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Study of genetic diversity in fertility restoring genotypes for *WA* cytoplasm in rice (*Oryza sativa* L.) through RAPD and SSR markers

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ABSTRACT: *Twenty five rice genotypes were used to study the genetic diversity and fertility restoration study with the help of RAPD and SSR markers. For genetic diversity study, sixty different RAPD primers were screened, out of which 15 gave satisfactory results. A total of 223 loci were observed, out of which 217 loci were found polymorphic with an average polymorphism of 97.83%. OPC-15 amplified maximum number of bands i.e. 265 bands. RAPD based analysis revealed high level of genetic variation among rice genotypes and led to the establishment of genetic relationship between them. The dendrogram based on pooled RAPD data has divided the genotypes into two major clusters 'A' and 'B', each of which comprised two sub cluster 'A1' and 'A2' and 'B1' and 'B2', respectively.*

The SSR analysis for fertility restorer genes with 10 microsatellite (SSR) primers generated 117 alleles. The highest allele length of 368bp was found in IR 24 with marker RM 171. The highest allelic frequency of 0.35 was recorded with RM 6737 and highest expected heterozygosity was recorded with RM 3873. The maximum similarity index of 0.278 was observed between the varieties Narmada and GR-102. The dendrogram generated has divided all 25 genotypes into two major clusters i.e. A and B, which were lateron sub divided into two sub clusters 'A1' and 'A2' and 'B1' and 'B2', respectively.

The results revealed that RAPD marker is effective in studying genetic diversity in intra-specific genotypes, which can be used by plant breeders in the breeding programme. The SSR study revealed that the genotypes such as GR3, GR6, GR8, GAR2, GR 103, GR 11, IR64, Pankhali203, GAR1, Narmada and Ashoka 200F shared a common band size with confirmed restorers such as IR 24, GR101, GR104 and GR7 indicating that these genotypes may also posses capacity for fertility restoration.

Key words: *Rice, genetic diversity, fertility restorer, RAPD, SSR*

INTRODUCTION

Rice (*Oryza sativa* L.) is the most important food crop plant in the world. Among the two cultivated species, *Oryza sativa* is most commonly grown throughout Asia, Australia, America and Africa, while *Oryza glaberrima* is grown on a small scale in Western Africa. More than 90% of the world's rice is grown and consumed in Asia. Among the rice growing countries in the world, India has the largest area under rice crop (about 45 million ha) and ranks second in production next to China. The India's rice production reached to a high record of 104.32 million tonnes in year 2011-2012.

India is a primary centre of origin of rice and has many local landraces. The most of them are not in cultivation while many are lost but few are still cultivated by resource-poor traditional farmers in areas practicing subsistence farming (Singh *et al.*, 2013). Rice, being a strictly self pollinated crop requires the use of male sterility system to develop commercial rice hybrids. The cytoplasmic male sterility (CMS) system is the most effective and partial way of exploiting heterosis in rice (Subudhi *et al.*, 1998). The fertility can be restored in CMS cytoplasm by nuclear genes and hence they are referred to fertility restorer genes or *Rf* genes (Eckardt, 2006). In rice, wild abortive (*WA*) type cytoplasmic male sterility (CMS) is commercially used for production of hybrid seeds in Asia. Most investigators tended to agree that restoration of *WA* type CMS is controlled by two nuclear genes (*Rf3*, *Rf4*) that are mapped on chromosome 1 and chromosome 10, respectively

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(Zhang *et al*., 2002). Characterization of restorers, maintainers and CMS lines with molecular markers will provide information on the molecular divergence of parental lines, which can be used for estimation of heterosis, selection of better parents and improvement of parental lines in rice. Markers linked to the gene can be used to select plants possessing the desired trait and markers throughout the genome can be used to select plants that are genetically similar to the recurrent parent. Due to advances in molecular biology techniques, large numbers of highly informative DNA markers such as RFLP, RAPD, AFLP, SSR and SCAR have been developed for the identification of genetic polymorphism.

The present study aims to study the genetic diversity through RAPD markers and analysis for fertility restorer genes in rice genotypes using microsatellite markers.

