Indian J. Genet., 79(1): 25-33 (2019)
DOI: https://doi.org/10.31742/IJGPB.79.1.4

Developing rice hybrids for temperate conditions using three line approach

Amir Rashid[1], Najeebul Rehman Sofi*, Asif Bashir Shikari[2], Gazala Hassan Khan, Showkat Ahmad Waza[2], Farooq Ahmad Sheikh, Ghulam Ahmad Parray, Mohd Ashraf Bhat[1], Mehrajudin Sofi and Ashaq Hussain

Mountain Research Centre for Field Crops, Khudwani, SKUAST-K, Jammu and Kashmir 192 102; [1]Division of Genetics and Plant Breeding, FOA Wadura, SKUAST-K; [2]Mountain Crop Research Station, Sagam, SKUAST-K, Jammu and Kashmir 192 102

(Received: July 2018; Revised: January 2019; Accepted: February 2019)

Abstract

Exploitation of heterosis in the form of hybrid rice is yet to be exploited at commercial level in the temperate regions of India. The present study is attempted to develop rice hybrids for the temperate conditions of Kashmir valley by utilizing the cytoplasmic genetic male sterility system (CGMS). Among the four CMS lines evaluated, the genotype SKUA-7A and SKUA-11A had desirable performances. The 24 test crosses developed involving these CMS lines were evaluated for spikelet fertility and pollen fertility. Moreover, the highest standard heterosis for grain yield and other yield attributes over the check varieties (Jehlum and Shalimar Rice-3) was recorded in the cross combination K15-34 followed by K15-31 and K15-40. Pollen parents of all the three test crosses on screening with RM-6100, DDRM-R*f*3-10 and DRCG-R*f*4-14 markers revealed presence of both R*f*3 and R*f*4 genes responsible for fertility restoration. Besides, 39 genotypes were screened for fertility restoration and the lines RL-2, RL-5, RL-6, RL-7, RL-10, Pusa Sugandh-3 and Kohsar were identified to carry homozygous alleles at both R*f*3 and R*f*4 loci. These genotypes can thus be used as promising restores for the development of temperate rice hybrids.

Key words: Hybrid rice, cytoplasmic male sterile lines, restorer lines, R*f*3, R*f*4

Introduction

Rice serves as a main staple food for more than half of the world population. The productivity of this crop has direct impact on global food security. Among the plethora of approaches for improving rice productivity, hybrid rice is a reliable option especially in irrigated rice growing areas. Hybrid rice exhibits superiority over high yielding purelines with respect to growth, yield and tolerance to stresses. The average yield of hybrid rice is at least 15-20 per cent more than that of inbred rice (Rather et al. 2001) and it has been anticipated that hybrid rice technology will play a key role in ensuring food security worldwide in the future decades (Sabar and Akhter 2003). Encouraged by the success of hybrid rice technology in enhancing rice productivity in China, India is the first country to exploit the hybrid rice technology on a commercial scale (Singh et al. 2013) and till date 102 rice hybrids developed both from public and private sector have been released for general cultivation (Anonymous 2018).

Although, research on the commercial utilization of heterosis in rice has made tremendous gains in tropical and subtropical regions of the country, it is still in its stage of infancy under temperate agro-ecological conditions. In the temperate areas, the phenomenon is yet to be exploited at commercial level. Kashmir valley is a part of greater Himalayas agro based system representing the typical temperate ecology (Najeeb et al. 2017). Rice serves as staple food for major portion of the population and is associated with economic, social and cultural heritage of the region. The current production of rice in the valley is about 0.4 million tonnes which is far behind from actual requirement of around 1.0 million tonnes (GoI 2016). Moreover, the demand of rice in Kashmir valley is at hike as the rate of increase in rice production is lower (1.5% per year) than the rate of increase in population (1.8% per year). Hybrid rice due to its high yield potential can serve as a solution to the problem.

