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Research Note

Identification of Effective Restorers and Maintainers for Development of Rice Hybrids in Temperate Ecology

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Abstract
Three cytoplasmic male sterile (CMS) lines with Wild Abortive (WA) CMS source were crossed with 9 rice genotypes to assess their restorer/maintainer behavior during 2011-12. Out of 27 test crosses evaluated, 10 restorers, 8 partial restorers and 9 partial maintainer cross combinations were categorized on the basis of pollen fertility and spikelet sterility. Three genotypes K-08-61, K-08-60 and Pusa Sughand-5 were found as effective restorers for all the three CMS lines with spikelet fertility above 83% in F_1s for data pooled over environments, besides one more genotype K-08-59 depicted restoration only with the introduced CMS line IR-68888A.
Key words
Rice, restorer, maintainer, pollen fertility, CMS lines

Hybrid rice offers an opportunity to boost the yield potential of rice. It has a yield advantage of 15-20% over conventional high-yielding varieties (Virmani *et al*., 1993). Exploitation of hybrid vigour is considered as one of the outstanding achievements of plant breeding during the 20th century. This has not only contributed to food security but has also benefited the environment (Duvick, 1999). The hybrids had a yield advantage of 1.0-1.5t ha^{-1} (20-30%) over the conventionally bred modern varieties (Yuan, 1998). Since then a tremendous progress has been achieved in terms of both enhancing heterosis and improving breeding approaches. Although a number of hybrid varieties have been developed and released for commercial cultivation in India but all these varieties have been released for tropical environmental conditions and could not perform well under temperate agroecological conditions of Kashmir. Hence developing hybrids for temperate environmental conditions holds a great importance. In Kashmir, the heterosis breeding of rice is still in its initial stages. The rice hybrids developed for tropical and subtropical situations in India and abroad lack adaptability to the specific agro-ecological conditions of the valley, thereby limiting the scope of direct introduction of rice hybrids and their parental material Therefore, an indigenous rice hybrid development programme was initiated at Mountain Research Centre for Field Crops, Khudwani of Sher-e-Kashmir University of Agriculture Sciences and Technology of Kashmir. This led to the development of two stable and well adapted CMS lines, namely SKAU-7A and SKAU-11A both carrying wild abortive (WA) cytosterility source. The identification of an effective restorer line proved to be a challenging task. Further, the plant type of these CMS lines needs to be improved to an appreciable extent for efficient hybrid seed production. Preliminary studies indicated that some rice genotypes maintained at the station carry the alleles conferring fertility restoration, where as some other lines showed nearly 100% pollen and spikelet sterility when crossed to the newly developed CMS lines. Hence this study was carried out with an objective to assess the extent of fertility restoring ability of pollen parents to identify effective restorer and maintainer lines among locally adopted germplasm.

The present investigation was conducted under two diverse environments *viz.* Mountain Research Centre for Field Crops, Khudwani(1580 m msl) [E1], and at the Experimental Farm of Krishi Vighyan Kandra (KVK) Pombay, Kulgam(2000m msl) [E2] during *Kharif* 2011 and 2012. In this study the lines namely Jhelum, Pusa Sughandh-5 (PS-5), China-988, Shalimar Rice-1 (SR-1), SKAU-382, SKAU-389, K-08-59, K-08-60 and K-08-61 were crossed with three CMS lines (SKAU-7A, SKAU-11A and IR-68888A) used as testers in a line x tester fashion to generate 27 cross combinations. In order to achieve good synchrony of the male and female parents staggered sowing was made of at 10 days interval. The genetic material was chosen from a broad assay of germplasm maintained at Mountain Research Centre for Field Crops, Khudwani. The crosses were attempted in Kharif 2011. A total of 27 F1s, 12 parents and two standard checks were evaluated separately during kharif 2012 using complete randomized block design with three replications at each location. Jhelum and SR-1 were used as standard checks. Each experimental plot in each replication had a single row of one meter length with inter row and intra row spacing of 20 cm and 15 cm respectively. Thirty days old seedlings were transplanted with single plant hill^{-1}. Recommended packages of practices were followed to raise a good crop. The observations were recorded on pollen fertility % and spikelet fertility %. Pollen fertility % was calculated as the mean percentage of fertile pollen grains to the total number of pollen grains in three random microscopic fields. Pollen fertility was observed under a light microscope using Iodine potassium iodide (1KI) [0.1%] staining method. Samples for pollen were collected from at least 10 florets from individual plants at the sixth growth stage (heading). For each microscopic field fertile pollen grains (dark stained round) and sterile pollen grains (unstained withered, unstained spherical and partially stained round) were counted. Pollen fertility was then calculated as ratio of fertile pollen to the total number of pollen grains. Spikelet fertility % was calculated as the number of filled spikelets of main panicles bagged before flowering of five randomly selected plants and accordingly mean was calculated. The percent spikelet fertility was calculated by dividing the number of fertile spikelets panicle^{-1} to the number of spikelets panicle^{-1} multiplied by 100.