MATERIALS AND METHODS

The present study on genetic diversity in fertility restoring genotypes for *WA* cytoplasm in rice (*Oryza sativa* L.) was carried out at Biotechnology Laboratory, Department of Genetics and Plant Breeding, B. A. College of Agriculture, Anand Agricultural University, Anand during 2012-2013.

Twenty five rice genotypes (including released varieties for different production systems) obtained from Main Rice Research Station, Anand Agricultural University, Nawagam were studied (Table 1). Seedlings of all the genotypes were raised in pots. Fresh leaves were collected and further utilized for isolating genomic DNA to study genetic diversity and polymorphism by RAPD and SSR analysis.

Total DNA was extracted from the fresh leaves of four weeks old seedling by Cetyl trimethyl ammonium bromide (CTAB) method as described by Ahmadikhah *et al.* (2007), with some modifications. To estimate quantity and quality (in terms of protein and RNA contamination) of isolated genomic DNA, spectrophotometry was performed and data were analyzed using Nanodrop N.D.1000 software (ver.3.3.0). The PCR reaction steps for RAPD are 94^0Cf or 5 minutes (Initial denaturation), 45 cycles each of 94^0 for 1 minute (Denaturation), 38 °C or 40 °C for 1 minute (Primer annealing), 72 °C for 2 minutes (Extension of annealed primer) and 72 °C for 10 minutes (Final Extension). For SSR analysis the PCR condition consisted of Initial denaturation at 94 °C for 7 minute, with 45 cycles of final denaturation at 94 °C for 45 sec, annealing at 55 °C for 1 minute and extension at 7 min, followed by final extension at 72 °C for 7 min. The amplified products for RAPD and SSR were analyzed electrophoretically using 1.8% and 2.5% agarose gel respectively. The separated bands were visualized under UV transilluminator and photographed using syngene gene snap-G-box (Alpha Ease FC4.0.0 gel Documentation system). Each amplified product was scored across all the genotypes for its presence or absence. The scores 1 and 0 indicate the presence or absence of bands, respectively. The data were entered in to binary matrix and subsequently analyzed using NTSYSpc version 2.02. Coefficients of similarity were calculated as Jaccard's similarity coefficient by SIMQUAL subroutine in SIMILARITY routine. The matrix of similarity was clustered using UPGMA algorithm under Sequential Agglomerative Hierarchical Nesting (SHAN) module of the NTSYS pc. Relationships among rice cultivars were graphically represented in the form of dendrograms. The cophenetic correlation analysis was carried out using COPH function of NTSYS pc. and dendrogram constructed based on the similarity coefficients. The PIC value for each locus was calculated on the basis of allele frequency (Anderson *et al*., 1993).

Table 1
List of genotypes used in the present study

Sr. No.	*Genotypes*	*Pedigree*
1	SK-20	Selection from local cultivar Sukhvel
2	GR-6	GR-3 x Pusa-33
3	GR-7	GR-3 x Basmati 370
4	GR-101	IR-8 x P-203
5	GR-102	IR-8 x P-203
6	GR-104	GR-101 x Basmati 370
7	Pankhali-203	Selection from local cultivar Pankhali
8	Narmada	TN-1 x Basmati 370
9	Basmati 370	Selection from Dehradun basmati
10	Pusa Basmati-1121	Pusa 614-1-2 x Pusa 614-2-4-3
11	GR-3	N-19 x IR-9-60
12	GR-11	Z-31 x IR-8-246
13	GR-12	GR-4 x IR-64
14	GAR-13	GR-11 x IET – 14726
15	GR-103	GR-11 x Masuri
16	GAR-1	Narmada x IET – 14708
17	GAR-2	Gurjari x IET – 14714
18	Sathi-34-36	Selection from local cultivar Sathi
19	GR-5	Selection from local cultivar NVS-18
20	GR-8	Selection from pure line Vyara-55
21	GR-9	Sathi-34-36 x CR-544
22	AAUDR-1	Sathi34-36 x Dadri Kolam
23	Ashoka-200F	Kalinga-III x IR 64
24	IR-24	IR-8 x IR-127-2-2
25	IR-64	Gam Pai-15 x TN-1