---

*Corresponding author's e-mail: najeeb_sofi@rediffmail.com

Published by the Indian Society of Genetics & Plant Breeding, A-Block, F2, First Floor, NASC Complex, IARI P.O., Pusa Campus, New Delhi 110 012; Online management by www.isgpb.org; indianjournals.com

Among the various approaches for commercial production of rice hybrids, three line system is the most effective way of developing rice hybrids. Though, number of rice CMS lines have been developed for three line hybrid developments in Kashmir valley, their characterization for various traits to ensure commercial exploitation of heterosis is yet to be carried out. The availability of fertility restorers is a major component of hybrid rice development. Several researchers have developed restorer lines suitable for harvesting actual potential of hybrids. Development of iso-cytoplasmic restorer lines is a novel approach to produce hybrids with minimum interaction between the cytoplasmic and nuclear gene (Kumar et al. 2017a, 2017b) to get potential yield. Moreover, the availability of restorers have been a major limitation for commercial development of hybrids under temperate conditions. The present study is an attempt to develop rice hybrids for Kashmir valley by utilizing the cytoplasmic-genetic male sterility and fertility restoration systems. In this study, the characterization of some CMS lines for allogamic, agro-morphological and fertility restoration related traits were carried out to identify the best performing lines. The superior CMS lines were further crossed with some elite genotypes to identify potential restorers. The estimations of heterosis for various traits of commercial importance were also carried out to identify best heterotic combinations. The male parents of the superior cross combinations along with some advanced breeding lines/ released varieties/ pre-released lines/ elite high altitude genotypes were screened with the help of molecular markers to identify the genes responsible for fertility restoration.

Materials and methods

Experimental details and field layout

Experimental field trials were carried out over the wet seasons of 2016 and 2017 at Mountain Research Centre for Field Crops (Khudwani), Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir (34^{o} N latitude and 74^{o}E longitude at 1680 amsl). During 2016, four wild abortive (WA) CMS lines along with their maintainers were characterized for different allogamy related and agro-morphological characters (Tables 1, 2 and 3). Test crosses were made during the same year between two selected CMS lines and 24 genotypes (including advanced breeding lines, released varieties and pre-released genotypes) (Table 4). In 2017, the seeds of F_1 hybrids were planted in the field to identify restorers and maintainers among the male parents based on pollen and spikelet fertility

Table 1. Analysis of variance for various allogamic and agro-morphological traits

S.No.	Characters	Mean squares		
		Replication (d.f.= 2)	Treatment (d.f.= 7)	Error (d.f.= 14)
1.	Duration of open floret	2.81390	154.66754***	1.82995
2.	Angle of open floret	1.42116	20.08716***	0.99677
3.	Ovary length	0.94522	10.67422***	1.76551
4.	Stigma length	1.45986	12.61162***	1.10872
5.	Style length	1.60987	27.87652***	1.03561
6.	Pistil length	0.03440	2.19223***	0.03361
7.	Anther length	1.45407	31.13118***	0.74243
8.	Filament length	0.34119	1.86328***	0.20313
9.	Panicle exsertion	0.97485	10.89213***	1.30474
10.	No. of tillers	0.23892	3.16543***	0.10140
11.	No. of productive tillers	1.11501	63.02783***	0.90461
12.	Days to 50% flowering	0.31505	5.54711***	0.14461
13.	Plant height	0.98119	8.86328***	0.43413
14.	Panicle length	1.61026	26.08306***	1.07087
15.	Flag leaf length	0.05632	0.18976***	0.03115
16.	Flag leaf width	0.00089	0.16543***	0.00140
17.	Flag leaf angle	0.65925	10.10449***	0.23285
18.	Days to maturity	1.24395	16.19876***	1.08882
19.	No. of total spikelets	1.42565	14.53662***	1.17804
20.	No. of filled spikelets	1.61455	107.30576***	1.24682
21.	Spikelet fertility (%)	2.13249	245.5678***	1.28976
22.	1000 seed weight (g)	0.10250	0.24711***	0.03461
23.	Pollen sterility	3.03030	171.61364***	1.87405
24.	Grain yield	2.010985	221.97664***	1.05438

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

Table 2. Comparison of allogamy related traits of CMS lines and their maintainers

