Genetics of fertility restoration of 'Wild Abortive' system based Cytoplasmic Male Sterility (CMS) in hybrid rice (*Oryza sativa* L.)

Mahalingam, A[1*] and R. Saraswathi[2]

[1]National Pulses Research Centre, Tamil Nadu Agricultural University, Vamban – 622 303. [2]Department of Rice, Centre for Plant Breeding and Genetics, Tamil Nadu Agricultural University, Coimbatore – 641 003.

*Corresponding author's E-mail: mahalingamcpbg2008@gmail.com

Received: Jan 2, 2016 Accepted: Jan 20, 2016 Published: March 28, 2016

ABSTRACT

Success in the development of rice hybrids largely depends on the availability of effective restorers and precise basic knowledge on the genetics of fertility restoration of CMS and restorer lines. In a study using twenty diverse restorers and five 'WA' type cytoplasmic genetic male sterile (CMS) line, revealed the fertility restoration to be governed by two major genes with epistatic interactions that differed from crosses to crosses. The inheritance of fertility restoration in CRMS 32A × AD 06084R and ten other cross combinations revealed an F_2 segregation ratio of 12:3:1 (FF: SF: CS), indicating the involvement of two dominant genes which exhibit dominant epistasis. In COMS 24A x IET 20899R hybrid, pollen fertility of F_2 segregation fell into the digenic ratio 9FF: 3SF: 4CS showed the involvement of digenic supplementary or an epistasis with recessive gene action. F_2 segregation ratio of 9:6:1 with two dominant genes which exhibiting epistasis with incomplete dominance was observed in COMS 24A × IET 20898 R and fourteen other cross combinations suggesting the two dominant genes Rf_3 and Rf_4 seem to control the fertility restoration. The differential mode of action of restorer genes could presumably be due to the influence of the female parent genotype or to the variable expression of the weaker gene in different genetic backgrounds. The differential segregation behaviour could also be due to the existence of certain modifiers influencing the penetrance and expressivity of the fertility-restorer genes.

Key words: hybrid rice, fertility restoration, dominant epistasis, incomplete dominance

Cytoplasmic male sterility (CMS) caused by lesions or rearrangements of mitochondrial genome is unable to produce functional pollens. But CMS can be restored by nuclear genes. The combination of cytoplasmic male sterility (CMS) in one parent and a restorer gene (*Rf*) to restore fertility in another parent are indispensable for the development of hybrid varieties. Therefore, the CMS systems are widely used for hybrid seed production (Yuan, 1992). The cytoplasm derived from wild rice, causes WA-type CMS in a sporophytic manner and is widely used for the production of rice hybrid seeds. Two fertility restorer genes *viz*., *Rf3* and Rf_4 are required for the production of viable pollen in WA-type CMS. These genes have been mapped to chromosomes 1 and 10, respectively (Jing *et al.*, 2001). Most investigators tended to agree that restoration of wild abortive (WA) type CMS in rice is controlled by two nuclear genes (Zhang *et al.*, 1997; Yao *et al.*, 1997). Boro type (BT) CMS is restored by nuclear fertility restorer gene Rf_1, which was mapped on chromosome 10 (Yokozeki *et al.*, 1996) and was finally cloned by several workers (Kazama and Toriyama, 2003; Komori *et al.*, 2004; Akagi *et al.*, 2004). HL type fertility restoration genes Rf_5 and Rf_6 were also mapped on chromosome 10 (Liu *et al.*, 2004)

The genetics of fertility restoration in WA-CMS lines has been shown to follow monogenic (Mishra *et al.*, 2003), digenic (Bharaj *et al.*, 1990), digenic with different types of interaction (Sarkar *et al.*, 2002), trigenic (Kumar and Chakrabarti, 1983) and trigenic interactions (Huang *et al.*, 1987). Nevertheless, most of the investigations tend to indicate that fertility restoration of the WA cytosterility system is controlled by two nuclear genes. There are many researchable issues still to be answered to put hybrid breeding programme on a sound footing for making sustained progress. Of

these, the nature of inheritance of the fertility restoring genes is an important aspect where knowledge is inadequate. In order to have a well - directed restorer breeding programme, adequate knowledge on genetic control of male fertility restoration is necessary, which is useful for transferring the fertility restoring genes to promising breeding lines to develop improved restorers. Hence, the present scientific investigation was taken up to study the magnitude of inheritance of fertility restoration genes in hybrid rice.