Results and Discussions: The present investigation was carried out in the Biotechnology Laboratory of Department of Genetics and Plant Breeding, B. A. College of Agriculture, Anand Agricultural University, Anand with a view to evaluate the genotypic variation present in 25 rice cultivars, utilizing the Random amplified polymorphic DNA (RAPD) markers and to tag the fertility restoring genes by Microsatellite (SSR) markers. In the present investigation, 60 arbitrary decamer oligonucleotide primers were screened sequentially until sufficient numbers of primers giving satisfactory amplification were obtained. Out of total primers tested, 15 RAPD primers from OPA, OPC and OPK series which gave satisfactory amplification were used for evaluating 25 rice genotypes (Table 2).

Table 2
List of RAPD primers used in the present study

Sr. No.	*Primer*	*Sequence (5'-3')*
1	OPA-7	GAAACGGGTG
2	OPA-8	GTGACGTAGG
3	OPA-10	GTGATCGCAG
4	OPA-16	AGCCAGCGAA
5	OPC-2	GTGAGGCGTC
6	OPC-6	GAACGGACTC
7	OPC-7	GTCCCGACGA
8	OPC-11	AAAGCTGCGG
9	OPC-15	GACGGATCAG
10	OPC-18	TGAGTGGGTG
11	OPK-7	AGCGAGCAAG
12	OPK-8	GAACACTGGG
13	OPK-16	GAGCGTCGAA
14	OPK-18	CCTAGTCGAG
15	OPK-20	GTGTCGCGAG

The results obtained using 15 primers revealed variety of RAPD fingerprints (Table 4). Amplification of total genomic DNA from different varieties produced a total of 223 fragments, of which 217 (97.43%) were polymorphic in nature. The number of amplification products produced by a primer ranged from 10 (OPA-16) to 18 (OPC-15) with an average of 15 bands per primer. The percentage of polymorphic bands shown by different primers ranged from 86.66 (OPK-8) to 100 (all except OPA-8, OPC-7, OPC-15, OPK-8 and OPK-20). One of the reasons for this high level of polymorphism could be due to extensive intra-specific variation in rice. Although the majority of primers produced polymorphic bands, no single primer could clearly distinguish all the genotypes. RAPD marker OPC-15 produced maximum numbers of 265 bands, while OPC-11 amplified minimum numbers of 133 bands. The PIC values ranged from 0.884 (OPA-16) to 0.934 (OPC-15) with an average of 0.91.

Jaccard's similarity coefficient which revealed the similarity matrix of all pair wise combinations of 25 genotypes is presented in Table 5. The similarity coefficient values ranged from 0.22 to 0.81. This indicated a wide range of variability in the similarity coefficient values, suggesting a fairly wide genetic base of 25 genotypes taken in the experiment. The highest value of similarity coefficient (0.81) was found between the varieties GR-101 and GR-102, while the lowest value of similarity coefficient (0.22) was observed between the genotypes Basmati-370 and AAUDR-1. Variety Basmati-370 showed less similarity with other genotypes.

In order to analyze the relatedness among the genotypes studied, the UPGMA-based dendrogram was constructed using paired matrix values for pooled RAPD data. As evident from the dendrogram, 25 genotypes formed two major clusters 'A' and 'B' (Figure 1). Major cluster 'A' was further divided into two 1sub-clusters 'A1' and 'A2'. Sub-cluster 'A1' consisted of three genotypes *viz.*, SK-20, GR-6 and GR-7, while sub-cluster 'A2' comprised of ten varieties *viz.*, GR-101, GR-102, Pusabasmati-1121, Narmada, GR-11, GR-12, GR-3, Pankhali-203, GR-104 and Basmati-370. Like major cluster 'A', cluster 'B' was also divided into two sub-clusters, 'B1' and 'B2'. Sub-cluster 'B1' was represented by four varieties *viz.*, GAR-13, GAR-2, GR-103 and GAR-1. Sub-cluster 'B2' was represented by eight varieties *viz.*, Sathi-34-36, GR-9, GR-5, GR-8, Ashoka-200F, IR-24, IR-64 and AAUDR-1. The results revealed a moderate level of genetic variation among rice genotypes and led to the establishment of genetic relationships between them. Varieties, GR-6 and GR-7 were clustered in the same sub-cluster 'A1' with close proximity due to common parent in their pedigree. Similarly, varieties GR-101 and GR-102 were found in the same sub-'A2' with highest similarity coefficient (0.81), which might be due to common parents in their pedigree. The aromatic varieties *viz.*, Narmada, Basmati-370, GR-101, GR-102, GR-104 and Pankhali-203 were found in the same sub- cluster 'A2'. Likewise, all drilled paddy varieties *viz.*, GR-9, GR-5, GR-8, Ashoka 200F, Sathi-34-36 and AAUDR-1 were clustering in a same sub-cluster 'B2'.

