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Research Note

Exploitation of heterosis in rice (*Oryza sativa* L.) using CMS system under temperate conditions

W. Hussain and G. S. Sanghera
SKUAST-K, Mountain Research Centre for Field Crops, Khudwani, Anantnag, 192102, Kashmir, India
Email: g_singh72@rediffmail.com

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Abstract:
The present study was conducted to evaluate 36 cross combinations developed by crossing 2 CMS lines with 18 testers in line x tester mating design to identify effective restores and maintainers from elite lines and work out best heterotic combinations in terms of yield and yield components. Assessment of standard heterosis based on standard checks (SR-1 and Jhelum) of effectively restored cross combinations showed that there was significant heterosis for all the traits except number of filled grains per panicle and the degree of heterosis varied from trait to trait. The entire cross combinations that showed superiority over standard checks for grain yield per plant also showed significant heterosis for majority of other traits. The best cross combination in terms of grain yield was SKAU7A x K-08-61-2 and for early maturity SKAU11A x SR-2 over check SR-1 only. In terms of restoration ability of genotypes 3 lines were categorized as effective restores viz. K-08-61-2, K-08-60-2 and SR-2 and 5 lines were categorized as maintainers viz. SKAU-405, Jhelum, SKAU-407, China-1007 and SKAU-391. The average proportion of restorers, partial restorers, partial maintainers and maintainers were 16:22:33:27, respectively.

Keywords: Rice, Temperate CMS lines, Restorers, Maintainers, Heterosis

The successful exploitation of hybrid vigour in rice using CMS system, identification of maintainers with higher adaptability and restorers with higher combining ability from elite breeding lines and landraces through test crossing (Ikehashi and Araki 1984) and their use in further breeding programme are the initial steps in three-line heterosis breeding (Siddiq 1996). Cytoplasmic male sterility and the fertility restoration system have been primarily used to develop heterotic rice hybrids in and outside China (Lin and Yuan 1980 and Virmani et al. 2003). Heterosis breeding is an important genetic tool that can facilitate yield enhancement of 15-20% over the conventional high yielding varieties and helps to enrich many other desirable quantitative and qualitative traits in crops (Srivastava 2000) even under fragile environmental conditions as hybrids are more adaptable under stress conditions. Presence of heterosis and SCA effects for yield and its related traits in rice using CMS lines has been reported by earlier workers (Roy and Mandal 2001; Sukhpal and Siddiq 2005; Neelam et al. 2009 and Tiwari et al. 2011). Though a good number of hybrids have been released in India (Mishra, 2009) but the initial evaluation of these hybrids and their parental lines has shown that hybrids developed from tropical and sub tropical areas as such were not suitable for cultivation under temperate condition of Kashmir (Sanghera et al. 2003). Recently, with the development of two well adapted cold tolerant CMS lines SKAU 7A and SKAU 11A based on WA cytosterility, can be utilized for development of medium bold rice hybrids with good grain quality for temperate conditions of Kashmir (Sanghera et al. 2010). Thus main objective of this study was to identify restorers and maintainers for newly developed CMS lines under the temperate conditions and to explore best heterotic combinations in terms of yield and yield contributing traits.

The experimental material for the present study was developed by crossing two temperate CMS lines viz. SKAU 7A and SKAU 11A used as females with 18 testers (SKAU-405, Jhelum, K-08-60-2, SKAU-403, SKAU-407, K-08-59-3, China-1007, SR-1, SKAU-389, K-08-59-1, SKAU-292, SKAU-391, SKAU-354, SKAU-46, Chenab, SKAU-406, K-08-61-2 and SR-2) used as males following Line x Tester mating design during the years 2010-2011 at Mountain Research Centre for Field Crops, Khudwani (34˚ N latitude and 74˚longitude) of Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir. The 36 cross combinations along with parents and 2 standard checks viz. SR-1 and Jhelum were evaluated in complete randomized block design in three replications during *Kharif* 2011. Thirty days old seedlings with single plant/hill were transplanted in a 5m long row with inter and intra row spacing of

20 and 15cm, respectively. One line of each entry was planted in each replication. All the recommended agronomic and plant protection practices were uniformly followed throughout the crop growth period for raising ideal crop stand. In each entry, five plants were selected randomly from each replication and biometrical observations were recorded on pollen fertility (%), spikelet fertility (%), number of spikelets per panicle, number of filled grains per panicle, number of chaff seed per panicle, panicle length (cm), number of tillers per plant, number of productive tillers / plant, plant height (cm), flag leaf area (cm^2), biological yield /plant (g), grain yield /plant(g), harvest index (%), grain length (mm), grain breadth (mm) and grain length/breadth ratio. Days to maturity and days to 50% flowering were recorded on plot basis. Based on pooled values, standard heterosis was calculated.

