rf4* (Table 2) located on chromosome 10, with the help of SSR marker RM6100 reported by Singh *et al.* (2005). Out of one hundred twenty five genotypes screened, fifty three lines/genotypes showed the presence of *Rf4* by amplifying 175-bp size fragment and seventy two lines showed the absence of *Rf4* by amplifying 165-bp size fragment compared with KMR3 restorer. Based on these results, it was confirmed that out of one hundred twenty five genotypes, fifty three are restorers, seventy two are non-restorers.

In same way, all these genotypes were screened with the help of SSR marker RM10313 linked to *Rf3* gene reported by Neeraja (2008). Out of 125 genotypes were also screened along with known restorer KMR3 and in that, seventy six lines showed the presence of *Rf3* by amplifying 215- bp size fragment, forty two showed the absence by amplifying 200-bp product size and seven showed the heterozygous amplification pattern. Based on molecular screening results, it could be know that out of one hundred twenty five lines screened, seventy six are restorers, forty two are non-restorers and seven lines may be partial restorers by comparing with known restorer KMR3R.

Similar study was conducted to screen one hundred breeding lines by Singh *et al.* (2014) utilizing the same SSR markers RM6100 and RM 10313 linked to *Rf4* and *Rf3*, respectively and identified that 61 lines to carry both Rf3 and Rf4 genes and these lines can be utilized in hybrid rice breeding as restorers. Revathi *et al.* (2013) stated that *Rf4* and *Rf3* genes showed 85 and 81 percentage efficiency, respectively in identifying restorer lines. Therefore, these markers are useful tool for evaluating large number of breeding lines to know about their fertility restoration in a short period of time without generating and evaluating large number of test crosses. The potential restorers may be identified with hundred percentage efficiency based on molecular screening itself, if candidate gene based markers are developed and validated for both *Rf4* and *Rf3* genes. Similar study was conducted by Cai *et al.* (2013), Singh *et al.* (2014) and Thippeswamy *et al*. (2014).

CONCLUSION

In comparison with both the *Rf3* and *Rf4* markers with 125 germplasm lines retreated that only 38 germplasm lines had

both *Rf3/Rf4* markers. Thus, these 38 germplasm lines were supposed to have best restoring ability, as such could be effectively utilized in development of hybrids in rice.

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Table 1: Primer sequence

Name of the Primer	Gene Tagged	Primer Sequence (5'- 3')	AT (ºC)
RM 6100	*Rf4*	F:TTCCCTGCAAGATTCTAGCTACACC R:TGTTCGTCGACCAAGAACTCAGG	55
RM 10313	*Rf3*	F: ACTTACACAAGGCCGGGAAAGG R: TGGTAGTGGTAACTCTACCGATGG	55

Table 2: Screening results of *Rf4* and *Rf3*

Sr. No.	Genotype	RM6100	RM10313	*Rf4* and *Rf3*	Sr. No.	Genotype	RM6100	RM10313	*Rf4* and *Rf3*
1	RNR-21240	No	*Rf3*	*Rf3*	**29**	RNR-17497	No	No	No
2	RNR-11718	No	*Rf3*	*Rf3*	**30**	MAS-29	No	No	No
3	RNR-21252	*Rf4*	*Rf3*	*Rf3*/*Rf4*	**31**	JGL-20171	No	No	No
4	RNR-17445	*Rf4*	*Rf3*	*Rf3*/*Rf4*	**32**	JGL-17004	*Rf4*	No	*Rf4*
5	RNR-21221	No	*Rf3*	*Rf3*	**33**	JGL-1798	No	No	No
6	RNR-21225	No	No	No	**34**	JGL-1118	No	No	No
7	RNR-21233	No	*Rf3*	*Rf3*	**35**	IRTON-7	No	No	No
8	RNR-19405	No	*Rf3*	*Rf3*	**36**	IIRON-57	*Rf4*	No	*Rf4*
9	RNR-19397	No	*Rf3*	*Rf3*	**37**	IURON-2	No	No	No
10	RNR-21280	*Rf4*	*Rf3*	*Rf3*/*Rf4*	**38**	IURON-5	No	No	No
11	RNR-19399	No	*Rf3*	*Rf3*	**39**	IURON-6	No	No	No
12	RNR-21271	No	*Rf3*	*Rf3*	**40**	IURON-8	No	No	No
13	RNR-21268	No	*Rf3*	*Rf3*	**41**	IURON-14	No	No	No
14	RNR-17368	*Rf4*	*Rf3*	*Rf3*/*Rf4*	**42**	IURON-15	No	No	No
15	RNR-17422	No	*Rf3*	*Rf3*	**43**	IURON-33	No	No	No
16	RNR-21304	*Rf4*	*Rf3*	*Rf3*/*Rf4*	**44**	IURON-38	No	No	No
17	RNR-19410	No	*Rf3*	*Rf3*	**45**	IURON-39	No	No	No
18	RNR19412	No	*Rf3*	*Rf3*	**46**	CSR-23	*Rf4*	No	*Rf4*
19	RNR-19411	No	*Rf3*	*Rf3*	**47**	CSR-27	*Rf4*	No	*Rf4*
20	RNR-21245	No	*Rf3*	*Rf3*	**48**	NLR-3349	No	No	No
21	MTU-1075	*Rf4*	*Rf3*	*Rf3*/*Rf4*	**49**	NLR-3242	No	No	No
22	Surekha	No	*Rf3*	*Rf3*	**50**	NLR-3353	No	No	No
23	Rajendra	No	*Rf3*	*Rf3*	**51**	KNM-605	No	No	No
24	MTU-1001	*Rf4*	*Rf3*	*Rf3*/*Rf4*	**52**	KNM-604	No	No	No
25	Bhadrakhali	No	*Rf3*	*Rf3*	**53**	SKAU-389	*Rf4*	*Rf3*	*Rf3*/*Rf4*
26	Erramallelu	No	No	NO	**54**	L-493	*Rf4*	*Rf3*	*Rf3*/*Rf4*
27	IR-64	No	No	No	**55**	GSR-2	*Rf4*	*Rf3*	*Rf3*/*Rf4*
28	Sughandhamathi	*Rf4*	No	*Rf4*	**56**	GSR-22	No	*Rf3*	*Rf3*

Table 2 contd….