The molecular analysis performed with 25 rice genotypes using ten microsatellite markers (SSR) generated a total number of 117 alleles (Table 3). The allele length ranged from 74 -368 bp. The highest allele length (368 bp) was found in IR 24 with the marker

Table 3
List of SSR primers used for Molecular Analysis of Fertility Restorer gene

Sr. No.	*Primer Name*	*Forward (5′-3′)*	*Reverse (5′-3′)*
1.	RM6100	TTCCCTGCAAGA TTCTAGCTA GCT ACACC	TGTTCGTCGACCAAGAACTCA GG
2.	RM171	AACGCGAGGACACGTACTTAC	ACGAGATACGTACGCCTT TG
3.	RM258	TGCTGTATGTAGCTCGCACC	TGGCCTTTAAAGCTGTCGC
4.	RM 216	GCATGGCCGATGGTAAAG	TGAATAAAACCACACGGCCA
5.	RM 6737	CATTGGGGGTGGATAAAGAG	TATCCTCTACTCCCTCGGCC
6.	RM 5359	CGTGATCTCGTGCATCCC	CCCTCAGGAGCTTCATGAAC
7.	RM 3233	GTGGTGAGTAAACAGTGGTGG	GAGAGCAGAGCAGAGGCA AC
8.	RM 3873	GCTAGCTAGGACCGACATGC	CCTCCTCCTTATCCTCCCTG
9.	RM 1	GCGAAAACACAATGCAAAAA	GCGTTGGTTGGACCTGAC
10.	RM 3530	GTAGATCCGGTCAGCTCCTC	CAAGGAGATTCCCTTCCATG

Table 4
Analysis of RAPD patterns generated using 15 arbitrary primers for rice genotypes

Sr. No.	*Name of primers*	*Maximum scorable band*	*Polymorphic loci (P)*	*Total loci (T)*	*Percentage Polymorphism (P/T)x100*	*PIC Value*
			OPA Series			
1	OPA-7	195	14	14	100	0.914
2	OPA-8	170	15	16	93.75	0.907
3	OPA-10	169	15	15	100	0.904
4	OPA-16	169	10	10	100	0.884
			OPC Series			
5	OPC-2	168	15	15	100	0.910
6	OPC-6	158	15	15	100	0.913
7	OPC-7	206	13	14	92.85	0.907
8	OPC-11	133	13	13	100	0.899
9	OPC-15	265	17	18	94.44	0.934
10	OPC-18	173	13	13	100	0.909
			OPK Series			
11	OPK-7	190	16	16	100	0.921
12	OPK-8	192	13	15	86.66	0.911
13	OPK-16	218	17	17	100	0.925
14	OPK-18	183	16	16	100	0.920
15	OPK-20	207	15	16	93.75	0.919
Range		133-265	10-17	10-18	86.66-100	0.884-0.934
Average		186.4	14.46	14.866	97.43	0.9118
Pooled		**2796**	**217**	**223**	**—-**	**—-**

RM 171. The allelic frequency ranged from 0.02-0.35. The highest allelic frequency (0.35) was recorded with GR-102, GR-103, GAR-2, Sathi-34-36, AAUDR-1 and GR- 5 with the marker RM 6737. The expected heterozygosity varied from 0.76-0.94, wherein the highest expected heterozygosity was recorded with the primer RM 3873. These data were utilized for calculating similarity coefficient and constructing dendrogram (Table 6 and Figure 2). The similarity coefficient values ranged from 0.042 to 0.278. The maximum similarity index of 0.278 was obtained between the varieties Narmada and GR-102.

