Evaluation of cultivars and land races of *Oryza sativa* for restoring and maintaining wild abortive cytoplasm

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Identification of restorers and maintainers from cultivars and landraces through test crossing and their use in further breeding programme are the initial steps in three-line heterosis breeding. Two experiments, one in the greenhouse for F_1 hybrid seeds production and another in the field for parental screening, were conducted during the 1999 rice growing season at the Institute of Agriculture and Animal Science (IAAS, TU), Rampur, Nepal. Three cytoplasmic male sterile (CMS) lines, eight improved cultivars and six landraces of rice were studied for their fertility restoring and sterility maintaining abilities. Pollen sterility was studied based on their stainability with potassium iodide iodine (I-KI) solution. On the basis of their interaction with I-KI, pollens were categorized as unstained withered sterile (UWS), unstained spherical sterile (USS), stained round sterile (SRS) and stained round fertile (SRF). For each hybrid, the percentage of spikelet fertility was estimated. The test lines were categorized as restorers, partial restorers, maintainers, and partial maintainers on the basis of pollen sterility and spikelet fertility. The male sterile lines had mostly UWS and USS types of pollen, whereas the restorer lines had more SRS and SRF types. There was no strong evidence for a relationship between pollen fertility and spikelet fertility. Five restorers, three partial restorers, two partial maintainers and four maintainers were identified. These restorers can be used to develop the hybrid seed while maintainers to maintain and/or to develop new CMS lines, because these are locally adapted cultivars. Pedigree analysis revealed that, for some of these test lines, TN-1 and CR94-13 might be the donors of maintainer and restorer gene(s), respectively.

Key words: CMS line, maintainer, restorer, rice

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Introduction

In Nepal, rice accounts for about 50% of the total cropped area and food production (Upadhaya 1996). Efforts to improve rice productivity in Nepal have resulted in the introduction of a large number of improved cultivars with varying yield potentials. To meet the demand created by increasing population and rising incomes, it is important to increase the yield potential of rice beyond that of semi-dwarf cultivars. Experiences in China, India, and Vietnam have established that hybrid rice offers an economically viable option to increase cultivar yield. The usual method for raising hybrids is to establish many inbred lines, perform inter-crosses and determine which hybrids are most productive in a given locality. As the female parents have to be male sterile, they should be maintained in every generation and male sterile lines have to be developed. They should be locally adapted and should perform well in hybrid combinations. The basic requisites for successful hybrid rice production are development of male sterile lines (A), maintainers (B) and restorers of fertility (R). Lin and Yuan (1980) reported the use of an effective restorer in China in commercial F_1 hybrids involving the wild aborted (WA) cytosterility system in 1973. Effective restorer lines for WA, Gam and Bt cytosterility systems have been identified among cultivated rice cultivars and elite breeding lines (Shinjyo 1969, 1972, Lin and Yuan 1980). For the CMS-WA system hundreds of effective restorer lines have been identified among cultivated rice cultivars and elite breeding lines bred in China (Lin and Yuan 1980, Yuan et al. 1994), International Rice Research Institute (IRRI 1983, Govinda Raj and Virmani 1988, Virmani 1994), Indonesia (Suprihatno et al. 1994), India (Rangaswamy et al. 1987, Siddiq et al. 1994), and the Philippines (Lara et al. 1994). The restorer lines for WA cytosterility were found more stable and their restoration ability was stronger (Virmani 1996). The frequency of restorer lines was higher among late maturing Indica cultivars and negligible among Japonica cultivars (Lin and Yuan 1980). The varieties IR24, IR26, IR661 and IR665, restorer of the most widely cultivated hybrids in China were developed at the IRRI (Virmani and Edwards 1983).

Identification of maintainers and restorers from elite breeding lines and landraces through test crossing (Ikehashi and Araki 1984, Virmani 1996) and their use in further breeding programme are the initial steps in three-line heterosis breeding (Siddiq 1996). The objectives of this study therefore, were to identify rice landraces and cultivars with fertility restoring ability and to identify maintainers of sterility among the test lines.

Materials and methods

Plant materials

This experiment was conducted in a greenhouse and experimental farm at the Institute of Agriculture and Animal Sciences (IAAS), Tribhuvan University, Rampur, Chitwan, Nepal, during the dry and wet seasons of 1999. The IAAS is located at 84º 29' E and 27º 37' N (224 m asl). Details of the 9 improved cultivars, 6 landraces and 3 wild aborted cytoplasmic male sterile (CMS) lines of rice used in this study are given in **Table 1**. The improved cultivars and landraces were obtained from the National Rice Research Program (NRRP), Hardinath, and IAAS, Rampur, respectively. The CMS lines were obtained from the IRRI, Philippines.