MATERIALS AND METHODS

A total of 51 tester lines (18 AICRIP parental lines, 13 recently stabilized breeding lines and 20 advanced cultures / lines under evaluation) and five cytoplasmic male sterile (CMS) lines COMS 23A, COMS 24A, COMS 25A, CRMS 31A and CRMS 32A possessing 'WA' cytoplasm formed the basic genetic materials for this study were raised during September 2009 at Tamil Nadu Rice Research Institute (TRRI), Aduthurai. All the 51 tester lines were crossed with five CMS lines in L x T mating design and a total of 255 test cross F_1 hybrids were synthesized. Resultant 255 hybrids were raised in a randomized block design with two replications during January 2010 using single seedling per hill at a spacing of 20 x 20 cm and observations were recorded on pollen and spikelet fertility. Thirty six F_1 hybrids with varied pollen fertility were selected, selfed and their F_2 generations were raised during July 2010.

The F_2 seedlings of each crosses were planted at a spacing of 20 x 20 cm with a population of 250 to 400 plants per cross. Genetics of fertility restoration was worked out through pollen fertility studies using 1% I_2-KI solution to identify fertile (stained) and sterile (non-stained) pollens, for which the anthers were collected from three randomly chosen spikelets (top and middle) and pollen grains were teezed out of the anther on a glass slide. The fertile and sterile pollen grains were counted in three microscopic fields under a binocular microscope. Pollen fertility was calculated as the ratio between the number of fertile pollen grains (stained round) and the total number of pollen grains in the microscopic field (*i.e*., fertile and sterile). Plants were classified into different fertility sterility groups as was done by Chaudhary *et al.* (1981). Plants with more than 60% fertile pollen were grouped as fully fertile (FF), those with 30–60% fertile pollen as partial fertile (PF), those having 1–30% fertile pollen as partial sterile (PS) and those which had 0% were grouped as completely sterile (CS). To fit into various Mendelian genetic ratios, the partially fertile and partially sterile plants were grouped into a single category as semi fertile (SF). The goodness of fit for various Mendelian genetic ratios in F_2 generation was tested using the χ^2 statistic.

RESULTS AND DISCUSSION

In the present investigation, segregation pattern for pollen and spikelet fertility of crosses involving twenty genetically diverse restorers and five WA type CMS lines was studied **(Table 1)**. Results showed that pollen fertility ranged between 64.55% (COMS 24A x IET 20899 to 100% (CRMS31A x IET20898R and CRMS31A x AD09525R). On the other hand, spikelet fertility ranged between 28.31% (COMS24A x IET20899R) and 95.56% (COMS23A x IET20898R) with a mean value of 86.08. The results revealed that fertility restoration is under dominant gene control and the degree of restoration varied with the restorers (Bharaj *et al*., 1991). In general, spikelet fertility count showed 8 to 10% higher values as compared to pollen fertility. IET 20898R showed the best restoring ability with the highest pollen and spikelet fertility counts. On the other hand, IET 20899R showed the lowest pollen and fertility of 64.55% and spikelet fertility count of 28.31%.

In F_2 generation data were recorded on both pollen and spikelet fertility. Since spikelet fertility data did not give any convincing pattern as it is influenced by several physiological and environmental factors, the data on pollen fertility were considered reliable for the study. The spikelet fertility is also influenced by pollen of partial stainability. Segregation pattern of pollen fertility in F_2 generation of test cross hybrids are presented in **Table 2**. In the present study, the inheritance of fertility restoration in the cross combination CRMS32A × AD06084R reveals an F_2 segregation ratio of 12:3:1 (FF: SF: CS), indicating the involvement of two dominant genes which exhibit dominant epistasis. This suggests that two dominant genes Rf_3 and Rf_4 seem to control the fertility restoration. The effect of one of the two dominant genes (Rf_3) in restoring fertility appears to be strong and as good as the two together (Rf_3Rf_4) while the other gene (Rf_4) showed weak restoration. The

homozygous or heterozygous plants for both the dominant genes (Rf_3Rf_3 Rf_4Rf_4 *or* $Rf_3rf_3Rf_4rf_4$) and those having homozygous or heterozygous dominant gene (Rf_3) and the other gene (Rf_4) as homozygous recessive gene ($Rf_3Rf_3rf_4rf_4$, $Rf_3rf_3rf_4rf_4$) were fully fertile. This indicated that the strong dominant gene Rf_3 alone could control the fertility restoration. While the plants homozygous for rf_3 (rf_3rf_3) and homozygous dominant ($rf_3rf_3Rf_4Rf_4$) or heterozygous dominant ($rf_3rf_3Rf_4rf_4$) at Rf_4 locus were semi fertile. The plants homozygous for recessive alleles of both the genes ($rf_3rf_3rf_4rf_4$) were completely sterile.