The dendrogram based on 25 SSR markers in this study revealed the presence of two major clusters *viz.*, 'A' and 'B'. The major cluster 'A' was further divided into two sub-clusters 'A1' and 'A2'. The sub-cluster 'A1' consisted of seven genotypes *viz.*,SK-20, GR-6, GR-11, GR-12, GR-7, Basmati 370 and Pankhali 203, while, sub-cluster 'A2' consisted of ten varieties *viz.*,GR-101, GR-104, GR-102, Narmada, Pusa Basmati

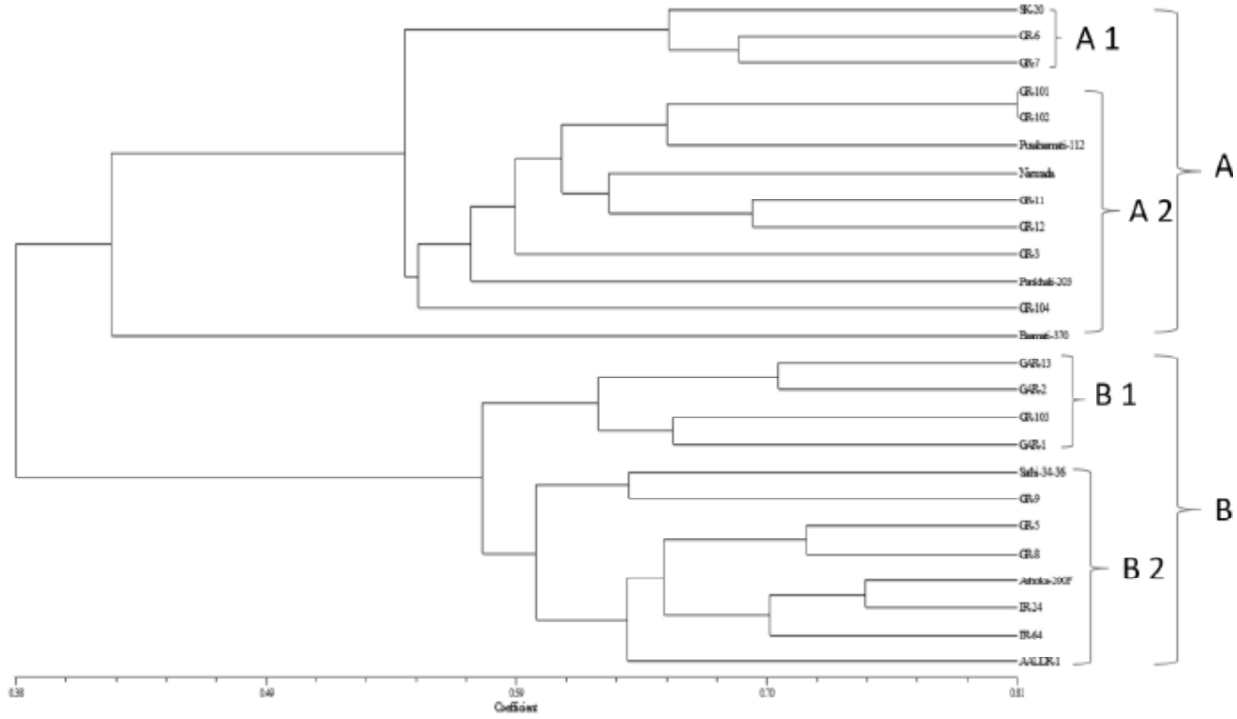

Figure 1: Dendrogram of 25 rice genotypes for genetic diversity with RAPD markers