Genotypes were classified into restorers, partial restorers, partial maintainers and maintainers based on pollen and spikelet fertility (Virmani *et al*., 1997).

Pollen fertility %	Category	Spikelet fertility %
0-1	Maintainer	0
1.1-5	Partial maintainer	0.1-50
50.1-80	Partial restore	50.1-75
>80	Restore	>75

Since the low frequency of restores among local rice cultivars is a serious problem in exploitation of hybrid rice. Hence in the present investigation, efforts were made to identify the restorers and maintainers among the local rice varieties for two newly developed, cold tolerant and well adapted CMS lines SKAU-7A, SKAU-11A and one introduction CMS line IR-68888A to develop potential rice hybrids for unique ecology of Kashmir valley. Out of 27 test crosses evaluated, 10 restorers, 8 partial restorers and 9 partial maintainer cross combinations were categorized on the basis of pollen fertility and spikelet sterility, the results of which are given in Table 1. The frequency of restorers, partial restorers and partial maintainers were 37, 29.63 and 33.32 % respectively. Three genotypes K-08-61, K-08-60 and PS-5 were found as effective restorers for all the three CMS lines with spikelet fertility above 83% in F_1's for data pooled over environments, besides this, one more genotype K-08-59 showed restoration with the introduced CMS line IR-68888A. The cross combinations which observed highest restoration ability were PS-5 x SKAU-11A (93.31%), K-08-61 x IR-68888A (92.88%), PS-5 x IR-68888A (92.45%), K-08-61 x SKAU-7A (92.28%), K-08-61 x SKAU-11A (91.69%), K-08-60 x SKAU-11A (90.91%), PS-5 x SKAU-7A (88.94%), K-08-60 x SKAU-7A (88.32%), K-08-60 x IR-68888A (87.28%), and K-08-59 x IR-68888A (85.75%). Rest of the lines behaved as partial maintainers or partial restorers as evident from the Table 1.

The frequency of restorers was lower than the total frequency of partial maintainers and partial restorers because the materials used in the present study was having tropical japonica background that lack fertility restoration system to WA cytoplasm. The low frequency of restoration has been reflected in various studies (Virmani and Kumar, 2004; Bisne and Motiramani, 2005; Saber *et al.*, 2007; Akhter *et al.*, 2007 and Ingale *et al.*, 2008). Three genotypes K-08-61, K-08-60 and PS-5 were found as effective restorers for all the three CMS lines with spikelet fertility above 83% in F_1s for data pooled over environments, besides one more genotype K-08-59 depicted restoration only with the introduced CMS line IR-68888A. Kumar and Reddy (2011) showed restoration of some parents with all the CMS lines, while others exhibited restriction for only one or two CMS lines.

Hence, these parents can be used as potential restores for the development of commercial hybrid for temperate agro ecological conditions particularly for Kashmir valley. Majority of lines turned out to be either partial restorers or partial maintainers. Neither of these can be effective restorers or maintainers for use in hybrid rice breeding programme.The variations in behavior of fertility restoration indicate that either the fertility restoring genes are different or that their penitrance and expressivity varied with the genotypes of the parents or the modifiers of female background. These observations are in support from Bisne and Motiramani (2005). Further, the present study also revealed that the promising hybrid combinations of K-08-60, K-08-61 and PS-5 with all the CMS lines have spikelet fertility more than 83% and were also found to be good general and specific combiners for grain yield and other traits. New CMS lines should be developed using the local germplasm. Several workers have reported that the pollen or spikelet fertility was highly influenced by environmental conditions (Sharma and Reinberg, 1978 and Zhou, 1988). Since all the F_1 hybrids of present study were evaluated over environments in the same year, hence effectiveness of these restorers has got confirmed to a great extent for future hybrid rice breeding.

