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IDENTIFICATION OF EFFECTIVE FERTILITY RESTORERS IN RICE UNDER TEMPERATE CONDITIONS OF KASHMIR VALLEY

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ABSTRACT

Exploitation of hybrid vigour is one of the most important applications of genetics in agriculture. It has highly contributed to food security, without affecting the environment. The availability of effective restorer lines that combines well with the CMS lines for various agro-morphological traits, determines the success of hybrid rice technology under temperate conditions like Kashmir Valley. To identify the best performing lines of rice in different cross combinations, four CMS lines and 34 genotypes were characterized for various agro-morphological and fertility restoration related traits. Out of 136 crosses, 43 (31.6%) were found to show the fertility restoration and 30 (22.06%) were found to be partial restorers. Sixty-three (46.32%) cross combinations were found to be partial maintainers and no cross combination was found to exhibit the maintainer behaviour. The genotypes RL-2, RL-3 and RL-8 were found to show restoration across all the CMS lines. Analysis of variance indicated the existence of significant differences among the genotypes, revealing that sufficient variability is present for different traits. The maximum positive value of st and ard heterosis for grain yield was recorded in SKUA-7A × RL-3. The marker-based assay using *Rf*3 and *Rf*4 linked markers was also carried out to validate the genes responsible for fertility restoration. The genotypes RL-1, RL-2, RL-5, RL-9, RL-11 and RL-12 showed polymorphism with all the markers. Sixteen hybrids showed fertility restoration behaviour with all the markers as well as under the field condition. Among these, 8 fall in the class of top 10 heterotic hybrids in the present study. The heterotic hybrids were evaluated for grain and cooking quality features. Out of the top 10 heterotic hybrids evaluated, nine were of medium to short grain types and only one SKUA-19A × RL-5 was of long grain type. Heterotic hybrids along with their parents showed intermediate amylose content which is preferable.

Key words: Hybrid rice, restorer lines, molecular markers, *Rf*3, *Rf*4, cooking quality

INTRODUCTION

Rice is one of the most consumed cereals with more than half of the world population depending upon this crop as staple food (Vanniarajan *et al.,* 2012). The crop possesses huge socio-economic relevance and thus enhancement in its yield potential has always been a major objective in almost all rice breeding programmes. The growth in rice yields must take place despite various challenges such as water scarcity, soil salinity, climate change, reduced arable l and and high input prices (Khush, 2005; Zhang, 2007; Najar *et al.,* 2018). Rice yields have witnessed a big leap of about 30% increment due to the development of semi dwarf varieties (Fang *et al.,* 2004). An additional increase of 20-25% can be achieved by exploiting heterosis in the form of hybrid rice (Waza *et al.,* 2016). Among various approaches for improving rice productivity, exploitation of heterosis has fared well and secured a good track record in eradicating the curse of yield stagnation. Hybrid rice has the potential to bridge the gap between production and dem and to meet the food requirements of the ever-increasing population while sustaining the natural resource base.

Rice hybrids were first commercialized in China during the late 1970s and gained huge success. With the success of hybrid rice cultivation in China, many countries including India adopted this technology. The first rice hybrids namely APHR 1 and APHR 2 were released in India in 1994. Gradually hybrid rice technology gained popularity in India with a total cultivated area of about 2.5 million hectares (Singh *et al.,* 2016) and till 2018 as many as 110 rice hybrids have been released by public and private sectors (AICRP Progress Report, 2019). Development of commercial rice hybrids needs a careful selection of parental lines to enable exploitation of maximum heterosis. Parental lines of heterotic cross combinations should complement each other for the important agronomic traits especially the grain yield. Though there are many approaches for the production of rice hybrids on commercial scale, the three-line (CGMS) system is the most productive way of exploiting heterosis in this crop. In CGMS, it is essential to identify the promising restorers against the available CMS lines. Although, a number of CMS lines have been developed which suit the mountainous areas of Kashmir, the availability of promising restorer lines remains to be the major limitation for hybrid development in rice. Effective restorer lines are mainly from the tropics where *indica* rice is exclusively grown.

The success of hybrid rice technology under temperate conditions mostly relies on the availability of potential restorers that combines well with the CMS lines for yield and yield attributes. The analysis of combing ability provides guidelines for selecting the potential parents and breeding methods. Evaluation of parents for combining ability also indicates relative magnitude of additive and non-additive variances for various characters like yield and its components. Line × tester mating design, due to their more manageability with a large number of parents have been widely used to underst and the gene effects in rice (Kempthorne, 1957; Alam *et al.,*2007). An added advantage of this method is that it gives an overall genetic picture of the materials under investigation in a single generation.