Lines	Duration of open floret (minutes)	Angle of open floret (^{0})	Ovary length (mm)	Stigma length (mm)	Style length (mm)	Pistil length (mm)	Anther length (mm)	Filament length (mm)	Panicle exsertion (%)
SKUA-7A	97.76^{e}	29.05^{ab}	0.56^{a}	1.63^{d}	0.54^{a}	2.70^{c}	1.95^{e}	7.72^{cd}	80.45^{g}
SKUA-7B	95.34^{d}	28.86^{a}	0.54^{ab}	1.65^{e}	0.53^{a}	2.71^{c}	1.94^{e}	7.71^{bc}	79.82^{g}
SKUA-11A	94.36^{d}	29.15^{ab}	0.57^{ab}	1.59^{b}	0.52^{a}	2.66^{ab}	1.85^{cd}	7.76^{d}	75.61^{e}
SKUA-11B	92.88^{c}	30.50^{cd}	0.57^{abc}	1.58^{bc}	0.52^{a}	2.66^{a}	1.86^{d}	7.76^{d}	76.11^{f}
SKUA-19A	92.42^{c}	33.50^{e}	0.61^{d}	1.55^{a}	0.63^{c}	2.68^{bc}	1.79^{ab}	7.60^{a}	74.81^{d}
SKUA-19B	90.10^{ab}	30.10^{c}	0.60^{cd}	1.54^{a}	0.59^{b}	2.67^{c}	1.80^{a}	7.61^{a}	73.98^{c}
SKUA-21A	90.44^{ab}	31.55^{cd}	0.59^{cd}	1.62^{d}	0.56^{a}	2.69^{bc}	1.82^{bc}	7.65^{b}	70.10^{a}
SKUA-21B	88.21^{a}	29.85^{bc}	0.58^{cd}	1.61^{bcd}	0.57^{b}	2.69^{c}	1.83^{bc}	7.67^{b}	71.51^{b}

Means followed by common letters are significantly not different by DMRT test at $p<0.05$

Table 3. Comparisons of agro-morphological characters of CMS lines and their maintainers

Lines	No. of tillers	No. of Productive tillers	Days to 50% flowering	Plant height (cm)	Panicle lenght (cm)	Flag leaf lenght (cm)	Flag leaf width (cm)	Flag leaf angle (^{0})
SKUA-7A	12.90^{e}	11.60^{d}	96^{d}	126.55^{g}	23.58^{a}	33.98^{ab}	1.14^{a}	32^{a}
SKUA-7B	14.10^{f}	12.50^{de}	96^{d}	123.6^{e}	24.85^{a}	43.19^{e}	1.37^{b}	33^{ab}
SKUA-11A	10.90^{c}	9.50^{ab}	97^{a}	125.82^{f}	23.92^{a}	35.09^{b}	1.38^{b}	36^{b}
SKUA-11B	11.80^{d}	10.40^{bc}	98^{a}	120.7^{d}	23.79^{a}	39.50^{d}	1.42^{b}	35^{ab}
SKUA-19A	10.40^{bc}	8.60^{a}	99^{b}	124.52^{e}	23.77^{a}	32.87^{a}	1.41^{b}	35^{ab}
SKUA-19B	9.50^{a}	9.10^{ab}	101^{b}	119.3^{c}	22.91^{a}	35.84^{b}	1.36^{b}	37^{b}
SKUA-21A	9.80^{ab}	9.90^{ac}	104^{c}	119.11^{b}	23.19^{a}	39.09^{c}	1.41^{b}	36^{ab}
SKUA-21B	10.70^{bc}	11.10^{cd}	100^{c}	113.67^{a}	23.76^{a}	37.78^{c}	1.40^{b}	36^{ab}

Means followed by common letters are significantly not different by DMRT test at $p<0.05$

Table 3. *(Contd...)*

Lines	Days to maturity	No. of total spikelets	No. of filled spikelets	Spikelet fertility (%)	1000-seed weight (g)	Pollen sterility (%)	Grain yield (t ha^{-1})
SKUA-7A	138^{bc}	145.6^{c}	0.0	0.0	0.0	99.50^{b}	0.0
SKUA-7B	139^{c}	144.8^{c}	140.2^{c}	96.8^{bc}	25.3^{a}	0.0	4.33^{d}
SKUA-11A	139^{a}	126.7^{a}	0.0-	0.0-	0.0	99.20^{b}	0.0
SKUA-11B	141^{a}	126.3^{a}	121.6^{a}	96.3^{b}	25.2^{a}	0.0	4.05^{c}
SKUA-19A	141^{ab}	137.4^{b}	0.0	0.0	0.0	98.50^{a}	0.0
SKUA-19B	142^{a}	136.6^{b}	130.6^{b}	95.6^{ab}	24.5^{b}	0.0	3.78^{b}
SKUA-21A	142^{bc}	165.7^{d}	0.0	0.0	0.0	98.00^{a}	0.0
SKUA-21B	143^{ab}	165.8^{d}	157.5^{d}	95.0^{a}	26.6^{c}	0.0	3.58^{a}