The pollen fertility was recorded at the time of panicle emergence. Half emerged panicles were taken from five randomly selected plants in individual 36 F_1's from the experimental field. Samples for pollen were collected from at least 10 florets from individual plants. Pollen fertility was observed under a light microscope using Iodine potassium iodide (1KI) [0.1%] staining method. The stain was prepared by dissolving of 1g iodine and 2g of potassium iodide in 100 ml distilled water and pollen fertility examined under the light microscope. The anthers were put on slide and gently crushed by using a needle to release the pollen grains. After removing the debris, a cover slip was placed and the slide was observed under the microscope. Pollen fertility was then calculated as the mean percentage of fertile pollen grains to the total number of pollen grains in three random microscopic fields. Unstained, half stained, shriveled and empty yellow pollen grains were classified as sterile while well filled, stained and dark round pollen grains were recorded as fertile. For spikelet fertility / sterility, 5 panicles of each testcross were covered with butter paper bags to avoid foreign pollen contamination and at maturity were harvested. The per cent spikelet fertility was then calculated by dividing the number of fertile spikelets per panicle to the number of spikelets per panicle multiplied by 100.The criteria for classifying the parental lines as maintainers and restorers were used as proposed by Virmani et al. (1997).

For exploitation of hybrid rice breeding under Kashmir valley, it is important to identify the potential restorers and maintainers from the existing rice germplasm. In the present study, among 36 cross combinations, 6 effective restorers, 8 partial restorers, 12 partial maintainers and 10 maintainers cross combinations were categorized on the basis of pollen fertility and spikelet fertility, the results of which are given in Table-1. The cross combinations which observed highest restoration ability was SKAU 7A x K-08-61-2 (93%), and minimum restoration ability was shown by SKAU7A x K-08-60-2 (88%). Regarding the restoration ability of elite lines towards the newly developed CMS lines (based on WA cytosterility) only three lines were found to be effective restorers namely K-08-61-2, K-08-60-2 and SR-2. Most of the lines behaved either partial restorers or partial maintainers and frequency of restorers, partial restorers, partial maintainers and maintainers were 16%, 22 %, 33 % and 27%, respectively. The frequency of restorers was much lower than the frequency of maintainers because the material used 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; Saber *et al.,* 2007; Akhter *et al.,* 2007 and Ingale *et al.,* 2008). The lines SKAU-405, Jhelum, SKAU-407, China-1007 and SKAU-391 were categorized as effective maintainers and thus can be used for the development of new CMS lines as they are locally adapted genotypes. Majority of lines that turned out to be either partial restorers or partial maintainers 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 penetrance 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 identified effective restorers and maintainers among the elite lines needs to be confirmed after screening of F_1's in various locations and seasons for future hybrid rice breeding as pollen or spikelet fertility is highly influenced by environmental conditions (Sharma and Reinberg 1978 and Zhou 1988).

<u>Heterosis for effectively restored cross combinations</u>
Standard heterosis over checks (SR-1 and Jhelum) was computed for six fully restored cross combinations for eighteen traits. The results revealed that heterosis varied from character to character and from cross to cross and none of the cross combination recorded significant heterosis for all the traits simultaneously (Table -2). These results are in agreement with the findings of Bagheri and Jelodar (2010); Rashid *et al.* (2007) and Singh *et al.* (2007). Heterosis for grain yield along with its components is very important consideration in heterosis breeding. Yield is a complex character and ultimate aim of plant breeding. All the effectively restored