Table 1. Pollen and spikelet fertility of F_1 hybrid and spikelet fertility of F_2 populations

Sl. No	Crosses	Pollen fertility (%)	Spikelet fertility (%)	Pollen fertility (%)
		F_1	F_1	F_2
1	**COMS 23A x IET 19863R**	90.53	90.21	68.05
2	**COMS 23A x IET 20881R**	91.24	94.19	70.67
3	**COMS 23A x IET 20885R**	90.31	90.69	65.86
4	**COMS 23A x IET 20888R**	97.41	81.60	72.53
5	**COMS 23A x IET 20897R**	93.33	92.27	69.53
6	**COMS 23A x IET 20898R**	93.17	95.56	73.22
7	**COMS 23A x IET 20937R**	95.98	92.54	69.84
8	**COMS 23A x AD 09194R**	96.41	92.01	72.88
9	**COMS 24A x IET 19863R**	91.58	95.16	71.66
10	**COMS 24A x IET 20885R**	85.05	82.99	66.88
11	**COMS 24A x IET 20897R**	95.66	93.74	76.92
12	**COMS 24A x IET 20898R**	93.21	92.92	68.04
13	**COMS 24A x IET 20899R**	64.55	28.31	68.09
14	**COMS 24A x IET 20945R**	91.96	91.33	75.85
15	**COMS 24A x AD 09525 R**	93.92	90.69	66.38
16	**COMS 24A x AD 09529 R**	92.03	82.95	70.28
17	**COMS 24A x AD 09530 R**	93.03	80.60	67.06
18	**COMS 25A x IET 19863R**	91.94	81.01	63.88
19	**CRMS 31A x IET 19863R**	91.78	88.56	55.87
20	**CRMS 31A x IET 20881R**	93.21	90.84	64.52
21	**CRMS 31A x IET 20897R**	95.99	90.97	68.57
22	**CRMS 31A x IET 20898R**	100.00	91.49	66.61
23	**CRMS 31A x AD 09525 R**	100.00	93.85	72.42
24	**CRMS 31A x AD 06084R**	90.06	93.16	71.88
25	**CRMS 31A x AD 07076R**	82.00	35.10	61.83
26	**CRMS 31A x AD 07158R**	86.88	82.72	72.51
27	**CRMS 31A x AD 08005R**	80.90	81.30	53.09
28	**CRMS 31A x AD 09194R**	90.84	92.31	70.73
29	**CRMS 32A x IET 19863R**	91.85	86.49	66.21
30	**CRMS 32A x IET 20897R**	92.19	82.10	68.93
31	**CRMS 32A x IET 20937R**	96.03	90.34	70.22
32	**CRMS 32A x AD 06084R**	91.05	90.35	74.52
33	**CRMS 32A x AD 07083R**	91.07	91.99	59.83
34	**CRMS 32A x AD 07309R**	81.21	93.42	69.38
35	**CRMS 32A x AD 08005R**	90.46	90.54	61.31
36	**CRMS 32A x AD 08010R**	83.21	84.70	46.83
	Mean	**90.83**	**86.08**	**67.58**
	CD at 5%	**4.78**	**4.82**	**5.02**
	CD at 1%	**6.42**	**6.59**	**6.73**

Table 2. Segregation pattern of pollen fertility in F_2 generation of test cross hybrids

Sl. No	Crosses	No. of plants observed						Genetic ratio	Chi – square value
		FF	PF	PS	SMS	FS	Total		
1	**COMS 24A x IET 20898R**	179	63	55	118	20	317	**9:6:1**	0.004^{ns}
2	**CRMS 32A x AD 06084R**	176	26	20	46	15	237	**12:3:1**	0.023^{ns}
3	**COMS 23A x IET 20898R**	182	25	18	43	15	240	**12:3:1**	0.033^{ns}
4	**COMS 23A x IET 20888 R**	172	33	14	47	15	234	**12:3:1**	0.095^{ns}
5	**COMS 24A x AD 09530 R**	183	75	40	115	21	319	**9:6:1**	0.110^{ns}
6	**CRMS 32A x IET 19863R**	74	30	16	46	8	128	**9:6:1**	0.063^{ns}
7	**COMS 24A x IET 20885R**	128	47	38	85	14	227	**9:6:1**	0.001^{ns}
8	**CRMS 31A x IET 20897R**	143	40	56	96	16	255	**9:6:1**	0.001^{ns}
9	**COMS 25A x IET 19863R**	206	73	70	143	23	372	**9:6:1**	0.061^{ns}
10	**CRMS 31A x IET 20898R**	184	46	80	126	20	330	**9:6:1**	0.034^{ns}
11	**CRMS 31A x AD 06084R**	169	27	14	41	14	224	**12:3:1**	0.009^{ns}
12	**COMS 24A x IET 19863R**	217	31	22	53	18	288	**12:3:1**	0.007^{ns}
13	**COMS 23A x IET 20937R**	147	52	48	100	16	263	**9:3:3:1**	0.011^{ns}
14	**CRMS 32A x AD 07309R**	215	30	27	57	18	290	**12:3:1**	0.045^{ns}
15	**COMS 24A x IET 20898R**	191	28	23	51	16	258	**12:3:1**	0.05^{ns}
16	**COMS 23A x IET 19863R**	185	70	48	118	20	323	**9:6:1**	0.064^{ns}
17	**COMS 24A x AD 09525 R**	158	53	49	102	17	279	**9:6:1**	0.030^{ns}
18	**CRMS 32A x IET 20897R**	165	56	53	109	18	292	**9:6:1**	0.003^{ns}
19	**COMS 24A x AD 09529 R**	110	37	34	71	12	193	**9:3:3:1**	0.020^{ns}
20	**CRMS 31A x IET 20881R**	172	60	60	120	20	312	**9:6:1**	0.069^{ns}
21	**COMS 23A x IET 20897R**	165	60	48	108	18	291	**9:6:1**	0.010^{ns}
22	**COMS 23A x AD 09194R**	195	25	24	49	16	260	**12:3:1**	0.001^{ns}
23	**COMS 24A x IET 20899R**	210	18	20	38	23	271	**9:3:4**	0.039^{ns}
24	**COMS 24A x IET 20945R**	215	28	30	58	18	291	**12:3:1**	0.077^{ns}
25	**CRMS 31A x AD 07076R**	170	65	44	109	18	297	**9:6:1**	0.049^{ns}
26	**CRMS 31A x AD 07158R**	158	19	21	40	13	211	**12:3:1**	0.001^{ns}
27	**CRMS 31A x AD 09194R**	191	24	25	49	16	256	**12:3:1**	0.007^{ns}
28	**CRMS 31A x AD 09525 R**	228	78	75	153	25	406	**9:3:3:1**	0.002^{ns}
29	**CRMS 32A x IET 20937R**	156	52	50	102	17	275	**9:3:3:1**	0.011^{ns}