F_1 seeds production

Crossing was performed in a greenhouse, using cylindrical crossing chambers made of 2.5 m plastic sheet. The top portion of the chamber was open. The pollen parents were seeded three times to ensure a continuous supply of pollen to the female parent during the period of flowering, while the CMS lines were seeded only once. Before crossing, each CMS plant was tested for pollen sterility. This was determined by staining pollen grains in 1% potassium iodide-iodine (I-KI) solution. At heading, about 10 spikelets from each plant were collected in the morning just prior to blooming and fixed in 70% alcohol. All the anthers from 6 spikelets were excised with the help of forceps and placed in the stain. The pollen grains were released with a needle and gently crushed. After the debris was removed, a cover slip was placed over the pollen material and it was observed under a microscope (10x). The method is similar to that described by Virmani et al. (1997) and Chaudhary et al. (1981). The CMS plants showing complete sterility were used for crossing. The F_1 seeds were produced in the greenhouse using the Approach method (Erickson 1970).

Screen nursery

A field experiment involving 14 F_1's, 14 pollen parents, and 3 CMS lines was conducted to screen the cultivars/landraces. The block was divided into 31 plots of 0.8 m^2 size each. The pollen parent was

TABLE 1. Improved rice cultivars, landraces and CMS lines used in this study

A. Improved cultivars

Cultivar	Pedigree	Parentage	Origin	Grain type	Reaction to diseases	
					Bl	BB
Bindeswari	IET1444	TN1/Co29	India	Medium	MR	MS
Chaite-6	NR274-7-3-3-1	NR6-5-46-50/IR28	Nepal	Medium	R	R
Janaki	BG90-2	Peta *3/TN1//Remadja	Sri Lanka	Coarse	R	MR
Sabitri	IR2071-124-6-4	IR 1561/IR1737// CR94-13	IRRI	Coarse	MR	MR
Radha-11	TCA80-4	Local selection	India	Medium	S	MR
Kanchan	IR39341-4PL-P28	CR 126-42-5/IR 2061-213	IRRI	Medium	MR	-
Khumal-4	NR10078-76-1-1	IR 28/Pokhreli Masino	Nepal	Fine	R	-
Khumal-7	IR7167-33-2-3-3-1	China1039DWF-MUT/Kn-1B-361-1-8-6-10	IRRI	Coarse	R	-

Bl-Blast, BB-Bacterial blight, MR- Moderately resistant, M- Moderately susceptible, R-Resistant, S- Susceptible

Source: NRRP 1997

B. Landraces

Landrace	Origin	Remarks
Deharadune	Nepal	All landraces are popular local cultivars of hilly area of Nepal and have intermediate stature. They mature earlier than local cultivars of the Tarai and are field resistant to blast and bacterial leaf blight
Ratodhan	Nepal	
Gogi	Nepal	
Kature	Nepal	
Chiunde	Nepal	
IAR-97-34	Nepal	

C. CMS lines of wild aborted type

CMS line	Origin	Parentage	Remarks
IR58025A	IRRI	IR4843A/8*Pusa167-120	Stable in sterility, best combiner for yield, has aromatic long slender grains; using this line more than 50 hybrids have been developed in India.
IR62829A	IRRI	IR46828A/8*IR29744-94	Stable in sterility, has functional male sterility, very good combiner; using this line more than 20 hybridshave been developed in India.
IR68888A	IRRI	IR62829A/6*IR62844-15//IR629744-94	Stable in sterility, good combiner

Source: DRR 1996

planted beside their F_1 and CMS planted after the pollen parent. The field was fertilized at the rate of 120 kg N, 60 kg P_2O_5 and 60 kg K_2O per ha. Half of the nitrogen was applied as a basal dose and half top-dressed one month after transplanting. The 21-day-old seedlings were transplanted in the field in two rows with 10 hills per row at spacing of 20 cm between rows and 20 cm between plants. A single seedling was planted in each hill. Pollen and spikelet fertility were measured from each plot.