In this study, the characterization of four CMS lines and 34 breeding lines for various agro-morphological and fertility restoration related traits have been carried out to identify the best performing lines. Heterosis frequency for different traits of commercial importance was estimated to identify the best heterotic combinations. The male parents of heterotic hybrids were validated using molecular markers linked to *Rf*3 on chromosome 1 and *Rf*4 on chromosome 10, and to identify the genes responsible for fertility restoration. Out of 136 hybrids obtained by crossing four CMS lines with 34 pollen parents, 10 highly heterotic hybrids along with their parents were subjected to cooking quality evaluation.

MATERIALS AND METHODS

Experimental site and layout: The experiment was accomplished during the Kharif seasons of 2017 and 2018 at MRCFC Khudwani, SKUAST of Kashmir (34° N latitude, 74°E longitude and 1680 AMSL) India. The experimental material consists of four wilds abortive (WA) CMS lines and 34 rice genotypes used as pollen parents in the hybridization programme. In 2017, test crosses were made between the CMS lines and pollen parents to generate the seed of 136 F_1 hybrids (Table 1). In 2018, the F_1 hybrids were raised to identify restorers, partial restorers, partial maintainers and maintainers among the male lines based on pollen and spikelet fertility studies (Virmani *et al.,* 1997). The CMS lines along with their maintainers were also characterized for different agro-morphological characters. Heterosis for various agro-morphological traits was estimated over the better parent, mid parent and over two st and ard check varieties (Shalimar Rice-3 and Shalimar Rice-4). During both the years of experimentation, the rice genotypes were planted in three replications using RCBD (r and omized complete block design). The genotypes were raised in 4 rows of 5 m length with a spacing of 20 × 15 cm, planting one seedling per hill. Recommended agronomic practices were adapted to ensure the establishment of a better crop st and . Ten plants, selected r and omly, from each replication were evaluated for various agro-morphological traits.

Evaluation for pollen and spikelet fertility: Pollen sterility/fertility of the CMS lines and F_1s was determined at flowering stage by fixing them in aceto-alcohol (1:3) solution for 24 hours and preserving in 70% ethanol. Afterwards, the pollen grains were stained in 1% potassium iodide-iodine (I-KI) solution and counted in three r and omly selected microscopic fields. The pollen grains were categorised as sterile or fertile based on their size, shape and staining behaviour (Virmani *et al.,* 1996). The spikelet fertility was estimated by bagging the 10 panicles from each replication before flowering. During maturity, the covered panicles were observed for seed set by counting the filled and unfilled grains (Choudhary *et al.,* 1981). Based on estimates of pollen and spikelet fertility, the male parental lines were categorised as restorers (R), partial restorers (PR), partial maintainers (PM) and maintainers (M) following the criteria by Govinda and Virmani (1988) and Virmani *et al.* (1997) (Table 2).

Table 2. Classification of fertility restoration (Govinda and Virmani 1988).

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

Cooking quality estimation: Out of the 136 cross combinations, 10 highly heterotic hybrids for grain yield were subjected to evaluation for various cooking quality features along with the parents. Data were recorded on various quality traits *viz.,* hulling (%), milling (%), head rice recovery (%), kernel length before cooking (mm), kernel breadth before cooking (mm), length breadth ratio, kernel length after cooking (mm), kernel breadth after cooking (mm), kernel elongation ratio, kernel elongation index, aroma, gelatinization temperature, amylose content

(%) and gel consistency (mm). Based on kernel length, the samples were classified as extra-long, long, medium and short. Further based on length breadth ratio, the genotypes were classified as slender, medium, bold and round (SES-IRRI, 2018). Kernel length before cooking (mm), kernel breadth before cooking (mm), kernel length after cooking (mm) and kernel breadth after cooking (mm) were calculated on graph paper with the help of magnifying glasses. Physiochemical properties like amylose content (%), gel consistency (mm) and alkali spread values were evaluated following the st and ard protocols along with some modifications as described and published by Juliano *et al.* (1971); Cagampang *et al.* (1973); Little *et al.* (1958), respectively. The presence of aroma was detected through the panel test as suggested by Sood and Sidiq (1978).