Table 3. Rf gene complex

Sl. No	Cross combination	CMS line	Restorer	Genetic constitution			Genetic Ratio
				FF	SF	CS	
1	COMS 24A x IET 20898R	$rf_3rf_3rf_4rf_4rf_e`rf_e`$	$Rf_3Rf_3Rf_4Rf_4Rf_e`Rf_e`$	Rf_3-Rf_4-	$3rf_3rf_3Rf_4$- $3Rf_3$-rf_4rf_4	$1rf_3rf_3rf_4rf_4$	**9:6:1**
2	CRMS 32A x AD 06084R	rf_3rf_3 rf_4rf_4	$Rf_3Rf_3Rf_4Rf_4Rf_e`Rf_e`$	$9\ Rf_3$— Rf_4— $3\ Rf_3$— $rf4rf_4$	$3\ rf_3rf_3Rf_4$—	$1rf_3rf_3rf_4rf_4$	**12:3:1**
3	COMS 23A x IET 20898R	rf_3rf_3 rf_4rf_4	$Rf_3Rf_3Rf_4Rf_4Rf_e`Rf_e`$	$9\ Rf_3$— Rf_4— $3\ Rf_3$— $rf4rf_4$	$3\ rf_3rf_3Rf_4$—	$1rf_3rf_3rf_4rf_4$	**12:3:1**
4	COMS 23A x IET 20888 R	rf_3rf_3 rf_4rf_4	$Rf_3Rf_3Rf_4Rf_4Rf_e`Rf_e`$	$9\ Rf_3$— Rf_4— $3\ Rf_3$— $rf4rf_4$	$3\ rf_3rf_3Rf_4$—	$1rf_3rf_3rf_4rf_4$	**12:3:1**
5	COMS 24A x AD 09530 R	$rf_3rf_3rf_4rf_4rf_e`rf_e`$	$Rf_3Rf_3Rf_4Rf_4Rf_e`Rf_e`$	Rf_3-Rf_4-	$3rf_3rf_3Rf_4$- $3Rf_3$-rf_4rf_4	$1rf_3rf_3rf_4rf_4$	**9:6:1**
6	CRMS 32A x IET 19863R	$rf_3rf_3rf_4rf_4rf_e`rf_e`$	$Rf_3Rf_3Rf_4Rf_4Rf_e`Rf_e`$	Rf_3-Rf_4-	$3rf_3rf_3Rf_4$- $3Rf_3$-rf_4rf_4	$1rf_3rf_3rf_4rf_4$	**9:6:1**
7	COMS 24A x IET 20885R	$rf_3rf_3rf_4rf_4rf_e`rf_e`$	$Rf_3Rf_3Rf_4Rf_4Rf_e`Rf_e`$	Rf_3-Rf_4-	$3rf_3rf_3Rf_4$- $3Rf_3$-rf_4rf_4	$1rf_3rf_3rf_4rf_4$	**9:6:1**
8	CRMS 31A x IET 20897R	$rf_3rf_3rf_4rf_4rf_e`rf_e`$	$Rf_3Rf_3Rf_4Rf_4Rf_e`Rf_e`$	Rf_3-Rf_4-	$3rf_3rf_3Rf_4$- $3Rf_3$-rf_4rf_4	$1rf_3rf_3rf_4rf_4$	**9:6:1**
9	COMS 25A x IET 19863R	$rf_3rf_3rf_4rf_4rf_e`rf_e`$	$Rf_3Rf_3Rf_4Rf_4Rf_e`Rf_e`$	Rf_3-Rf_4-	$3rf_3rf_3Rf_4$- $3Rf_3$-rf_4rf_4	$1rf_3rf_3rf_4rf_4$	**9:6:1**