Means followed by common letters are significantly not different by DMRT test at $p<0.05$

studies. Heterosis estimates over better parent and over two standard check varieties (Jhelum and Shalimar Rice-3) for various traits of commercial importance were carried out from the test crosses to identify best performing hybrids. During both years of the study, planting material was raised in a randomized complete block design (RCBD) with three replications. Each genotype was raised in two rows of 3 m length,

Table 4. Evaluation of test crosses for identification of restorers and maintainers

Test cross	Pedigree	Spikelet fertility (%)	Pollen fertility (%)	Inference
K15-27	SKUA-7A/K10-3-6-1	96.78	95.43	Effective restoration
K15-28	SKUA-7A/K10-3-7-2	94.68	95.15	Effective restoration
K15-29	SKUA-7A/ K10-31-5-1	94.11	93.22	Effective restoration
K15-30	SKUA-7A/ K10-31-7-3	91.81	86.45	Effective restoration
K15-31	SKUA-7A/ K10-32-4-1	86.51	88.35	Effective restoration
K15-32	SKUA-7A/ K10-3-1-5	76.27	77.56	Partial restoration
K15-33	SKUA-7A/ K10-3-7-2	96.13	97.05	Effective restoration
K15-34	SKUA-7A/ K10-19-8-1	94.46	92.88	Effective restoration
K15-35	SKUA-7A/Pusa Sugandh-3	96.61	95.8	Effective restoration
K15-36	SKUA-7A/SKUA-403	59.49	60.56	Partial restoration
K15-37	SKUA-7A/SKUA-408	82.41	80.15	Effective restoration
K15-38	SKUA-7A/Pusa Basmati 1509	60.61	60.77	Partial restoration
K15-40	SKUA-11A/Pusa Sugandh-3	94.07	95.13	Effective restoration
K15-41	SKUA-11A/ SKUA-408	19.23	19.87	Partial maintenance
K15-42	SKUA-11A/Pusa Sugandh-3	93.18	94.52	Effective restoration
K15-43	SKUA-11A/SKUA-407	18.75	20.65	Partial maintenance
K15-44	SKUA-11A/ SKUA-420	19.54	21.61	Partial maintenance
K15-45	SKUA-11A/Shalimar Rice-2	94.41	94.1	Effective restoration
K15-46	SKUA-11A/ SKUA 292	18.15	22.88	Partial maintenance
K15-47	SKUA-11A/ SKUA -406	18.33	23.12	Partial maintenance
K15-48	SKUA-11A/ K10-3-2-2	93.61	93.56	Effective restoration
K15-49	SKUA-11A/ K10-19-2-1	95.08	94.78	Effective restoration
K15-50	SKUA-11A/ K10-19-4	95.16	94.55	Effective restoration
K15-51	SKUA-11A/ K10-19-1-3	96.07	97.04	Effective restoration

planting single seedling per hill. Recommended package of practices were followed to establish a good crop stand. Five plants of each genotype were randomly selected to record data on allogamic and agro-morphological traits.

Screening for pollen and spikelet fertility

Pollen sterility of the CMS lines and F_1s was determined by staining pollen grains in 1% potassium iodide- iodine (I-KI) solution. The pollens were counted in three random microscopic fields and classified as sterile or fertile based on their shape, size and staining pattern (Dalmacio et al. 1995; Virmani et al. 1996). For spikelet fertility, five panicles from each experimental line were bagged before flowering and at maturity the bagged panicles were examined for seed set. Spikelet fertility was determined as percentage by counting the total number of filled grains in proportion to the total number of spikelets. On the basis of pollen and spikelet fertility percentage, the genotypes were classified based on the criteria proposed by Govinda and Virmani (1988) and Virmani et al. (1997) i.e., restorers (pollen fertility >80%; spikelet fertility >75%), partial restorers (pollen fertility: 50.1-80%; spikelet fertility: 50.1-75%), partial maintainers (pollen fertility: 1.1-50%; spikelet fertility: 0.1-50%) and maintainers (pollen fertility: 0-1%; spikelet fertility: 0%).