Pollen sterility

Pollen sterility of the F_1s was determined by staining pollen grains in 1% I-KI solution (Dalmacio et al. 1995, Virmani et al. 1997, Chaudhary et al. 1981, Sohu and Phul 1995, Young et al. 1983). The pollen grains in 3 randomly selected microscopic fields were counted. The pollen grains were classified based on their shape, size and extent of staining (Virmani et al. 1997, Young et al. 1983, Chaudhary et al. 1981) as shown in **Box 1**.

In the case of CMS lines and some hybrids, the patterns of pollen abortion were classified as follows (Chaudhary et al. 1981):

Type 1: Almost all pollen grains appear as UWS and USS.

Type 2: The majority of pollen grains appear as USS (51%), followed by SRS (36%) and UWS (14%).

Type 3: The majority of pollen grains are SRS (52%); UWS and USS are 20-25%.

Spikelet fertility

Five panicles from each experimental unit were bagged before flowering for spikelet fertility analysis. At maturity, the bagged panicles were examined for seed set. Spikelet fertility was determined by dividing the total number of seeds by the total number of spikelets. Test lines were classified on the basis of pollen fertility and spikelet fertility **(Table 2)**.

F_1s were also classified on the basis of seed set as male parent or weaker than male parent, anthers whether plumpy yellow or white shriveled.

Results and discussion

The pollen and spikelet fertility of hybrids are given in **Table 3**. In hybrids, pollen fertility ranged from 1 to 82% and spikelet fertility varied from 0 to 87%. Pollen fertility varied from 28 to 97%, while spikelet fertility ranged from 73 to 91% in pollen parents **(Table 4)**.

Our data indicates that pollen's susceptibility to staining with I-KI solution does not correlate with spikelet fertility. This may be due to the ability of single fertile pollen to fertilize a spikelet. It

BOX 1. Categories of rice pollen and their features

Category of pollen	Shape and staining behaviour	Classification
Unstained withered sterile (UWS)	Withered and undeveloped, unstained	Sterile
Unstained spherical sterile (USS)	Spherical and smaller, unstained	Sterile
Stained round sterile (SRS)	Round and small, lightly or incompletely stained, rough surface	Sterile
Stained round fertile (SRF)	Round and large, darkly stained, smooth surface	Fertile

TABLE 2. Classification of test lines into maintainers and restorers

Pollen fertility (%)	Category	Spikelet fertility (%)
0-1	Maintainer	0
1.1-50	Partial maintainer	0.1-50
50.1-80	Partial restorer	50.1-75
>80	Restorer	>75

Source : Virmani et al. 1997

TABLE 3. Pollen and spikelet fertility of hybrids

SN	Hybrid	Pollen fertility (%)	Spikelet fertility (%)	Seed set as	F/S	Test line	Inference on test line
1	IR68888A/Radha-11	80	87	MP	F	Radha-11	R
2	IR58025A/Janaki	49	33	W	F	Janaki	PM
3	IR58025A/Kanchan	81	75	MP	F	Kanchan	R
4	IR58025A/Khumal-4	32	57	MP	F	Khumal-4	PR
5	IR58025A/Sabitri	82	84	MP	F	Sabitri	R
6	IR58025A/Chaite-6	55	58	W	F	Chaite-6	PR
7	IR68888A/Bindeswari	1	0	W	F	Bindeswari	M
8	IR68888A/Khumal-7	1	0	W	S	Khumal-7	M
9	IR62829A/Deharadune	1	0	W	F	Deharadune	M
10	IR62829A/Ratodhan	82	79	MP	F	Ratodhan	R
11	IR68888A/Gogi	59	26	W	F	Gogi	PM
12	IR62829A/Kature	81	76	MP	F	Kature	R
13	IR68888A/Chiunde	1	0	W	F	Chiunde	M
14	IR58025A/IAR-97-34	56	49	MP	F	IAR-97-34	PR
	Range	1-82	0-87				
	Mean	47	45				
	SE	9.04	9.15				

MP-male parent, W-weaker than MP, F-plumpy yellow anthers, S-white shriveled anthers on visual basis, R-restorer, PR-partial restorer, PM-partial maintainer, M-maintainer suggests that pollen fertility is independent of the spikelet fertility. Therefore even a low number of fertile pollen counted in this study can give a higher seed set. However, the sterility of the inter-varietal rice hybrids is due primarily to pollen sterility. Guiquen et al. (1994) reported that sterility in the inter-varietal hybrids of cultivated rice is caused by the allelic interaction at the F_1 pollen sterility loci. Six loci of genes controlling F_1 pollen sterility in rice have been reported (Guiquen et al. 1994). Our study is in agreement with Guiquen et al. (1994) in that among F_1 hybrids, the higher the incidence of the heterozygote S^i/S^j at the six loci, the higher the incidence of pollen sterility and spikelet sterility.