Table 2 contd….

Sr. No.	Genotype	RM6100	RM10313	*Rf4* and *Rf3*	Sr. No.	Genotype	RM6100	RM10313	*Rf4* and *Rf3*
57	GSR-34	No	No	No	**87**	TCP-10246	No	*Rf3*	*Rf3*
58	GSR-40	No	*Rf3*	*Rf3*	**88**	SV-315-081R	*Rf4*	*Rf3*	*Rf3*/*Rf4*
59	IET-24151	*Rf4*	*Rf3*	*Rf3*/*Rf4*	**89**	RPHR-1004	*Rf4*	*Rf3*	*Rf3*/*Rf4*
60	IET-24342	*Rf4*	*Rf3*	*Rf3*/*Rf4*	**90**	ABU-10-82R	*Rf4*	*Rf3*	*Rf3*/*Rf4*
61	IET-24356	*Rf4*	*Rf3*	*Rf3*/*Rf4*	**91**	RPHR-517	No	*Rf3*	*Rf3*
62	IET-24297	No	No	No	**92**	SG 26-120	*Rf4*	No	*Rf4*
63	NDR359(NC)	No	*Rf3*	*Rf3*	**93**	Akshayadhan	*Rf4*	*Rf3*	*Rf3*/*Rf4*
64	RP5715-323-3-1-1	No	*Rf3*	*Rf3*	**94**	KMP-175	*Rf4*	*Rf3*	*Rf3*/*Rf4*
65	IET-23227	No	*Rf3*	*Rf3*	**95**	BI-33	No	No	No
66	RTN 605-111-1-2	*Rf4*	*Rf3*	*Rf3*/*Rf4*	**96**	KMP-153	No	No	No
67	PAU 3835-12-1-1-2	*Rf4*	*Rf3*	*Rf3*/*Rf4*	**97**	KMP-128	*Rf4*	*Rf3*	*Rf3*/*Rf4*
68	Culture kaumk 157	No	*Rf3*	*Rf3*	**98**	RNR-20110	*Rf4*	No	*Rf4*
69	NDR3308	*Rf4*	*Rf3*	*Rf3*/*Rf4*	**99**	RNR-20115	*Rf4*	No	*Rf4*
70	UPR-3841-3-2-1	No	No	No	**100**	RNR-20595	*Rf4*	H	*Rf4*, *Rf3*(H)
71	CRR 484-2-1-1-1-1	No	*Rf3*	*Rf3*	**101**	RNR-20601	No	H	*Rf3*(H)
72	RP5892-32-9-5-4-3-2	No	*Rf3*	*Rf3*	**102**	RNR-20611	*Rf4*	H	*Rf4*, *Rf3*(H)
73	HKR 10-34	No	*Rf3*	*Rf3*	**103**	RNR-20710	No	H	*Rf3*(H)
74	R1641-914-1-400-1	*Rf4*	*Rf3*	*Rf3*/*Rf4*	**104**	RNR-20715	No	H	*Rf3*(H)
75	RP 5883-IR-83142-B-57-B	No	*Rf3*	*Rf3*	**117**	RNR-20819	*Rf4*	No	*Rf4*
76	KPH-466	*Rf4*	No	*Rf4*	**118**	RNR-20824	*Rf4*	*Rf3*	*Rf3*/*Rf4*
77	HRR 08-29	*Rf4*	*Rf3*	*Rf3*/*Rf4*	**119**	RNR-20829	*Rf4*	H	*Rf4*,*Rf3*(H)
78	RP 4978-60-3-2-2	*Rf4*	*Rf3*	*Rf3*/*Rf4*	**120**	RNR-20831	No	*Rf3*	*Rf3*
79	HKR 09-104	No	*Rf3*	*Rf3*	**121**	RNR-20847	No	*Rf3*	*Rf3*
80	CR2274-2-3-3-1	*Rf4*	*Rf3*	*Rf3*/*Rf4*	**122**	RNR-20879	No	H	*Rf3*(H)
81	AAGP9772	*Rf4*	*Rf3*	*Rf3*/*Rf4*	**123**	RNR-21042	No	*Rf3*	*Rf3*
82	AYT-21	*Rf4*	No	*Rf4*	**124**	Anjali	*Rf4*	*Rf3*	*Rf3*/*Rf4*
83	L2-182	*Rf4*	*Rf3*	*Rf3*/*Rf4*	**125**	Vandana	*Rf4*	*Rf3*	*Rf3*/*Rf4*
84	SVHR-3005	*Rf4*	*Rf3*	*Rf3*/*Rf4*	**C**	MTU-1010	No	*Rf3*	*Rf3*
85	NH12-144X	No	No	No	**C**	CRdhan-201	*Rf4*	*Rf3*	*Rf3*/*Rf4*
86	NH12-103R	*Rf4*	*Rf3*	*Rf3*/*Rf4*	**C**	MAS-946	No	*Rf3*	*Rf3*

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