Statistical analysis

The data generated was subjected to statistical analysis using SPSS (Statistical Package for the Social Sciences) version 16. To estimate significance of differences among the genotypes (hybrids, parents and checks), the mean data for each character was

Table 5. Estimates of heterosis of test crosses over better parent and standard varieties

Test cross	Productive tillers plant^{-1}			Spikelets per panicle^{-1}			Days to maturity			1000-grain weight (g)			Grain yield (t ha^{-1})		
	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C
K15-31	-19.51**	-16.81**	-14.66**	18.11**	10.34**	2.10	-0.73	0.00	-0.73	6.45**	8.83**	16.63**	9.21**	10.71**	06.33**
K15-34	20.24**	-15.13**	-12.93**	10.37**	10.45**	2.20	-2.17	-0.74	-1.46	-6.56**	2.12**	9.44**	9.44**	15.52**	11.01**
K15-40	14.12**	-18.49**	-16.38**	29.74**	6.90**	-1.09	6.47**	8.82**	8.03**	6.82**	1.26**	8.53**	17.63**	12.02**	7.60**
SE(±)	0.31	0.31	0.31	1.21	1.21	1.21	0.93	0.93	0.93	0.13	0.13	0.13	0.73	0.73	0.73

*P<0.05, ** <0.01; Check 1= Jehlum; Check 2= Shalimar Rice-3; A = Hererosis over better parent; B = Heterosis over check 1; C = Heterosis over Check 2

subjected to Analysis of Variance (ANOVA) as suggested by Steel and Torrie (1980). Duncan's Multiple Range test was also carried out for mean comparison. The *t* test was applied to determine significant difference of F_1 hybrids from respective better parents and standard checks using the formulae suggested by Wynne et al. (1970).

Molecular evaluation

The pollen parents (K10-32-4-1, K10-19-8-1 and Pusa Sugandh-3) of three test crosses (K15-31, K15-34 and K15-40) along with 14 advanced breeding lines and 25 other genotypes (Table 6) were screened for the presence of fertility restorer gene R*f*3 using marker DDRM-R*f*3-10. These genotypes were also screened with the help of R*f*4 linked SSR marker RM6100 and gene based marker DRCG-R*f*4-14. The R*f*3 and R*f*4 genes has been reported to restore male fertility for WA CMS system in rice and mapped on chromosomes 1 and 10, respectively (Ahmadikhah and Karlov 2006; Alavi et al. 2009).

Results and discussion

Evaluation of allogamic, agro-morphological and fertility restoration traits

In the present investigation, the ANOVA revealed considerable amount of variation among the genotypes for all the traits under study (Table 1). The CMS line SKUA-7A followed by SKUA-11A recorded desirable performance for most of the allogamy related characters such as duration of open florets, stigma length and panicle exsertion (Table 2). Duration of open floret is an important character which influences out-crossing in rice and its longer duration is desirable. Duration of open floret depends much on the pace of pollination as any delay or failure in pollination prolongs the duration of open floret (Parmar 1979; Virmani 1994). Similar results were recorded in this study as florets remained open for longer duration in CMS lines as compared to their respective maintainer lines. Duration of floret opening was recorded longest in CMS line SKUA-7A (97.7 minutes). Similarly, large feathery stigmas present a desirable character in CMS lines as it can receive pollens more efficiently resulting in better seed set on natural out-crossing. The stigma length of more than 0.8 mm is desirable (Gireesh et al. 2010) and all the CMS lines in present study recorded higher value of stigma length with highest value for SKUA-7A (1.63 mm). Larger style length helps in better stigma exsertion and thus improves the chances of receiving more pollen grains (Vidyachandra et al. 1997). In the present study, style length varied from 0.52 to 0.63 mm with highest value in SKUA-19A. Longer filament aids in better dispersal of pollen and in the present study, it ranged from 7.60 mm to 7.76 mm with highest value for SKUA-11A. Likewise, complete panicle exsertion in male-sterile lines would expose all spikelets for out-crossing. Incomplete panicle exsertion in a pollen parent would result in lower pollen release into the air. Therefore, a good panicle exsertion in both seed and pollen parent is essential to attain high outcrossing rates (Ingale et al. 2004; Gopinath and Raghava 2005). CMS line SKUA-7A recorded the highest panicle exsertion (80.4%) in the present study.