Three CMS lines had a higher percentage of UWS and USS than that of rest lines. IR68888A had no SRF at all while the other two had some fertile pollen **(Table 4)**. The higher percentage of SRS in hybrids IR68888A/Bindeswari, IR68888A/Khumal-7, IR62829A/Deharadune and IR68888A/Chiunde was associated, on average, with 1% SRF. The hybrids having higher SRS were associated with high frequency of SRF as in IR68888A/Radha-11, IR58025A/Janaki, IR58025A/Kanchan, IR58025A/Khumal-4, IR58025A/Sabitri, IR58025A/Chaite-6, IR62829A/Ratodhan, IR62829A/Kature and IR58025A/IAR-97-34. **Table 4** shows that hybrids with some SRF pollen had fewer filled grains in the panicles. It indicates that hybrids having higher UWS and USS will be more useful for developing new CMS lines from their sterile hybrids.

The hybrids were classified as semi-sterile on the basis of spikelet fertility of 40-80%. The male parents of these hybrids were designated as partial restorers. In these hybrids, SRS had dominated the other pollen categories. The partial restorer IAR-97-34 had

TABLE 4. Pollen categories and types of male sterility in male sterile lines, hybrids and test lines

SN	CMS/ hybrid/test line	Total pollen examined	Frequency (%) UWS	USS	SRS	SRF	Type	Pollen sterility (%)	Spikelet fertility (%)
1	IR68888A	238	47.27	50.77	1.96	0.00	I	100.00	0.00
2	IR58025A	385	29.67	68.25	0.69	1.38	I	98.62	0.00
3	IR62829A	268	27.77	38.85	21.67	11.70	I	88.30	0.00
4	IR68888A/Radha-11	455	4.94	5.49	31.14	58.43		41.57	86.82
5	Radha-11	493	0.14	1.49	24.36	74.02		25.98	83.96
6	IR58025A/Janaki	374	12.13	18.11	20.79	48.97		51.03	32.98
7	Janaki	521	3.78	2.18	14.08	79.96		20.04	75.34
8	IR58025A/Kanchan	426	2.35	4.77	27.91	64.97		35.03	75.00
9	Kanchan	427	0.86	0.86	14.74	83.54		16.46	73.13
10	IR58025A/Khumal-4	289	3.92	15.11	51.10	29.87	III	70.13	57.27
11	Khumal-4	553	0.00	0.00	20.51	79.26		20.74	88.92
12	IR58025A/Sabitri	440	1.29	7.65	21.05	70.02		29.98	84.43
13	Sabitri	587	0.57	2.44	29.51	67.48		32.52	86.34
14	IR58025A/Chaite-6	304	4.93	8.88	31.36	54.83		45.17	57.54
15	Chaite-6	283	1.18	0.71	3.53	94.59		5.41	86.02
16	IR68888A/Bindeswari	288	12.49	38.27	48.25	1.00	III	99.00	0.00
17	Bindeswari	468	0.00	2.99	0.36	96.66		3.34	82.94
18	Masuli (check)	266	0.63	16.44	54.96	27.98	III	72.02	84.23
19	IR68888A/Khumal-7	193	10.02	28.67	60.32	1.00	III	99.00	0.00
20	Khumal-7	457	0.22	1.31	21.79	76.68		23.32	79.26
21	IR62829A/Deharadune	401	4.91	21.36	73.15	1.00	III	99.00	0.00
22	Deharadune	338	4.14	17.24	9.07	69.56		30.44	81.84
23	IR62829A/Ratodhan	462	0.22	2.74	19.48	77.56		22.44	78.73
24	Ratodhan	455	0.37	3.59	12.68	83.36		16.64	78.15
25	IR68888A/Gogi	394	4.82	22.51	13.96	58.71		41.29	26.15
26	Gogi	440	0.30	2.20	16.00	81.50		18.50	66.99
27	IR62829A/Kature	352	7.76	10.89	25.00	56.34		43.66	76.00
28	Kature	547	0.43	4.20	32.66	62.71		37.29	87.45
29	IR68888A/Chiunde	228	26.28	48.18	25.50	0.50	II	99.50	0.00
30	Chiunde	403	0.99	12.33	31.02	55.67		44.33	73.45
31	IR58025A/IAR-97-34	451	6.35	13.52	23.71	56.43		43.57	49.34
32	IAR-97-34	496	0.60	6.51	25.52	67.36		32.64	90.56
	Range	193-587	0-47.27	0-68.25	0.36-73.15	0-96.66		3.34-100	0-90.56
	Mean	396.31	6.92	14.95	25.25	52.9		47.09	57.59
	SE	18.12	1.93	3.01	3.01	5.48		5.49	6.07