Statistical analysis: The data obtained was analysed statistically following Line × tester mating design (Kempthorne, 1957), using *Windostat* Software version 9.1. To analyse the worth of differences among the genotypes, the mean values for each trait was subjected to Analysis of Variance. The variability parameters for various traits were also computed to estimate the differences among the genotypes.

Table 1. Restoration ability of various rice genotypes for (WA) CMS lines.

S. No	Cross Combination	SKUA-7A			SKUA-11A			SKUA-19A			SKUA-21A		
		PF (%)	SF (%)	Class	PF (%)	SF (%)	Class	PF (%)	SF (%)	Class	PF (%)	SF (%)	Class
1	RL-1	25.58	23.42	PM	47.72	44.28	PM	92.06	88.66	R	75.62	71.66	PR
2	RL-2	94.22	91.17	R	90.08	87.17	R	91.28	89.15	R	88.29	85.00	R
3	RL-3	96.31	92.34	R	88.28	84.05	R	92.66	88.93	R	86.59	83.64	R
4	RL-4	71.88	69.28	PR	83.11	77.56	R	58.39	56.23	PR	80.07	75.00	R
5	RL-5	93.96	87.77	R	91.84	88.83	R	94.11	92.44	R	41.66	39.44	PM
6	RL-6	57.16	52.05	PR	92.99	90.72	R	93.97	91.25	R	71.92	68.19	PR
7	RL-7	94.16	88.28	R	90.61	87.22	R	94.71	92.39	R	76.16	73.11	PR
8	RL-8	92.04	90.41	R	91.79	88.82	R	91.74	88.72	R	84.66	80.45	R
9	RL-9	44.00	41.04	PM	66.45	63.72	PR	90.94	86.56	R	91.65	87.89	R
10	RL-10	90.11	87.72	R	72.46	70.08	PR	90.67	85.16	R	82.45	78.28	R
11	RL-11	89.32	85.27	R	75.55	68.32	PR	73.84	68.88	PR	93.09	90.66	R
12	RL-12	56.13	50.76	PR	81.52	76.12	R	79.05	75.66	PR	94.62	91.61	R
13	RL-13	88.39	84.27	R	16.32	13.62	PM	88.96	85.95	R	75.88	72.31	PR
14	RL-14	90.31	85.67	R	81.02	79.02	R	52.46	52.12	PR	88.16	84.11	R
15	SKUA-403	2.00	1.55	PM	8.51	6.21	PM	48.78	46.18	PM	48.95	47.21	PM
16	SKUA-408	5.24	3.11	PM	10.56	8.31	PM	52.23	48.76	PM	48.32	45.54	PM
17	SKUA-412	91.67	88.51	R	84.17	77.61	R	44.61	42.73	PM	46.73	44.28	PM
18	SKUA-415	26.71	22.86	PM	43.05	41.11	PM	46.16	44.12	PM	47.36	42.17	PM
19	SKUA-420	52.19	47.38	PM	63.31	64.06	PR	47.31	46.01	PM	80.94	74.95	PR
20	SKUA-483	41.91	36.91	PM	49.16	46.77	PM	49.16	47.92	PM	2.00	1.40	PM
21	SKUA-484	56.28	53.39	PR	24.65	21.08	PM	44.96	42.45	PM	47.71	45.61	PM
22	SKUA-486	1.42	0.66	PM	16.05	11.15	PM	45.44	43.15	PM	6.06	4.07	PM
23	SKUA-487	61.16	58.14	PR	18.81	15.24	PM	41.6	38.31	PM	29.67	28.11	PM
24	SKUA-488	7.81	6.01	PM	47.79	44.67	PM	45.67	42.38	PM	49.51	47.06	PM
25	SKUA-491	61.47	57.72	PR	48.86	47.17	PM	56.62	51.72	PR	41.55	37.13	PM
26	SKUA-494	62.68	58.98	PR	37.05	32.32	PM	47.78	45.32	PM	60.23	58.93	PR
27	SKUA-495	36.16	33.51	PM	47.88	45.63	PM	47.62	41.86	PM	56.43	50.35	PR
28	SKUA-496	14.51	14.65	PM	49.11	45.77	PM	44.07	40.66	PM	25.15	24.00	PM
29	SKUA-497	46.73	43.52	PM	36.21	31.53	PM	50.02	45.61	PM	53.06	50.66	PR
30	SKUA-498	42.96	41.28	PM	36.68	33.62	PM	47.68	41.98	PM	65.28	59.74	PR
31	SKUA-499	55.26	53.51	PR	59.23	54.41	PR	49.88	46.92	PM	74.51	77.71	PR
32	SKUA-500	29.28	26.72	PM	55.21	50.34	PR	47.83	46.15	PM	1.77	1.48	PM
33	Pusa sug and h-3	54.99	52.68	PR	32.68	30.92	PM	87.81	84.16	R	86.83	82.82	R
34	Basmati-1509	45.73	42.42	PM	48.84	44.94	PM	81.71	77.16	R	83.06	79.92	R