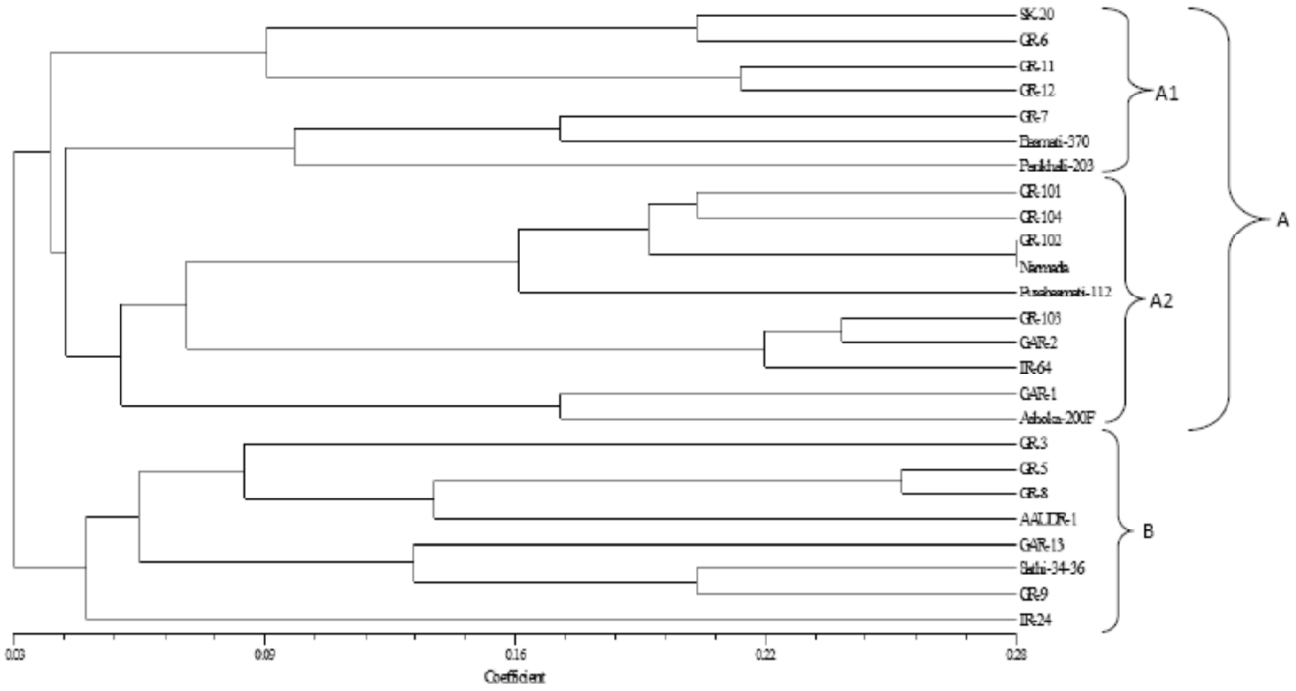

Figure 2: Dendrogram of 25 rice genotypes for fertility restorer genes with SSR marke

1121, GR-103, GAR-2, IR-64, GAR-1 and Ashoka-200F. The cluster 'B' consisted of genotypes GR-3, GR-5, GR-8, AAUDR-1, GAR-13, Sathi-34-36, GR-9 and IR-24. Majority of the drilled varieties of Gujarat are grouped in the same major cluster 'B'. Similarly, confirmed fertility restoring genotypes GR-101 and GR-104 were found in the same sub-cluster 'A2' whereas, confirmed non-restorer SK-20 and GR-6

