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Short Communication

Molecular screening for the presence of wide compatibility gene *S5* neutral allele in the parental lines of hybrid rice

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Rice is the most important food crop in India occupying 44 m ha, the largest area in the world. The country's population of more than a billion is growing at 1.7% per year [1] outpacing the 1.4% annual growth rate of rice production. To maintain self-sufficiency, annual production needs to increase by 2 million tons every year. Moreover, drastic depletion of land and water resources due to population explosion and urbanization demands for a promising and economically viable technology to achieve higher productivity. Hybrid rice appears to be the most feasible and readily adoptable technologies to meet the future demands of rice production. Hybrid rice has a yielding advantage of about 15 to 20 % over the best commercial rice varieties. Exploitation of inter-subspecific heterosis in rice is recognized as one of the innovative approaches to further enhance genetic yield potential. A major difficulty encountered in the development of such inter-subspecific hybrid is the partial hybrid sterility (HS) frequently observed in most *indica*/*japonica* crosses [2].

In contrast, a special group of rice germplasm referred to as wide-compatibility varieties (WCVs), is able to produce highly fertile hybrids when crossed to both *indica* and *japonica* varieties [3]. More than 28 genes were reported to control HS and of them *S5* locus was considered to be the major locus controlling this trait [4]. A genetic model to account for wide compatibility in rice was proposed by Ikehashi *et al*. [5]. According to this model, single *S5* locus controls the wide compatibility. There are three alleles at the *S5* locus: a neutral allele (wide compatibility allele/gene) *S5n*; an *indica* allele *S5i*; and a japonica allele, *S5j*. *S5nS5i* and *S5nS5j* plants would be fully fertile, while *S5iS5j* plants would be partially sterile. They also determined, using morphological markers chromogen colour (*C*) and waxy endosperm (*wx*), that the *S5* locus was located on chromosome 6.

Until now screening and identification of wide compatible genotypes is accomplished by measuring the level of spikelet fertility in the progeny derived from crosses with appropriate testers utilizing morphological markers linked to a few WC genes. As these approaches based on morphological markers are difficult, time consuming and often inconclusive, there is a need for more efficient and robust techniques to identify and select specific WC gene/genotype(s) [4]. Use of PCR based DNA markers, which are abundant, phenotypically neutral and not influenced by environment [6], can help overcome many of the limitations being encountered in the morphological marker-based screening of wide compatible genotypes. Recently, SSR markers have been used extensively to identify WC genes or HS loci through genome-wide analysis [7, 8]. Singh *et al*. [4] reported that five SSR markers RM276, RM50, RM225, RM253 and RM136 linked to S5 locus useful in identification and deployment of WC genes for development of *indica-japonica* hybrids. Recently Chen *et al*. [9] cloned *S5*, a major locus for *indica–japonica* hybrid sterility and wide compatibility, using a map-based cloning approach and they showed that *S5* encodes an aspartic protease conditioning embryo-sac fertility and a discontinuous 136-bp deletion

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that was separated by a TAAT motif in the first exon of the gene encoding aspartic protease was reported in wide-compatible varieties carrying the neutral allele compared to the *indica* and *japonica* alleles. By targeting 136 bp deletion of *S5* neutral allele Sundaram *et al.* [10] developed a PCR-based the multiplex marker system (S5-MMS) suitable for identification of allelic status at the major wide compatibility gene locus *S5* in rice germplasm.

In this study we used S5-InDel ((F:CCTACG TTTGACTGCCTGCCTG R:CTACACGCGGCTTC GGGAAAGC) primer reported by Sundaram *et al.* [10] which targets neutral allele specific deletion. One hundred and three breeding lines (Table 1) were raised in the field and leaves collected from 20-30 day old seedlings were used for DNA isolation. Genomic DNA was extracted by a modified potassium acetate method

Table 1.