combinations out yield the standard checks SR-1 and Jhelum by 25.88% to 55.70%, respectively. Among these the best cross combinations was SKAU 7A x K-08-60-2 (44.70% over SR-1 and 55.70% over Jhelum) and SKAU 7A x K-08-60-2 (25.88% over SR-1 and 35.45% over Jhelum) revealed minimum heterosis. Rahimi *et al.* (2010) and Tiwari *et al.* (2011) also reported more than 25% yield increase over standard variety in rice. Cross combinations SKAU 7A x K-08-61-2, SKAU 11A x K-08-61-2, SKAU 7A x SR-2 and SKAU 11A x SR-2 recorded significant heterosis for the biological yield over both the checks. Earlier rice workers (Peng and Virmani 1990 and Tiwari *et al.* 2011) have also reported significant heterosis for this trait. The per cent of heterosis for these combinations ranged from (8.9% to 13.14%) over check SR-1 and (10.14% to 14.33%) over check Jhelum. The most desirable cross combination SKAU 7A x K-08-061-2 for yield also revealed significant standard heterosis for pollen fertility, spikelet fertility, number of tillers per plant, number of productive tillers per plant, panicle length, flag leaf area, biological yield, harvest index, grain length and grain length/breadth ratio. Negative heterosis is desirable for breeding early hybrids and varieties. Two cross combinations SKAU 7A x SR-2 and SKAU 11A x SR-2 manifested superiority for days to 50% flowering (6.58% and 7.23%) and days to early maturity (9.49% and 9.89%) over the check SR-1. This suggests the possibility of developing early maturity hybrids from these cross combinations that is a desperate need under temperate condition. Heterosis for early maturity and other important yield components were both in positive and negative direction. Neelam *et al.* (2009) and Tiwari *et al.* (2011) also reported both positive and negative heterosis for various yield components and other traits.

Taller plant height is desirable and needed phenotypic acceptability of the hybrid genotypes in valley. In the present study, 4 cross combinations SKAU 7A x K-08-60-2, SKAU 11A x K-08-60-2, SKAU 7A x K-08-61-2 and SKAU 11A x K-08-61-2 were found desirable for taller height in rice genotypes. The heterosis in these cross combinations ranged from (23.53% to 28.71%) over check SR-1 and (26.83% to 32.15%) over check Jhelum. Generally, large panicle length is associated with high number of grains per panicle which results into higher product, therefore positive heterosis for panicle length is desirable. In the present study, all cross combinations were found to have significant heterosis in this regard except SKAU 7A x SR-2 and SKAU 11A x SR-2 over check SR-1. The spectrum of variation for heterotic combinations was 16.66% to 30.15% over SR-1 and 3.11% t0 38.23% over Jhelum. Singh *et al*. (1998) reported both positive and negative heterosis for panicle length. Pollen fertility and spikelet fertility are the important traits which directly influence the ultimate product i.e. grain yield. For these traits SKAU 7A x K-08-61-2 (5.27% over SR-1and 6.20% over Jhelum) and SKAU 11A x K-08-61-2 (4.38% over SR-1 and 4.45% over Jhelum) were the desirable heterotic combinations. Superiority of these traits has also been reported by Panwar *et al.* (2002) and Singh (2000).

Virmani *et al.* (1981, 1982) have reported positive heterosis for number of spikelets per panicle, they concluded that standard heterosis in yield was primarily due to increased number of spikelets per panicle. This conclusion is in justification with our own findings as the superior cross combinations were SKAU 7A x K-08-60-2 (12.07% over SR-1 and 9.56% over Jhelum), SKAU 11A x K-08-60-2 (10.6% over SR-1 and 8.31% over Jhelum) and SKAU11A x SR-2 (3.15% over SR-1 and 5.446% over Jhelum) that revealed desirable heterosis for this trait. However, for number of filled grains per panicle no significant heterosis was observed for the entire cross combinations in the present study. Furthermore, panicle bearing tillers per plant is believed to be closely associated with high yield potential. Desirable combination for this trait was SKAU 7A x K-08-61-2 (21.20% over SR-1 and 29.03% over Jhelum). Positive heterosis for productive per tillers plant has been revealed by Shanthala *et al.* (2009) and Neelam *et al.* (2009).