10	CRMS 31A x IET 20898R	$rf_3rf_3rf_4rf_4rf_e`rf_e`$	$Rf_3Rf_3Rf_4Rf_4Rf_e`Rf_e`$	Rf_3-Rf_4-	$3rf_3rf_3Rf_4$- $3Rf_3$-rf_4rf_4	$1rf_3rf_3rf_4rf_4$	**9:6:1**
11	CRMS 31A x AD 06084R	rf_3rf_3 rf_4rf_4	$Rf_3Rf_3Rf_4Rf_4Rf_e`Rf_e`$	$9\ Rf_3$— Rf_4— $3\ Rf_3$— $rf4rf_4$	$3\ rf_3rf_3Rf_4$—	$1rf_3rf_3rf_4rf_4$	**12:3:1**
12	COMS 24A x IET 19863R	rf_3rf_3 rf_4rf_4	$Rf_3Rf_3Rf_4Rf_4Rf_e`Rf_e`$	$9\ Rf_3$— Rf_4— $3\ Rf_3$— $rf4rf_4$	$3\ rf_3rf_3Rf_4$—	$1rf_3rf_3rf_4rf_4$	**12:3:1**
13	COMS 23A x IET 20937R	rf_3rf_3 rf_4rf_4	$Rf_3Rf_3Rf_4Rf_4Rf_e`Rf_e`$	$9\ Rf_3$— Rf_4— $3\ Rf_3$— $rf4rf_4$	$3\ rf_3rf_3Rf_4$—	$1rf_3rf_3rf_4rf_4$	**9:3:3:1**
14	CRMS 32A x AD 07309R	rf_3rf_3 rf_4rf_4	$Rf_3Rf_3Rf_4Rf_4Rf_e`Rf_e`$	$9\ Rf_3$— Rf_4— $3\ Rf_3$— $rf4rf_4$	$3\ rf_3rf_3Rf_4$—	$1rf_3rf_3rf_4rf_4$	**12:3:1**
15	COMS 24A x IET 20898R	rf_3rf_3 rf_4rf_4	$Rf_3Rf_3Rf_4Rf_4Rf_e`Rf_e`$	$9\ Rf_3$— Rf_4— $3\ Rf_3$— $rf4rf_4$	$3\ rf_3rf_3Rf_4$—	$1rf_3rf_3rf_4rf_4$	**12:3:1**
16	COMS 23A x IET 19863R	$rf_3rf_3rf_4rf_4rf_e`rf_e`$	$Rf_3Rf_3Rf_4Rf_4Rf_e`Rf_e`$	Rf_3-Rf_4-	$3rf_3rf_3Rf_4$- $3Rf_3$-rf_4rf_4	$1rf_3rf_3rf_4rf_4$	**9:6:1**
17	COMS 24A x AD 09525 R	$rf_3rf_3rf_4rf_4rf_e`rf_e`$	$Rf_3Rf_3Rf_4Rf_4Rf_e`Rf_e`$	Rf_3-Rf_4-	$3rf_3rf_3Rf_4$- $3Rf_3$-rf_4rf_4	$1rf_3rf_3rf_4rf_4$	**9:6:1**
18	CRMS 32A x IET 20897R	$rf_3rf_3rf_4rf_4rf_e`rf_e`$	$Rf_3Rf_3Rf_4Rf_4Rf_e`Rf_e`$	Rf_3-Rf_4-	$3rf_3rf_3Rf_4$- $3Rf_3$-rf_4rf_4	$1rf_3rf_3rf_4rf_4$	**9:6:1**