PF: Pollen fertility, SF: Spikelet Fertility, PM: Partial Maintainer, PR: Partial Restorer, R: Restorer

Molecular evaluation: The pollen parents in the present study were validated for fertility restorer genes *Rf*3 (using markers DDRM-RF3-10, RM1 and RM3148) and *Rf*4 (using marker RM6100). *Rf*3 and *Rf*4 genes have earlier been described to restore fertility in Wild Abortive based CMS lines of rice (Ahmadikhah and Karlov, 2006; Alavi *et al.,* 2009).

RESULTS AND DISCUSSION

Evaluation for fertility restoration: The 136 test crosses derived from four CMS lines and 34 breeding lines were evaluated for pollen fertility at flowering stage and spikelet fertility at maturity. Pollen and spikelet fertility related traits form the important criteria for identifying restorer and maintainer lines at test cross nursery stage (Ikehashi and Araki, 1984; Virmani *et al.,* 1996). The fertility restoration behaviour of the pollen parents for the four CMS lines is presented in Table 1. Out of 136 possible cross combinations, 43 (31.6%) were found to show the fertility restoration and 30 (22.06%) were found to be partial restorers. Sixty-three (46.32%) cross combinations were found to be partial maintainers and no cross combination was found to exhibit the maintainer behaviour. It is clear from the data that the fertility restoration ability of the pollen parents varied with the genotype of the female parent. The genotypes RL-2, RL-3 and RL-8 were found to show restoration for all the CMS lines. It was evident that the frequency of restorers was higher than the frequency of maintainers for all CMS lines. The most probable reason may be that the male parents used in the crossing program are *indica* background derivatives from the crosses and one of the parents was a potential restorer. High frequency of restorers than the maintainers had also been reported by Upadhyay and Jaiswal (2012); Khan *et al.* (2012); Reddy *et al.* (2012) and Waza and Jaiswal (2016).

Estimation of Heterosis: Analysis of variance indicated the existence of significant differences among the genotypes studied (Table 3), revealing that sufficient variability was present for different traits and selection would be effective in developing the varieties with desirable features. Heterosis (%) over the better parent, mid parent and standard checks (Shalimar Rice-3 and Shalimar Rice-4) for different traits under study was observed for each and every cross combination. All the crosses possessed a variable degree of heterosis for different traits. For the traits, days to 50% flowering and kernel breadth, negative heterotic value is considered to be desirable. However, for rest of the characteristics, positive heterotic estimates are usually preferable. The top-performing hybrids in the present study have been presented in Table 4. The maximum positive value of standard heterosis in case of grain yield was recorded in SKUA-7A × RL-3. The hybrid, was also a top performer for flag leaf area, number of effective tillers per plant, panicle length and number of spikelets per panicle. The study is supported by the finding of previous workers Sravan *et al.* (2016); Priyanka *et al.* (2014a) and Rashid *et al.* (2019).

Table 3. Analysis of variance for different traits.