CMS lines SKUA-7A and SKUA-11A also exhibited good performance for other agro-morphological traits particularly number of tillers, plant height and pollen sterility (Table 3). The lines also recorded lowest number of days to 50% flowering and maturity than other CMS lines. Large number of productive tillers is a desirable trait and in this study highest number of productive tillers among CMS lines were recorded in SKUA-7A (11.6). Among the CMS lines, the highest number of spikelets per panicle was observed in SKUA-21A (165.7). Generally CMS lines with less than 95% pollen sterility are not desirable for use in hybrid production (Sawanath et al. 2003; Waza et al. 2016). All the CMS lines in the present study showed greater pollen sterility with the highest value in SKUA-7A (99.5%) followed by SKUA-11A (99.2%). High seed yield is the final aim of any crop improvement programme. Seed yield for CMS lines was recorded in their respective maintainer lines. In this study, the highest yield was recorded in SKUA-7B (4.33 t ha^{-1}) followed by SKUA-11B (4.05 t ha^{-1}). Based on the overall performance of CMS lines, SKUA-7A followed by SKUA-11A may be considered as most promising for development of hybrids under temperate conditions.

The 24 test crosses derived from the agro-morphologically superior CMS lines (SKUA-7A and SKUA-11A) were evaluated for spikelet fertility and pollen fertility. The pollen and spikelet fertility traits are important criteria at test cross nursery stage for identifying restorer and maintainer lines (Ikehashi and Araki 1984; Virmani 1996). Based on the estimates, male parents were classified into 16 effective restorers, five partial maintainers and three partial restorers (Table 4). Most of the lines behaved as restorers and the higher frequency of restorers may be due to the reason that all the lines belonged to indica background which possess good fertility restoration ability towards WA cytoplasm. High frequency of restorers than the maintainers have also been reported earlier (Virmani and Edwards 1983; Jaiswal and Parveen 2009; Das et al. 2013; Waza et al. 2016). Contrary to the present findings, Ali and Khan (1996) reported nearly four times higher frequency of maintainers than restorers in the material they studied. In this study, the cross combination which had the highest restoration ability was SKUA-7A/K10-3-6-1 and minimum restoration ability was exhibited by SKUA-11A/SKUA-408.

Estimation of heterosis

Heterosis (%) over better parent and over standard checks (Jhelum and Shalimar Rice-3) for each trait under study was estimated for all the 24 cross combinations. The heterosis varied from cross to cross and trait to trait. None of the test crosses recorded significantly desirable heterosis for all the traits. For the traits where increasing trend is desirable, the hybrids with significant and positive heterotic effects were considered superior. For the characters where decreased expressions are favoured, the crosses with significant negative heterosis were considered

Table 6. Marker based characterization for fertility restoration

Genotype	Gene	Status**
RL-1	R*f*3	Restorer
RL-2	R*f*3, R*f*4	Restorer
RL-3	R*f*4 (Heterozygous)	Non-Restorer
RL-4	R*f*4 (Heterozygous)	Non-Restorer
RL-5	R*f*3, R*f*4	Restorer
RL-6	R*f*3, R*f*4	Restorer
RL-7	R*f*3, R*f*4	Restorer
RL-8	R*f*3 (Heterozygous), R*f*4	Restorer
RL-9	R*f*3, R*f*4*	Restorer
RL-10	R*f*3, R*f*4	Restorer
RL-11	R*f*3, R*f*4*	Restorer
RL-12	R*f*4	Restorer
RL-13	R*f*3, R*f*4*	Restorer
RL-14	R*f*4*	Restorer
Shalimar Rice-1	None	Non-Restorer
Shalimar Rice-2	R*f*4	Restorer
Shalimar Rice-3	None	Non-Restorer
Shalimar Rice-4 (SKUA-408)	None	Non-Restorer
SKUA-412	None	Non-Restorer
SKUA-415	None	Non-Restorer
SKUA-420	None	Non-Restorer
SKUA-402	R*f*3, R*f*4*	Restorer
K09-12-13-1	None	Non-Restorer
K10-1-1-1	None	Non-Restorer
K08-1-11	None	Non-Restorer
K08-23-2-1-2	None	Non-Restorer
PVT-V3	None	Non-Restorer
Pusa Sugandh-3	R*f*3, R*f*4	Restorer
Pusa Basmati-1509	R*f*4	Restorer
K-332	None	Non-Restorer
K-10-33	R*f*4	Restorer
Kamad	R*f*3 (Heterozygous)	Non-Restorer
MushkBudji	R*f*3 (Heterozygous), R*f*4	Restorer
Zag	R*f*3	Restorer
Kohsar	R*f*3, R*f*4	Restorer
SKUA-403	None	Non-Restorer
SKUA-406	None	Non-Restorer
SKUA-407	None	Non-Restorer
SKUA-292	None	Non-Restorer