S.No.	Genotype	*S5* allele status	Source	S.No	Genotype	*S5* allele status	Source
1	RPHR118	I/J	DRR	51	IRGC 6309	N	IRRI
2	RPHRGQ 25	I/J	DRR	52	IRGC 3394	I/J	IRRI
3	RPHRSG-25-74	N	DRR	53	IRGC 10658	N	IRRI
4	RPHR124	N	DRR	54	IRGC 3223	I/J	IRRI
5	RPHRSG-26-120	I/J	DRR	55	IRGC 27129	N	IRRI
6	RPHR SG22-23-1	I/J	DRR	56	IRGC 39111	N	IRRI
7	RPHRNRI 38	I/J	DRR	57	RPHR 1124	HET	IRRI
8	RPHR972	N	DRR	58	IRGC 43402	N	IRRI
9	RPHR Shrabahi	I/J	DRR	59	IRGC 2052	I/J	IRRI
10	RPHRSC5 28-4-1-1	N	DRR	60	IRGC 1789	I/J	IRRI
11	RPHR628-2	N	DRR	61	IRGC 2245	I/J	IRRI
12	TCP 9288	N	DRR	62	IRGC 47216	N	IRRI
13	TCP 9289	N	DRR	63	IRGC 47248	N	IRRI
14	TCP9290	N	DRR	64	IRGC 8193	I/J	IRRI
15	RPHRPNR 2-49	N	DRR	65	TCP 9243	HET	IRRI
16	RPHR695	N	DRR	66	IRGC 50399	N	IRRI
17	RPHRTG-70	N	DRR	67	IRGC 50596	N	IRRI
18	RPHRTG-64	I/J	DRR	68	RPHRGQ-70	N	DRR
19	RPHRTG-23	I/J	DRR	69	RPHRGQ-86	HET	DRR
20	RPHRB-95-12	I/J	DRR	70	RPHRGQ-58	HET	DRR
21	RPHRB-95-16	I/J	DRR	71	RPHRGQ-54	N	DRR
22	RPHRB-95-91	I/J	DRR	72	RPHR998	N	DRR
23	RPHRB-95-95	I/J	DRR	73	RPHRGQ-64-1	N	DRR
24	TCP-9211	N	DRR	74	ICRD 16-9-2-1	N	DRR
25	TCP-9213	HET	DRR	75	ICRD 16-1-4-2-1	N	DRR
26	TCP-9215	HET	DRR	76	DR-714-1-2 R	N	DRR
27	TCP-9220	I/J	DRR	77	RPHR-945-1-2	I/J	DRR
28	TCP-9221	I/J	DRR	78	RPHRSG22-289-3	N	DRR
29	TCP-9225	I/J	DRR	79	RPHRIBL52-1	N	DRR
30	TCP-9348	I/J	DRR	80	RPHRSG27-118-3	N	DRR
31	TCP-9349	N	DRR	81	RPHR255	N	DRR
32	TCP-9352	N	DRR	82	TCP3699	I/J	DRR

33	TCP-9353	N	DRR	83	RPHRSG-25-74	N	DRR
34	TCP-9354	N	DRR	84	RPHRVG-175	I/J	DRR
35	TCP-9356	N	DRR	85	RPHRVG-269	I/J	DRR
36	TCP-9357	I/J	DRR	86	RPHRVG-294	I/J	DRR
37	TCP-9358	N	DRR	87	RPHRBR827-35	I/J	DRR
38	TCP-9360	N	DRR	88	RPHRSG26-120	I/J	DRR
39	TCP-9363	I/J	DRR	89	RPHRSG27-177	I/J	DRR
40	TCP-9365	I/J	DRR	90	TCP 9410	I/J	DRR
41	TCP-9368	N	DRR	91	TCP 9401	N	DRR
42	Tropical japonica lines IRGC 1943	N	DRR	92 93	TCP 9415 TCP 9403	I/J N	DRR DRR
43	IRGC 5441	I/J	IRRI	94	TCP 9416	I/J	DRR
44	IRGC 6937	I/J	IRRI	95	TCP 9493	I/J	DRR
45	IRGC 4105	I/J	IRRI	95	TCP 9497	I/J	DRR
46	IRGC 3849	I/J	IRRI	97	RPHR 517	HET	DRR
47	IRGC 3370	I/J	IRRI	98	RPHR410-5	I/J	DRR
48	IRGC 3388	I/J	IRRI	99	TCP 9528	N	DRR
49	IRGC 3255	I/J	IRRI	100	TCP 9433	I/J	DRR
50	IRGC 5766	N	IRRI	101	TCP 9435	N	DRR
				102	TCP 9437	I/J	DRR
				103	TCP 9434	N	DRR