Besides these yield contributing traits, heterosis was manifested by entire cross combinations for grain length. The range of heterosis was 3.77% to 18.91% over SR-1 and 4.85% to 20.14% over Jhelum. Both negative and positive heterosis was recorded in cross combinations for grain breadth, and desirable heterotic combinations for this trait were SKAU7A x SR-2 (17.29% over SR-1 and 18.03% over Jhelum) and SKAU 11A x SR-2 (16.6% over SR-1 and 17.40% over Jhelum) which is in accordance with the findings of Rahimi *et al.* (2010). Harvest index is not directly a yield contributing trait but is considered important parameter for genetic improvement of genotypes. Heterosis for this trait was revealed by all the combinations except SKAU 7A x SR-2 and SKAU 11A x SR-2 over check SR-1 only. The heterosis ranged from -1.75% to 14.45% over check SR-1 and 12.75% to 31.77% over check Jhelum. Heterosis for this trait was also found by Peng and Virmani (1990) and Tiwari *et al.* (2011).

The presence of restorers and maintainers among the elite lines infers that hybrids and new CMS lines can be developed under temperate conditions of Kashmir. The acceptable amount of heterosis for yield and other yield contributing traits indicates that hybrids can be commercially exploited in the present conditions.

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Table-1. Rice (*Oryza sativa* L.) genotypes identified as restorers and maintainers based on pollen and spikelet fertility of various cross combinations.

Crosses	Pollen fertility (%)	Spikelet fertility (%)	*Inference
SKAU 7A x SKAU-405	0	0	M
SKAU 11A x SKAU-405	0	0	M
SKAU 7A x Jhelum	3	1	M
SKAU 11A x Jhelum	2	0	M
SKAU 7A x K-08-60-2	88	84	R
SKAU 11A x K-08-60-2	92	84	R
SKAU 7A x SKAU-403	40	38	PM
SKAU 11 x SKAU-403	43	41	PM
SKAU 7A x SKAU-407	1	0	M
SKAU 11A x SKAU-407	1	0	M
SKAU 7A x K-08-59-3	78	73	PR
SKAU 11A x K-08-59-3	78	71	PR
SKAU 7A x China-1007	0	0	M
SKAU 11A x China-1007	0	0	M
SKAU 7A x SR-1	33	31	PM
SKAU 11A x SR-1	30	28	PM
SKAU 7A x SKAU-389	62	60	PR
SKAU 11A xSKAU-389	61	56	PR
SKAU 7A x K-08-59-1	76	70	PR
SKAU 11A x K-08-59-1	74	72	PR
SKAU 7A x SKAU-292	10	4	PM
SKAU 11A x SKAU-292	13	6	PM
SKAU 7A x SKAU-391	1	0	M
SKAU 11A x SKAU-391	1	0	M
SKAU 7A x SKAU-354	19	14	PM
SKAU 11A x SKAU-354	22	15	PM
SKAU 7A x SKAU-46	62	55	PR
SKAU 11A x SKAU-46	64	53	PR
SKAU 7A x Chenab	19	18	PM
SKAU 11A x Chenab	16	13	PM
SKAU 7A x SKAU-406	30	27	PM
SKAU 11A x SKAU-406	28	26	PM
SKAU 7A x K-08-61-2	93	89	R
SKAU 11A x K-08-61-2	92	90	R
SKAU 7A x SR-2	90	86	R
SKAU 11A x SR-2	91	88	R

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

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Table- 2.Estimation of heterosis (%) for fully restored cross combinations over standard checks for various agro-morphological traits in rice (*Oryza sativa* L.)