UWS, unstained withered sterile, USS, unstained spherical sterile, SRS, stained round sterile, SRF, stained round fertile, Type I- almost all pollen appears as UWS and USS, II-majority of pollen as USS (51%) followed by SRS (36%) and UWS (14%), III-majority of pollen SRS followed by USS and UWS

TABLE 5. Restorers and maintainers for three CMS lines

CMS line	Restorers	Maintainers
IR58025A	Kanchan, Sabitri	-
IR62829A	Kature, Ratodhan	Deharadhune
IR68888A	Radha-11	Bindeswori, Khumal-7, Chiunde
Frequency (%)	36	29

more spikelet sterility than the other two partial restorers, Khumal-4 and Chaite-6. Spikelet fertility percentage varied widely among hybrids, and many hybrids had a lower spikelet fertility percentage than the high-yielding cultivars. Therefore, it is of practical importance to understand the causes of high spikelet sterility in hybrids for possible increase in spikelet fertility.

Restorers and maintainers identified in the study are summarized in **Table 5**. Among these lines, five were restorers, three were partial restorers, four were maintainers and two were partial maintainers. Radha-11 was found to be an effective restorer for IR68888A, Kanchan and Sabitri for IR58025A and Ratodhan and Kature for IR62829A. Bindeswari and Khumal-7 were found to be maintainers for IR68888A, and Deharadune for IR62829A. No maintainer for IR58025A was found. With respect to maintaining ability, all maintainers appeared to function effectively in maintaining sterility. All F_1 of these pollen parents with CMS showed a rate of 0% spikelet fertility and 0.5 to 1% pollen fertility. The frequency of restorers (36%) was higher than that of maintainers (21%). The frequency of restorer lines was higher among rice cultivars originating in lower latitudes. Virmani and Edwards (1983) reported that effective restorer cultivars were mainly distributed in the tropics where Indica rice was exclusively grown. Virmani (1996) found a lower incidence of restorer lines in northern China, eastern Europe, Japan, and Korea. The restoring ability of rice cultivars has been found to be, to some extent, related to their origin (Govinda Raj and Virmani 1988). Among Indica rice cultivars the frequency of R gene is higher in late maturing cultivars than in early maturing ones (Ahmed 1996). The restorer frequency is very low in typical Japonica rice cultivars (Lin and Yuan 1980, Virmani et al. 1981). It suggests that origin and pedigree of test lines are important characters to be considered in evaluating the rice genotypes for restoring and maintaining WA cytoplasm. Maintainer line, Bindeswari had been derived from the Taichun Native 1 (TN-1). Therefore, Bindeswari may have received its maintaining property from TN-1. Similarly the restorer gene in Sabitri might have come from CR94-13. Since the restorers and maintainers identified here are locally adapted, these cultivars and landraces may have value in heterosis breeding. Restorers can be improved (Liu et al. 1998) by using various procedures. Among the approaches used in developing new restorers, recombination breeding is the most common (Ahmed 1996). New restorers can be developed through cross breeding, which can enlarge the genetic base of R lines by pyramiding complementary traits from various sources in order to meet the breeding objectives. The CMS-WA system has been used extensively to transfer cytoplasmic male sterility traits in various genotypes both within and outside of China. The intensive use of a single source of male sterile cytoplasm in developing hybrid cultivars was found disastrous in the cases of Texas cytoplasm in maize and Tift cytoplasm in pear millet (Pokhriyal et al. 1974). It was therefore, considered wise to diversify sources of the cytoplasm. The maintainer and restorer lines identified here may be useful in increasing genetic diversity. The restorers can be used to develop hybrids and the maintainers to maintain and/or to develop new CMS lines. ■

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