19	COMS 24A x AD 09529 R	*rf_3rf_3 rf_4rf_4*	*$Rf_3Rf_3Rf_4Rf_4Rf_e$`$Rf_e$`*	*9 Rf_3— Rf_4— 3 Rf_3— $rf4rf_4$*	*3 $rf_3rf_3Rf_4$—*	*1$rf_3rf_3rf_4rf_4$*	**9:3:3:1**
20	CRMS 31A x IET 20881R	*$rf_3rf_3rf_4rf_4rf_e$`$rf_e$`*	*$Rf_3Rf_3Rf_4Rf_4Rf_e$`$Rf_e$`*	*Rf_3-Rf_4-*	*3$rf_3rf_3Rf_4$- 3Rf_3-rf_4rf_4*	*1$rf_3rf_3rf_4rf_4$*	**9:6:1**
21	COMS 23A x IET 20897R	*$rf_3rf_3rf_4rf_4rf_e$`$rf_e$`*	*$Rf_3Rf_3Rf_4Rf_4Rf_e$`$Rf_e$`*	*Rf_3-Rf_4-*	*3$rf_3rf_3Rf_4$- 3Rf_3-rf_4rf_4*	*1$rf_3rf_3rf_4rf_4$*	**9:6:1**
22	COMS 23A x AD 09194R	*rf_3rf_3 rf_4rf_4*	*$Rf_3Rf_3Rf_4Rf_4Rf_e$`$Rf_e$`*	*9 Rf_3— Rf_4— 3 Rf_3— $rf4rf_4$*	*3 $rf_3rf_3Rf_4$—*	*1$rf_3rf_3rf_4rf_4$*	**12:3:1**
23	COMS 24A x IET 20899R	*rf_3rf_3 rf_4rf_4*	*$Rf_3Rf_3Rf_4Rf_4Rf_e$`$Rf_e$`*	9 *Rf_3-Rf_4-*	3 *Rf_3-rf_4rf_4*	3 *$rf_3rf_3Rf_4$-* 1$rf_3rf_3rf_4rf_4$	**9:3:4**
24	COMS 24A x IET 20945R	*rf_3rf_3 rf_4rf_4*	*$Rf_3Rf_3Rf_4Rf_4Rf_e$`$Rf_e$`*	*9 Rf_3— Rf_4— 3 Rf_3— $rf4rf_4$*	*3 $rf_3rf_3Rf_4$—*	*1$rf_3rf_3rf_4rf_4$*	**12:3:1**
25	CRMS 31A x AD 07076R	*$rf_3rf_3rf_4rf_4rf_e$`$rf_e$`*	*$Rf_3Rf_3Rf_4Rf_4Rf_e$`$Rf_e$`*	*Rf_3-Rf_4-*	*3$rf_3rf_3Rf_4$- 3Rf_3-rf_4rf_4*	*1$rf_3rf_3rf_4rf_4$*	**9:6:1**
26	CRMS 31A x AD 07158R	*rf_3rf_3 rf_4rf_4*	*$Rf_3Rf_3Rf_4Rf_4Rf_e$`$Rf_e$`*	*9 Rf_3— Rf_4— 3 Rf_3— $rf4rf_4$*	*3 $rf_3rf_3Rf_4$—*	*1$rf_3rf_3rf_4rf_4$*	**12:3:1**
27	CRMS 31A x AD 09194R	*rf_3rf_3 rf_4rf_4*	*$Rf_3Rf_3Rf_4Rf_4Rf_e$`$Rf_e$`*	*9 Rf_3— Rf_4— 3 Rf_3— $rf4rf_4$*	*3 $rf_3rf_3Rf_4$—*	*1$rf_3rf_3rf_4rf_4$*	**12:3:1**
28	CRMS 31A x AD 09525 R	*rf_3rf_3 rf_4rf_4*	*$Rf_3Rf_3Rf_4Rf_4Rf_e$`$Rf_e$`*	*9 Rf_3— Rf_4— 3 Rf_3— $rf4rf_4$*	*3 $rf_3rf_3Rf_4$—*	*1$rf_3rf_3rf_4rf_4$*	**9:3:3:1**
29	CRMS 32A x IET 20937R	*rf_3rf_3 rf_4rf_4*	*$Rf_3Rf_3Rf_4Rf_4Rf_e$`$Rf_e$`*	*9 Rf_3— Rf_4— 3 Rf_3— $rf4rf_4$*	*3 $rf_3rf_3Rf_4$—*	*1$rf_3rf_3rf_4rf_4$*	**9:3:3:1**

The F_2 population of the crosses COMS23A × IET20898R, COMS23A × IET20888R, CRMS31A × AD06084R, COMS24A × IET19863R, CRMS32A × AD07309R, COMS24A × IET20898R, COMS23A × AD09194R, COMS24A × IET20945R, CRMS31A × AD07158R and CRMS31A × AD09194R exhibited a similar segregation ratio of 12:3:1 of FF:SF:CS type of plants, also indicating the epistasis with dominant gene action controlled by two dominant genes. Epistasis with dominant gene action in the inheritance of fertility restoration of WA-CMS system has been reported by earlier workers (Sarkar *et al*., 2002 and Hossain *et al*., 2010). When the CMS line COMS24A crossed with the restorer line IET 20899R, the F_2 segregation for pollen fertility fell into the digenic ratio 9FF: 3SF: 4CS. The results indicated the involvement of the digenic supplementary or epistasis with recessive gene action. Assuming that Rf_3 and Rf_4 were the dominant alleles of the two restorer genes, the fertility restoring action of Rf_3 seemed to be stronger than Rf_4. The segregation pattern in the cross combination indicated that when both dominant genes were present together in heterozygous ($Rf_3Rf_3Rf_4rf_4$ or $Rf_3rf_3Rf_4rf_4$ or $Rf_3rf_3Rf_4Rf_4$) or homozygous condition ($Rf_3Rf_3Rf_4Rf_4$) the plants were fully fertile. The homozygous rf_4rf_4 plants with homozygous dominant (Rf_3Rf_3) or heterozygous dominant (Rf_3rf_3) for the Rf_3 gene fell in the semi-fertile group. The homozygous rf_3rf_3 plants with homozygous dominant (Rf_4Rf_4) or heterozygous dominant (Rf_4rf_4) for Rf_4 locus were completely sterile. The dominant allele of Rf_4 gene did not show any effect of fertility restoration in the absence of the other dominant allele of the Rf_3 gene. Thus, the two genes appeared to have additive effects in imparting full fertility restoration. The plants homozygous for recessive alleles of both the genes (rf_3rf_3 rf_4rf_4) were completely sterile. The F_2 ratio of 9:3:4 involving supplementary or epistasis with recessive gene action has been reported earlier by Shoud and Phul (1995), Govinda Raj and Virmani (1988), Ramalingam *et al*. (1992), Sarkar *et al*. (2002) and Hossain *et al*. (2010)