Crosses	Days to 50% flowering		Days to maturity		Pollen fertility (%)	
	SR-1	Jhelum	SR-1	Jhelum	SR-1	Jhelum
SKAU 7A x K-08-60-2	2.975**	0.95	5.09**	0.24	1.10	1.29
SKAU 11A x K-08-60-2	3.29**	0.32	2.77**	2.68**	1.144	1.56
SKAU 7A x K-08-61-2	5.26**	-1.32	2.54**	1.93	4.81**	4.93**
SKAU 11A x K-08-61-2	3.61**	1.13	2.08**	1.47	4.72**	4.83**
SKAU 7A x SR-2	-6.58**	1.24	-9.49*	2.40**	1.44	0.56
SKAU 11A x SR-2	-7.23**	1.41	-9.81*	2.01*	-0.725	0.36
Crosses	**Spikelet fertility (%)**		**Spikelets panicle^{-1}**		**Filled grains panicle^{-1}**	
	SR-1	Jhelum	SR-1	Jhelum	SR-1	Jhelum
SKAU 7A x K-08-60-2	-12.15*	-12.09*	12.07*	9.56*	-22.14*	-22.83*
SKAU 11A x K-08-60-2	-11.35*	-11.28*	10.60*	8.13*	-21.12*	-22.83*
SKAU 7A x K-08-61-2	5.27**	6.20**	-7.17*	-7.60*	-5.28*	-7.33**
SKAU 11A x K-08-61-2	4.38**	4.45**	-5.05**	-7.71*	-2.21	-2.33
SKAU 7A x SR-2	-9.50*	-9.44*	-1.91	0.32	-9.19*	-11.16*
SKAU 11A x SR-2	-8.04*	-7.97*	3.15**	5.446**	-5.79**	-7.83**
Crosses	**Chaff seed panicle^{-1}**		**Panicle length(cm)**		**No. of tillers plant^{-1}**	
	SR-1	Jhelum	SR-1	Jhelum	SR-1	Jhelum
SKAU 7A x K-08-60-2	23.76*	21.82*	16.6*	23.8*	-9.09*	-6.25*
SKAU 11A x K-08-60-2	21.52*	20.00*	20.45*	27.94*	-23.63*	-21.25*
SKAU 7A x K-08-61-2	0.00	-1.70	30.15*	38.23*	21.20*	24.99*
SKAU 11A x K-08-61-2	-11.53*	-14.81*	22.61*	30.23*	0.60	2.49
SKAU 7A x SR-2	21.36*	20.37*	1.61	3.11**	30.30*	34.36*
SKAU 11A x SR-2	18.45*	17.77*	-0.77	4.39**	33.33*	37.49*
Crosses	**No. productive tillers plant^{-1}**		**Plant height (cm)**		**Flag leaf area (cm^2)**	
	SR-1	Jhelum	SR-1	Jhelum	SR-1	Jhelum
SKAU 7A x K-08-60-2	-9.97**	-3.87	23.53*	26.83*	29.96*	33.36*
SKAU 11A x K-08-60-2	-28.01*	-20.06*	24.48*	27.48*	5.13**	7.13**
SKAU 7A x K-08-61-2	21.20*	29.03*	28.71*	32.15*	29.00*	20.29*
SKAU 11A x K-08-61-2	-9.09**	-3.221	26.07*	29.44*	21.13*	25.23*
SKAU 11A x SR-2	-13.33*	-7.74**	-0.03	-2.71	1.8	1.55
SKAU 11A x SR-2	-6.66**	-0.63	-0.03	-2.91	1.52	1.77
Crosses	**Biological yield plant^{-1}(g)**		**Grain yield plant^{-1}(g)**		**Harvest index (%)**	
	SR-1	Jhelum	SR-1	Jhelum	SR-1	Jhelum
SKAU 7A x K-08-60-2	0.69	1.74	25.88*	35.45*	10.83*	27.20*
SKAU 11A x K-08-60-2	0.34	1.39	27.06*	36.72*	14.81*	31.77*
SKAU 7A x K-08-61-2	13.14*	14.33*	44.70*	55.70*	14.45*	19.88*
SKAU 11A x K-08-61-2	15.22*	16.43*	41.17*	51.19*	11.02*	14.80*
SKAU 7A x SR-2	7.26**	8.39**	36.47*	46.84*	2.79	19.12*
SKAU 11A x SR-2	8.99**	10.14*	27.06*	36.72*	-1.75	12.75*
Crosses	**Grain length (mm)**		**Grain breadth (mm)**		**Grain L/B ratio**	
	SR-1	Jhelum	SR-1	Jhelum	SR-1	Jhelum
SKAU 7A x K-08-60-2	18.91*	20.14*	-2.96**	-2.34**	22.58**	23.09**
SKAU 11A x K-08-60-2	17.62*	18.22*	0.74	1.37	16.79*	17.28*
SKAU 7A x K-08-61-2	17.01*	18.22*	-2.96**	-2.34**	20.18*	21.18*
SKAU 11A x K-08-61-2	16.10*	17.30*	0.74	1.37	15.49*	15.00*
SKAU 7A x SR-2	3.77**	4.85**	17.29*	18.03*	-11.46*	-11.09*
SKAU 11A x SR-2	5.30**	6.39**	16.66*	17.40*	-9.68*	-9.30*

*, ** Significant at 5 and I per cent level, respectively