S. No	Characters	Mean squares						
		Replications (d.f= 2)	Crosses (d.f=135)	Females (d.f= 3)	Males (d.f= 33)	Female × male (d.f.= 99)	Error (d.f=270)	Total (d.f.=407)
1	Days to 50 per cent flowering	4.43**	40.75**	6.44**	78.43**	29.23**	0.35	13.77
2	Pollen fertility	4.15	2052.13**	1644.02	4216.04**	1343.19**	2.10	682.10
3	Plant height	97.03**	140.45**	37.46	313.95 **	85.80**	4.09	49.74
4	Flag leaf area	5.00**	25.63**	8.81*	50.01**	18.02**	0.33	8.75
5	Spikelet fertility	14.90**	1980.42**	1604.1	4110.57**	1281.77**	1.50	657.97
6	Number of tillers per plant	3.63*	18.52**	65.19*	32.56**	14.24**	1.12	6.90
7	Panicle length	0.04	3.63**	0.72	7.03**	2.59**	0.33	1.43
8	Number of spikelets per panicle	0.26	1342.80**	473.40	2992.85**	819.14**	7.18	450.17
9	1000 grain weight	0.25	5.87**	11.25**	7.79	5.07**	0.16	2.06
10	Grain yield per plant	0.53**	15.38**	6.54**	34.08**	9.41**	0.05	5.14
11	Biological yield per plant	0.003	77.68**	39.20**	161.22***	51.00**	0.09	25.83
12	Harvest Index	44.29**	58.54**	24.89**	81.44*	51.93***	3.44	21.92
13	Grain length	0.03	0.47*	0.26	0.16	0.498*	0.01	0.16
14	Kernel length	0.02	0.45**	0.12	0.20	0.37**	0.01	0.16
15	Grain breadth	0.001	0.03**	0.02	0.025	0.08**	0.002	0.010
16	Kernel breadth	0.003	0.04**	0.09	0.035	0.04**	0.004	0.015

*, **, *** = Significant at 0.05, 0.01 and 0.001 levels, respectively

Table 4. Best performing hybrids for various yield and yield attributes over the commercial checks.

	Over Shalimar Rice-3	Over Shalimar Rice-4
Days to 50 percent flowering	SKUA-21A × SKUA-499	SKUA-21A × SKUA-499
Plant height	SKUA-21A × RL-9	SKUA-21A × RL-9
Flag leaf area	SKUA-7A × RL-3	SKUA-7A × RL-3
Number of tillers per plant	SKUA-7A × RL-3	SKUA-7A × RL-3
Panicle length	SKUA-7A × RL-3	SKUA-7A × RL-3
Number of spikelets per panicle	SKUA-7A × RL-3	SKUA-7A × RL-3
1000 grain weight	SKUA-7A × RL-14	SKUA-7A × RL-14
Grain yield per plant	SKUA-7A × RL-3	SKUA-7A × RL-3
Biological yield per plant	SKUA-19A × RL-8	SKUA-19A × RL-8
Harvest Index	SKUA-7A × RL-6.	SKUA-7A × RL-6.
Kernel length	SKUA-21A × Basmati-1509	SKUA-21A × Basmati-1509.
Kernel breadth	SKUA-11A × SKUA-420	SKUA-11A × SKUA-420

Combining ability evaluation: None of the parental lines exhibited significant desirable values of GCA effects for all the traits studied. Similar results have been reported by Tiwari *et al.* (2011); Latha *et al.* (2013); Priyanka *et al.* (2014b) and Waza *et al.* (2015). Moreover, none of the crosses exhibited significant and desirable SCA effects for all the traits, showing that no specific cross combination was desirable for all the characters. Similar results have been reported by Tiwari *et al*. (2011) and Ghara *et al*. (2012). Among the female parents, SKUA-7A possessed significant desirable estimate of GCA effects for most of the characters under study. However, among the male parental lines RL-1, RL-2, RL-11, SKUA-497, SKUA-494, SKUA-496 and SKUA-420 showed significant desirable estimate of GCA effect for most of the traits studied. Out of the 136 hybrids, SKUA-19A × RL-13 revealed the maximum value of SCA effects for grain yield followed by SKUA-19A × RL-1, SKUA-7A × RL-3 and SKUA-11A × RL-4. For the top heterotic hybrids, the female parent SKUA-7A showed desirable value of GCA effect for grain yield, while 16 male parents also showed desirable GCA effect for the trait. Chakraborty *et al.* (2009) and Tiwari *et al.* (2011) have reported that to obtain heterotic hybrids, it is desirable to select at least one parent possessing higher GCA and other with either lower, average or high GCA. This is in accordance with the present findings.

Marker based screening of fertility restoration genes: Pollen parents were evaluated for the fertility restorer gene *Rf*3 using markers DDRM-RF3-10, RM1 and RM3148. Further, these parental lines were also validated through *Rf*4 linked SSR marker RM6100 (Table 5). The lines RL-1, RL-2, RL-5, RL-9, RL-11 and RL-12 showed polymorphism with all the markers. These molecular markers have already been reported as relating to fertility restoration in WA based CMS lines of rice (Singh *et al.,* 2005; Suresh *et al.,* 2012; Revathi *et al.,* 2013 and Waza *et al.,* 2013).

Table 5. Restoration behaviour of parental lines using molecular markers.