*The lines amplified restorer specific allele with respect to either one of the markers RM6100 or DRCG-R*f*4-14 and carried non-restorer allele for the other. **The grouping for restorers was done on the basis of at least one dominant homozygous allele for either of the genes (R*f*3 or R*f*4); DDRM-R*f*3-10 (R*f*3); RM6100 (R*f*4) promising (Dar et al. 2015; Waza et al. 2016). The highest positive standard heterosis for grain yield over both the checks was recorded in K15-34 followed by K15-31 and K15-40. These crosses also revealed significant heterosis for number of productive tillers per plant, number of spikelets per panicle and 1000 seed weight (Table 5). All the three crosses may be considered as most desirable ones keeping in view their overall performance for yield and yield attributes. Among these cross combinations, K15-34 revealed the highest estimates of standard heterosis of 15.5% and 11% over checks Jhelum and Shalimar Rice 3, respectively. Heterosis for earliness in the present study was found to be in both negative and positive directions, which has also been reported earlier (Neelam et al. 2009; Waza et al. 2016).

Molecular screening of genotypes for fertility restoring genes

Male parents (K10-32-4-1, K10-19-8-1 and Pusa Sugandh-3) of all the three most desirable cross combinations were screened for the presence of fertility restorer gene R*f* 3 using marker DDRM-R*f* 3-10. These genotypes were also screened for R*f*4 linked SSR marker RM6100 and gene based marker DRCG-R*f*4-14. Based on marker assisted screening, all three lines were identified as homozygous for both the restorer genes. These markers have earlier been validated for fertility restoration in rice WA-based CMS lines (Singh et al. 2005; Suresh et al. 2012; Revathi et al. 2013).

In addition, 14 advanced breeding lines were screened for fertility restorer gene R*f* 3 with the help of marker DDRM-R*f* 3-10. Lines RL-1, RL-2, RL-5, RL-6, RL-7, RL-9, RL-10, RL-11 and RL-13 amplified restoration specific allele (210 bp), while RL-4, RL-12 and RL-14 showed alternate allele of 180 bp size that is linked to non-restorer trait. The line RL-8 revealed heterozygous behavior at DDRM-R*f* 3-10 (Fig. 1). All the lines were screened for fertility restorer R*f*4 gene using linked SSR marker RM6100 and gene based marker DRCG-R*f*4-14. The lines RL-2, RL-5, RL-6, RL-7, RL-8, RL-10, RL-12 and RL-14 amplified homozygous restoration specific allele (175bp) for RM6100. All the 14 lines except RL-1 and RL-14 were found to carry homozygous restoration allele at DRCG-R*f*4-14 marker locus. However, lines RL-3 and RL-4 revealed heterozygous behavior at both the marker loci RM6100 and DRCG-R*f*4-14 (Table 6).

A set of 25 lines (including important released varieties, pre-released lines and elite high altitude genotypes) were also screened with the help of molecular markers (Table 6). For marker DDRM-R*f* 3-10, all the genotypes amplified non-restoration specific allele except for SKUA-402, Pusa Sugandh-3, Zag and Kohsar which amplified the homozygous restoration specific allele. Kamad and Mushk Budji amplified heterozygous fragment at R*f* 3 locus. Shalimar Rice-2, SKUA-402, Pusa Sugandh-3, Pusa Basmati-1509, K-10-33, Mushk Budji and Kohsar amplified restoration specific allele for both RM6100 and DRCG-R*f*4-14 markers. Rest of the genotypes revealed non-restorer allele at both the markers of R*f*4.