In COMS24A × IET20898R cross combination, fertility restoration study reveals a F_2 segregation ratio of 9:6:1 (FF: SF: CS), indicating the involvement of two dominant genes which exhibit epistasis with incomplete dominance. This suggests that two dominant genes Rf_3 and Rf_4 seem to control the fertility restoration. The effect of one of the two dominant genes (Rf_3) in restoring fertility appears to be strong and as good as the two together (Rf_3Rf_4) while the other gene (Rf_4) showed weak restoration. When both genes (Rf_3 and Rf_4) are separate, then two dominant alleles have similar effect. The homozygous or heterozygous plants for both the dominant genes ($Rf_3Rf_3Rf_4Rf_4$ or $Rf_3Rf_3Rf_4rf_4$ or $Rf_3rf_3Rf_4Rf_4$ or $Rf_3rf_3Rf_4rf_4$) were fully fertile and those plants having homozygous recessive gene for Rf_3 or Rf_4 ($rf_3rf_3Rf_4Rf_4$ or $rf_3rf_3Rf_4rf_4$ or $Rf_3Rf_3rf_4rf_4$ or $Rf_3rf_3rf_4rf_4$) were semi sterile (**Table 3**). This indicated the necessity of any one of the dominant gene for fertility restoration, when both the dominant genes are present together then fertility restoration was so stronger. The plants homozygous for recessive alleles of both the genes ($rf_3rf_3rf_4rf_4$) were completely sterile. The F_2 population of the crosses COMS24A × IET 20898R, COMS24A × AD09530R, CRMS32A × IET19863R, COMS24A × IET20885R, CRMS31A × IET20897R, COMS25A × IET19863R, CRMS31A × IET20898R, COMS23A × IET19863R, COMS24A × AD09525R, CRMS32A × IET20897R, CRMS31A × IET20881R, COMS23A × IET20897R and CRMS31A × AD07076R exhibited a similar segregation ratio of 9:6:1 of FF:SF:CS type of plants, thus indicating the epistasis with incomplete dominance type of gene action controlled by two dominant genes. An epistasis with dominant type of gene action in the inheritance of fertility restoration of WA-CMS system has also been reported by earlier workers (Ramalingam *et al*., 1992 and Sarkar *et al*., 2002).

CONCLUSION

The differential mode of action of restorer genes could presumably be due to the influence of the female parent genotype or to the variable expression of the weaker gene in different genetic backgrounds. The differential segregation behaviour could also be due to the existence of certain modifiers influencing the penetrance and expressivity of the fertility-restorer genes. The explanation of obtaining different ratios of the same restorer is that the two F_1 hybrids differ from each other in respect of nuclear genetic contribution from CMS lines which are obviously different. After one cycle of meiosis, the genomic contribution from the CMS and restorer lines is randomly distributed to different F_2 plants and, unlike the F_1 plants, the F_2 plants are likely to have the

different genetic constitution. This change in genetic background as a result of recombination is likely to have an influence on the genetics of fertility restoration.

REFERENCES

Akagi, H., A. Nakamura, Y. Yokozeki, A. Inagaki, H. Takahashi, K. Mori and T. Fujimura. 2004. Positional cloning of the rice *Rf-1* gene, a restorer of BT-type cytoplasmic male sterility that encodes a mitochondria targeting PPR protein. *Theor. Appl. Genet.* 108: 1449–1457.

Bharaj T. S., S. S. Bains, G. S. Sidhu and M. R. Gagneja. 1991. Genetics of fertility restoration of 'wild abortive' cytoplasmic male sterility in rice *(Oryza sativa* L.). *Euphytica,* 56: 199-203.