S. No	Genotypes/ Male parents	DDRM-RF3-10		RM1		RM3148		RM6100	
		Bp	Class	Bp	Class	Bp	Class	Bp	Class
1	RL-1	210	R	116	R	155	NR	165	NR
2	RL-2	210	R	116	R	170	R	175	R
3	RL-3	180	NR	116	R	155	NR	175	R
4	RL-4	180	NR	116	R	155	NR	175	R
5	RL-5	210	R	116	R	170	R	175	R
6	RL-6	210	R	116	R	155	NR	175	R
7	RL-7	210	R	116	R	170	R	175	R
8	RL-8	180	NR	116	R	155	NR	175	R
9	RL-9	210	R	116	R	170	R	175	R
10	RL-10	210	R	116	R	155	NR	175	R
11	RL-11	210	R	116	R	170	R	175	R
12	RL-12	210	R	116	R	170	R	175	R
13	RL-13	210	R	116	R	155	NR	175	R
14	RL-14	180	NR	116	R	170	R	175	R
15	SKUA-403	180	NR	100	NR	170	R	175	R
16	SKUA-408	180	NR	100	NR	170	R	175	R
17	SKUA-412	180	NR	100	NR	170	R	175	R
18	SKUA-415	180	NR	100	NR	170	R	165	NR

19	SKUA-420	180	NR	116	R	155	NR	165	NR
20	SKUA-483	180	NR	100	NR	155	NR	175	R
21	SKUA-484	180	NR	100	NR	170	R	175	R
22	SKUA-486	180	NR	116	R	170	R	175	R
23	SKUA-487	180	NR	116	R	170	R	175	R
24	SKUA-488	180	NR	100	NR	155	NR	175	R
25	SKUA-491	180	NR	100	NR	155	NR	175	R
26	SKUA-494	210	R	116	R	155	NR	175	R
27	SKUA-495	180	NR	100	NR	155	NR	175	R
28	SKUA-496	180	NR	100	NR	155	NR	175	R
29	SKUA-497	180	NR	100	NR	155	NR	165	NR
30	SKUA-498	180	NR	100	NR	155	NR	175	R
31	SKUA-499	180	NR	100	NR	155	NR	175	R
32	SKUA-500	180	NR	116	R	155	NR	175	R
33	Pusa sug and h-3	210	R	116	R	155	NR	175	R
34	Basmati-1509	180	NR	100	NR	170	R	175	R
CMS Lines									
1	SKUA-7A	180	NR	100	NR	155	NR	165	NR
2	SKUA-11A	180	NR	100	NR	155	NR	165	NR
3	SKUA-19A	180	NR	100	NR	155	NR	165	NR
4	SKUA-21A	180	NR	100	NR	155	NR	165	NR

Bp: Base pair, NR: Non-Restorer, R: Restorer

Sixteen hybrids showed fertility restoration behaviour with all the markers as well as under the field condition (Table 6). Among these, 8 crosses fall in the class of top 10 heterotic hybrids in the present study. PCR assay with DDRM-RF3-10, RM1 and RM3148 showed 210bp, 116bp and 170bp fragments specific to the fertility restorer allele *Rf*3, respectively. Similarly, 180bp, 100bp, 155bp fragments correspond to their non-restorer allele. Likewise, marker RM6100 depicts allele size of 175bp for restorer and 165bp for non-restorer phenotypes concerning the *Rf*4 locus. Sheeba *et al.* (2009) have reported the presence *Rf*4 gene at 1.2 cM from an SSR marker RM6100. The marker RM3148 has been reported to be flanked with *Rf*3 gene at a genetic distance of 19.7 cM (Nematzadeh and Kiani 2010). The marker RM1 has been reported to be flanked with *Rf*3 gene at a genetic distance of 6.49 cM (Alavi *et al.,* 2009).

Table 6. Hybrids showing fertility restoration behaviour under field conditions as well as with *Rf*3 and *Rf*4 markers.