Table 5
Similarity matrix of 25 rice genotypes for RAPD primers

	1.	*2.*	*3..*	*4.*	*5.*	*6.*	*7.*	*8.*	*9.*	*10.*	*11.*	*12.*	*13.*	*14.*	*15.*	*16.*	*17.*	*18.*	*19.*	*20.*	*21.*	*22.*	*23.*	*24.*	*25.*
1,	1.00																								
2.	0.66	1.00																							
3.	0.66	0.69	1.00																						
4.	0.58	0.53	0.63	1.00																					
5.	0.61	0.56	0.63	0.81	1.00																				
6.	0.60	0.54	0.50	0.49	0.59	1.00																			
7.	0.53	0.45	0.48	0.54	0.55	0.53	1.00																		
8.	0.56	0.52	0.56	0.60	0.63	0.62	0.62	1.00																	
9.	0.42	0.42	0.38	0.37	0.37	0.54	0.39	0.45	1.00																
10.	0.58	0.53	0.55	0.64	0.68	0.55	0.63	0.63	0.41	1.00															
11.	0.55	0.53	0.53	0.54	0.57	0.56	0.57	0.60	0.42	0.64	1.00														
12.	0.51	0.50	0.50	0.55	0.56	0.52	0.55	0.61	0.44	0.59	0.63	1.00													
13.	0.55	0.57	0.57	0.61	0.65	0.55	0.56	0.66	0.43	0.67	0.59	0.70	1.00												
14.	0.43	0.38	0.38	0.41	0.43	0.35	0.40	0.44	0.26	0.42	0.39	0.38	0.42	1.00											
15.	0.37	0.35	0.33	0.34	0.38	0.33	0.31	0.32	0.29	0.38	0.33	0.34	0.35	0.63	1.00										
16.	0.41	0.37	0.39	0.40	0.39	0.38	0.33	0.39	0.28	0.38	0.37	0.36	0.37	0.64	0.66	1.00									
17.	0.39	0.38	0.38	0.39	0.43	0.34	0.38	0.42	0.24	0.43	0.38	0.37	0.40	0.71	0.60	0.64	1.00								
18.	0.38	0.33	0.35	0.36	0.40	0.35	0.36	0.41	0.26	0.38	0.33	0.32	0.35	0.50	0.46	0.61	0.54	1.00							
19.	0.41	0.40	0.39	0.41	0.43	0.35	0.35	0.41	0.26	0.42	0.37	0.36	0.41	0.55	0.52	0.61	0.61	0.67	1.00						
20.	0.42	0.41	0.40	0.41	0.43	0.33	0.37	0.39	0.24	0.42	0.37	0.39	0.40	0.62	0.51	0.57	0.60	0.59	0.72	1.00					
21.	0.40	0.37	0.38	0.38	0.39	0.41	0.39	0.43	0.28	0.41	0.38	0.39	0.40	0.55	0.50	0.66	0.59	0.64	0.64	0.66	1.00				
22.	0.38	0.37	0.36	0.36	0.40	0.42	0.39	0.40	0.22	0.40	0.35	0.35	0.39	0.60	0.48	0.50	0.65	0.52	0.64	0.67	0.61	1.00			
23.	0.42	0.40	0.39	0.39	0.39	0.41	0.36	0.40	0.27	0.43	0.39	0.37	0.42	0.61	0.54	0.66	0.62	0.59	0.69	0.65	0.62	0.60	1.00		
24.	0.42	0.40	0.40	0.40	0.42	0.43	0.36	0.41	0.25	0.42	0.37	0.39	0.41	0.58	0.53	0.60	0.72	0.55	0.63	0.66	0.59	0.65	0.74	1.00	
25.	0.43	0.39	0.40	0.40	0.43	0.46	0.40	0.46	0.25	0.46	0.41	0.39	0.43	0.66	0.51	0.58	0.68	0.57	0.66	0.66	0.61	0.65	0.67	0.74	1.00

Where

1. SK-20	2- GR-6	3- GR-7	4- GR-101	5-G-102	6- GR-104
7-Pankhali-203	8-Narmada	9-Basmati-370	10- Pusa Basmati-1121	11- GR-3	12- GR-11
13- GR-12	14-GAR-13	15-GR-103	16-GAR-1	17-GAR-2	18-Sathi-34-36
19- GR-5	20- GR-8	21- GR- 9	22- AAUDR- 1	23- Ashoka 200 -F	24- IR-24
25- IR-64					