Bharaj, T. S., G. S. Sidhu and S. S. Gill. 1990. Ease of fertility restoration in CMS lines. *Int. Rice Res. Newsl.,* 15: 4 – 5.

Chaudhary, R. C., S. S. Virmani, and G.S. Khush .1981. Patterns of pollen abortion in some cytoplasmic genetic male sterile liens of rice. *Oryza,* 18: 140–2.

Govinda Raj, K. and E. A. Siddiq. 1985. Genetics of fertility restoration and biochemical basis of male sterility – fertility system in rice. *Rice. Genet. Newsl.,* 2: 103 – 104.

Govindaraj, K. and Virmani, S.S. 1988. Genetics of fertility restoration of 'WA' type cytoplasmic male sterility in rice. *Crop Sci.*, 28:787-792.

Huang, C. S., T. Tseng and C. G. Shen. 1987. Gentic analysis of fertility restoration in cytoplasmically male sterile indica rice. J. *Agric. Res. China.* 36: 137 – 150.

Jing, R., X. Li, P. Yi, Y. Zhu. 2001. Mapping fertility restoring genes of rice WA cytoplasmic male sterility using SSLP markers. Bot Bull Acad Sin., 42:167–171.

Kazama, T. and K. Toriyama. 2003. A pentatricopeptide repeat-containing gene that promotes the processing of aberrant apt6 RNA of cytoplasmic male-sterile rice. *FEBS Lett.*, 544: 99-102.

Komori, T., T.Yamamoto, N. Takemori, M. Kashihara and H. Matsushima. 2003. Fine genetic mapping of the nuclear gene, Rf-1, that restores the BT-type cytoplasmic male sterility in rice (*Oryza sativa* L.) by PCR-based markers. *Euphytica,* 129: 241-247.

Kumar, S. and S. N. Chakrabarti. 1983. Genetic and cytogenetic analysis of spikelet sterility in indica_japonica crosses in *Oryza sativa* L. Indian J Genet Plant Breed., 60: 441–50.

Liu, X.Q., X. Xu, Y.P. Tan, S.Q. Li and J. Hu. 2004. Inheritance and molecular mapping of two fertility-restoring loci for Hongolian gametophytic cytoplasmic male sterility in rice (*Oryza sativa* L.). *Mol. Genet. Genom.*, 271: 586-94.

Md. Sarowar Hossaina, Ashok Kumar Singh and Fasih-uz-Zaman. 2010. Genetics of fertility restoration of 'WA'-based cytoplasmic male sterility system in rice (Oryza sativa) using indica / japonica derivative restorers. *Science Asia,* 36: 94–99.

Mishra, G.P., R. K, Singh, T. Mohapatra, A.K, Singh, K.V, Prabhu, F.U, Zaman and R.K, Sharma. 2003. Molecular mapping of a gene for fertility restoration of wild abortive cytoplasmic male sterility using a Basmati rice restorer line. J Plant Biochem Biotech. 12:37–42.

Ramalingam, J., N. Nadarajan, P. Rangasamy and C. Vanniarajan. 1992. Genetic analysis of fertility restoration in hybrid rice (*Oryza sativa* L.). *Ann. Agric. Res.*, 13: 221 – 223.

Sarkar, C.K.G., F.U. Zaman and A.K. Singh. 2002. Genetics of fertility restoration of 'WA' based cytoplasmic male sterility system in rice (Oryza sativa L.) using basmati restorer lines. *Indian J Genet Plant Breed*., 62: 305–8.

Sohu, V.S. and P.S. Phul. 1995. Inheritance of fertility restoration of three source of

cytoplasmic male sterility in rice. J Genet *Breed*., 49: 93–6.

Yao, F.Y., C.G. Xu, S.B. Yu, X.J. Li, Y.J. Gao, X.H. Li and Q. F. Zhang. 1997. Mapping and genetic analysis of two fertility restorer loci in the wildabortive cytoplasmic male sterility system of rice (*Oryza sativa* L.). *Euphytica*, 98: 183-187.

Yokozeki, Y., A. Nakamura, A. Inagaki, N. Ichikawa, Y. Koshino, H. Akagi, H. Shimada, T. Tsuchiya, C. Shinjyo and T. Fujimura. 1996. DNA markers of a fertility restoration gene *Rf*1 in rice (*Oryza sativa* L). *Breeding Sci.*, 46 (1):73

Yuan, L.P. 1992. Development and prospect of hybrid rice breeding. In: You CB and Chen ZL (eds.) Agricultural Biotechnology. Proc. Asian-Pacific Conf. Agric. Biotechnol., China Sciences and Technology Press, Beijing, 97-105.

Zhang, G., T.S. Bharaj, S.S. Virmani and N. Huang. 1997. Mapping of the *Rf3* nuclear fertility restoring gene for WA cytoplasmic male sterility in rice using RAPD and RFLP markers. *Theor. Appl. Genet.*, 94: 27-33.