S. No	Hybrids	S. No	Hybrids	S. No	Hybrids
1	SKUA-7A × RL-2	7	SKUA-11A × RL-7	12	SKUA-19A × RL-9
2	SKUA-7A × RL-5	8	SKUA-11A × RL-12	13	SKUA-21A × RL-2
3	SKUA-7A × RL-7	9	SKUA-19A × RL-2	14	SKUA-21A × RL-9
4	SKUA-7A × RL-11	10	SKUA-19A × RL-5	15	SKUA-21A × RL-11
5	SKUA-11A × RL-2	11	SKUA-19A × RL-7	16	SKUA-21A × RL-12
6	SKUA-11A × RL-5				

Cooking quality evaluation: The heterotic hybrids were evaluated for grain and cooking quality features. Out of the 10 heterotic hybrids evaluated, nine *viz.,* SKUA-7A × RL- 2, SKUA-7A × RL-3,SKUA-7A × RL-7,SKUA-11A × RL-7,SKUA-11A × RL-5, SKUA-21A × RL-14,SKUA-21A × RL-12, SKUA-21A × RL-11 and SKUA-21A × RL-9 were of medium to short-grain types and only one SKUA-19A × RL-5 was of long-grain type (Fig 1). The pattern may be attributed to dominance of short grain phenotype over the long-grain. Eight hybrids *viz.,* SKUA-7A × RL-7, SKUA-11A × RL-7, SKUA-11A × RL-5, SKUA-19A × RL-5, SKUA-21A × RL-14, SKUA-21A × RL-12, SKUA-21A × RL-11 and SKUA-21A × RL-9 showed hard gel consistency. However, one cross combination (SKUA-7A × RL-2) showed medium and another one (SKUA-7A × RL-3) as soft scores of the gel consistency. The hard gel consistency is dominant over medium which in turn is dominant over the soft (Tang *et al.,* 1991). Soft gel consistency is desirable especially in the Indian Subcontinent (Majid *et al.,* 2019). Heterotic hybrids along with their parents showed intermediate amylose content which is preferable. Besides, most of these hybrids showed intermediate alkali spread value.

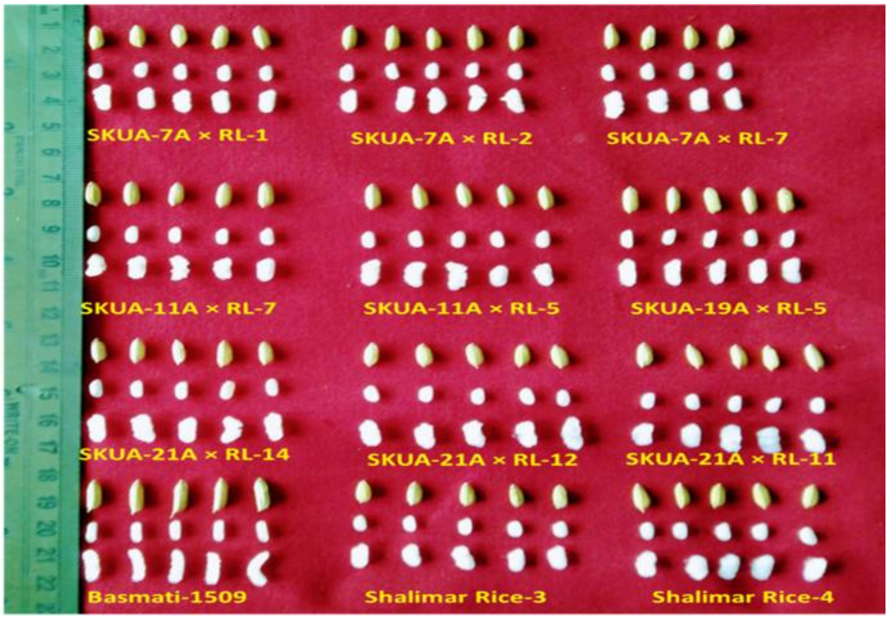

Fig 1. Grain type of the highly heterotic hybrids (Ist 3 rows) along with the check varieties (last row).

Conclusion: The present study encourages the commercial exploitation of heterosis in rice under temperate conditions where hybrids are not yet commercialized in this crop. Since the CMS lines in rice under temperate conditions have already been developed, the availability of effective restorers will boost the hybrid rice breeding under such regions. The genotypes homozygous for the restorer alleles may be used as parental lines for the development of heterotic three-line temperate hybrids after a thorough validation of heterosis for various traits. Knowledge regarding the genotypes possessing restorer genes shall enhance hybrid rice breeding under temperate conditions of Kashmir valley. Besides, the use of molecular markers has turned out to be the potential tool for enhancing the selection efficiency and thus the phenomenon of hybrid rice breeding. The highly heterotic hybrids so developed can be a way to breach the yield potential of rice, which may, in turn, ensure the food security and enhance the income of farmers.

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