The presence of restorers among elite genotypes reveals the possibility of developing rice hybrids for temperate agroecologies and more particularly for Kashmir valley. Also new CMS lines in diverse genetic backgrounds can be developed in locally adapted germplasm that is prerequisite for hybrid rice breeding. Restorer genes found in exotic genotypes can be transferred to elite high yielding and desirable genotypes through appropriate backcross breeding programme to develop new restorer lines.

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Table 1. Estimated pollen/spikelet fertilities in rice (*Oryza sativa* L.)

Crosse Combinations	Pollen fertility (%)			Spikelet fertility (%)			Inference
	E1	E2	mean	E1	E2	mean	
SKAU-7A x K-08-59	72.44	70.43	71.43	69.12	67.45	68.28	PR
SKAU-11A x K-08-59	71.21	69.32	70.26	67.54	65.74	66.64	PR
IR-68888A x K-08-59	87.32	83.72	85.52	83.43	80.32	81.87	R
SKAU-7A x Jhelum	3.73	1.34	2.53	1.66	1.23	1.45	PM
SKAU-11A x Jhelum	2.11	1.65	1.88	1.07	0.87	0.97	PM
IR-68888A x Jhelum	2.32	2.14	2.23	1.57	1.22	1.39	PM
SKAU-7A x SKAU-382	58.44	53.56	56.0	50.55	47.77	49.16	PR
SKAU-11A x SKAU-382	64.33	61.23	62.78	58.34	55.76	57.05	PR
IR-68888A x SKAU-382	55.23	52.13	53.68	49.66	45.51	47.58	PR
SKAU-7A x SKAU-389	67.38	66.21	66.79	60.22	60.65	60.43	PR
SKAU-11A x SKAU-389	71.33	66.2	68.76	63.21	59.86	61.53	PR
IR-68888A x SKAU-389	65.24	60.4	62.82	58.23	54.32	56.27	PR
SKAU-7A x Ch-988	18.42	17.87	18.14	9.43	8.45	8.94	PM
SKAU-11A x Ch-988	17.54	15.32	16.43	13.54	12.97	13.25	PM
IR-68888A x Ch-988	13.23	10.21	11.72	7.22	7.12	7.17	PM
SKAU-7A x SR-1	42.11	38.95	40.53	38.64	34.66	36.65	PM
SKAU-11A x SR-1	34.53	29.58	32.05	12.45	11.54	11.99	PM
IR-68888A x SR-1	17.44	12.54	14.99	5.6	4.78	5.19	PM
SKAU-7A x K-08-60	90.21	89.32	89.76	85.34	82.87	84.10	R
SKAU-11A x K-08-60	93.33	90.64	91.98	86.22	84.54	85.38	R
IR-68888A x K-08-60	87.65	87.54	87.59	85.23	81.67	83.45	R
SKAU-7A x K-08-61	93.87	93.24	93.55	90.54	86.45	88.49	R
SKAU-11A x K-08-61	91.47	90.32	90.89	89.66	89.43	89.54	R
IR-68888A x K-08-61	94.76	92.67	93.71	88.43	86.09	87.26	R
SKAU-7A x PS-5	88.21	87.68	87.94	86.45	80.43	83.44	R
SKAU-11A x PS-5	93.34	93.65	93.49	88.45	85.89	87.17	R
IR-68888A x PS-5	92.65	90.12	91.38	87.64	87.12	87.38	R

M= maintainer, PM= Partial Maintainer, R= Restorer, PR= Partial Restorer

Table 2. Potential restores and maintainers identified in rice (*Oryza sativa* L.)

Parent	Pollen fertility (%)	Spikelet fertility (%)	Inference
K-O8-60	89.77	84.31	Restorer
K-O8-61	92.72	88.43	Restorer
Pusa Sugandh -5	90.94	86.00	Restorer
Jhelum	2.21	1.27	Partial Maintainer