Table 6
Similarity matrix of 25 rice genotypes for SSR primers

	1	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*	*12*	*13*	*14*	*15*	*16*	*17*	*18*	*19*	*20*	*21*	*22*	*23*	*24*	*25*
1	1.00																								
2	0.20	1.00																							
3	0.05	0.11	1.00																						
4	0.18	0.18	0.10	1.00																					
5	0.05	0.05	0.04	0.16	1.00																				
6	0.00	0.06	0.05	0.20	0.18	1.00																			
7	0.00	0.05	0.04	0.04	0.09	0.11	1.00																		
8	0.05	0.05	0.04	0.22	0.27	0.17	0.09	1.00																	
9	0.00	0.05	0.16	0.00	0.05	0.05	0.15	0.00	1.00																
10	0.05	0.00	0.00	0.10	0.15	0.17	0.14	0.20	0.04	1.00															
11	0.00	0.00	0.05	0.05	0.05	0.05	0.04	0.15	0.11	0.10	1.00														
12	0.11	0.05	0.10	0.05	0.00	0.05	0.00	0.15	0.05	0.09	0.05	1.00													
13	0.16	0.05	0.00	0.00	0.09	0.05	0.04	0.14	0.00	0.09	0.04	0.21	1.00												
14	0.00	0.00	0.10	0.00	0.05	0.05	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00											
15	0.05	0.00	0.05	0.05	0.05	0.12	0.04	0.04	0.00	0.10	0.00	0.05	0.00	0.05	1.00										
16	0.05	0.00	0.00	0.11	0.11	0.00	0.05	0.05	0.00	0.00	0.05	0.00	0.00	0.05	0.18	1.00									
17	0.00	0.00	0.10	0.10	0.04	0.11	0.00	0.09	0.00	0.04	0.05	0.04	0.00	0.05	0.23	0.05	1.00								
18	0.00	0.00	0.04	0.00	0.14	0.00	0.00	0.00	0.04	0.04	0.04	0.04	0.00	0.09	0.04	0.00	0.09	1.00							
19	0.00	0.00	0.00	0.00	0.05	0.00	0.00	0.00	0.00	0.00	0.11	0.00	0.00	0.11	0.05	0.18	0.05	0.21	1.00						
20	0.05	0.05	0.05	0.11	0.00	0.05	0.00	0.15	0.00	0.04	0.05	0.10	0.04	0.11	0.05	0.11	0.16	0.04	0.25	1.00					
21	0.00	0.00	0.10	0.00	0.04	0.05	0.00	0.00	0.00	0.00	0.00	0.00	0.04	0.16	0.05	0.00	0.04	0.20	0.05	0.00	1.00				
22	0.00	0.00	0.04	0.05	0.04	0.00	0.00	0.04	0.00	0.00	0.10	0.00	0.04	0.00	0.05	0.05	0.10	0.09	0.16	0.10	0.10	1.00			
23	0.05	0.00	0,04	0.10	0.09	0.00	0.00	0.04	0.04	0.00	0.00	0.00	0.00	0.00	0.00	0.16	0.00	0.00	0.04	0.00	0.04	0.04	1.00		
24	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.05	0.00	0.00	0.00	0.00	0.05	0.04	0.00	0.05	0.00	0.15	0.10	0.04	1.00	
25	0.00	0.00	0.10	0.09	0.15	0.14	0.04	1.00																	

Where

1.SK-20	2- GR-6	3- GR-7	4- GR-101	5-G-102	6- GR-104
7-Pankhali-203	8-Narmada	9-Basmati-370	10- Pusa Basmati-1121	11- GR-3	12- GR-11
13- GR-12	14-GAR-13	15-GR-103	16-GAR-1	17-GAR-2	18-Sathi-34-36
19- GR-5	20- GR-8	21- GR- 9	22- AAUDR- 1	23- Ashoka -200 F	24- IR-24
25- IR-64					

were found in the same sub-cluster 'A1'. Marker assisted selection (MAS) is being explored as an important supplement to phenotypic selection in rice breeding. PCR based markers offers great potential to enhance the efficiency of MAS, genetic variability (Gami *et al*., 2013). SSR markers have the advantages of rapidity, straight, and simplicity of RAPD, and the stability, reliability, and repeatability of RFLP. The results presented here clearly indicate that the microsatellite markers RM258, RM171 and RM3148 will be facilitating MAS of restorer lines in CMS-WA system from large source nurseries to avoid routine testcrosses in hybrid breeding programs. It is also expected that the use of these microsatellite markers in MAS integrated with backcross breeding will produce near isogenic lines (NILs) of fertility restorer lines for genetic research (Nematzadeh and Kiani, 2010). The identification of closely linked markers would be valuable for use in MAS strategy and finally help in map-based cloning of the fertility restorer gene in near future (Alavi *et al*. 2009).

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