I/J-*Indica/Japonica*, N-*S5* neutral allele, HET-Heterozygous

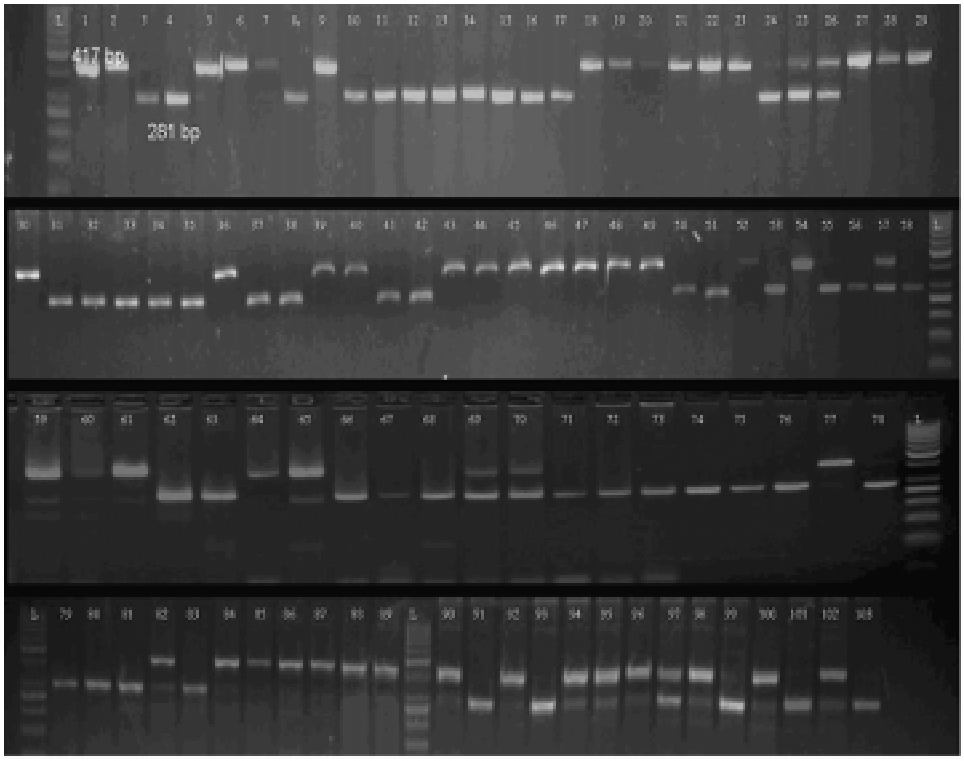

Fig. 1. Screening for the presence of wide compatibility locus *S5* neutral allele

[11]. S5-inDel amplification reaction was carried out using 10 µl volume containing 25–30 ng of template DNA, primers (F&R) 0.25 µM, 10 x PCR buffer with 1.5 mM $MgCl_2$, 2.5 mM dNTPs, 1 U *Taq* DNA polymerase.The touchdown PCR profile was: an initial denaturation at 95ºC for 5 min followed by 10 cycles of denaturation for 30 s at 94°C, 30 s annealing at 72–63°C (1°C decrease per cycle), 1 min extension at 72°C and 30 cycles of denaturation for 30 s at 94°C, 30s annealing at 60°C, 1 min extension at 72°C, with a final extension of 72°C for 7 min. The amplified PCR products were separated on a 1.5% agarose gel and stained with Ethidium Bromide and visualized under a UV transilluminator (Alpha Innotech, USA). The gels were scored based on the banding pattern as *indica*/*japonica* type, neutral and heterozygous. A 417-bp fragment was amplified in *indica* and *japonica* alleles and a 281-bp fragment in genotypes carrying the neutral allele at *S5* (Fig. 1). 0ut of 103 lines screened 48 lines showed the presence of *S5* neutral allele 48 showed *indica/japonica* type 7 lines showed heterozygous pattern. The lines which were identified to possess *S5* neutral allele could be utilized in inter subspecific hybridization to exploit higher level of heterosis. The present study clearly demonstrated the utility of primer S5 InDel in identifying wide compatible genotypes possessing S5 neutral allele.

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