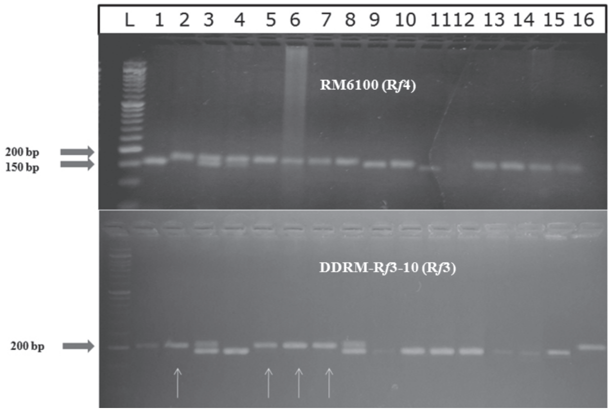

Fig. 1. Marker based screening of different rice genotypes for fertility restorer genes R*f*4 and R*f* 3. Lanes-1: RL-1, 2: RL-2, 3: RL-3, 4 : RL-4, 5: RL-5, 6: RL-6, 7: RL-7, 8: RL-8, 9: Shalimar Rice-1, 10: Shalimar Rice-2, 11: Shalimar Rice-3, 12: Shalimar Rice-4, 13: SKUA-412, 14: SKUA-415, 15: SKUA-420, 16: SKUA-402. Green arrows reveal the presence of homozygous restoration specific alleles for both R*f*4 and R*f* 3

In the present study, the classification of a genotype for being a restorer was done considering at least one dominant homozygous allele for either of genes R*f* 3 and R*f*4. This is in accordance with the work carried out by Katara et al. (2017). Since, the two markers RM6100 or DRCG-R*f*4-14 were used to screen the gene R*f*4, only one of them amplified restoration specific alleles across the genotypes RL-9, RL-11, RL-13, RL-14 and SKUA-402. This may be due to the possibility of a recombination event between the marker and the gene during the development of these restorers. The markers validated in this study are co-dominant and thus capable of discriminating the homo and heterozygotes. These markers can be used to accelerate the process of breeding restorer lines in rice (Suresh et al. 2012; Revathi et al. 2013; Waza and Jaiswal 2016; Katara et al. 2017). The lines RL-2, RL-5, RL-6, RL-7, RL-10, Pusa Sugandh-3 and

Kohsar were identified to carry homozygous alleles for fertility restoration at both R*f*3 and R*f*4 loci. Marker based evaluation for R*f* genes can be useful in generating information on alleles harboured in test germplasm lines that can subsequently be used as promising restores for the development of rice hybrids.

The present study opens up a new avenue for commercial exploitation of heterosis in rice under temperate agro-ecosystems. The introduction of rice hybrids need to be backed up with robust and durable hybrid seed production system. This is possible only at the availability of adaptable, reliable and stable set of parental lines. The development of temperate CMS lines in an adaptable background along with the availability of suitable and effective fertility restorers is indispensible for the development of three line rice hybrids. Moreover, the characterization of well adapted elite genotypes for R*f* loci and their subsequent exploitation for the development of highly heterotic hybrids can ensure food security under the temperate conditions. Besides, molecular markers have the potential to enhance the selection efficiency and thereby speed up the hybrid rice breeding. These lines can be useful as parents for the development of successful three-line temperate rice hybrids.

Authors' contribution

Conceptualization of research (AR, NRS, ABS); Designing of the experiments (AR, NRS, ABS); Contribution of experimental materials (NRS, FAS, GAP, MAB); Execution of field/lab experiments and data collection (NRS, GHK, SAW, MS); Analysis of data and interpretation (NRS, SAW, AH); Preparation of manuscript (AR, NRS, SAW, AH).

Declaration

The authors declare no conflict of interest.

References

Anonymous. 2018. Progress Report 2017. Vol.1, Varietal Improvement, All India Coordinated Rice Improvement Project, ICAR-Indian Institute of Rice Research, Rajendranagar, Hyderabad.

Ahmadikhah A. and Karlov G. I. 2006. Molecular mapping of the fertility restoration gene R*f*4 for WA cytoplasmic male sterility in rice. Plant Breed., **125**: 363-367.

Alavi M., Ahmadikhah A., Kamkar B. and Kalateh M. 2009. Mapping R*f*3 locus in rice by SSR and CAPS markers. Int. J. Genet. Mol. Biology, **1:** 121-126.

Ali S. S. and Khan M. G. 1996. Maintainers and restorers identified in some rice cultivars of Pakistan. Int. Rice Res. Newslett., **21**: 31.

Dalmacio R. D., Brar D. S., Ishii T. and Virmani S. S. 1995. Identification and transfer of a new cytoplasmic male sterility source from *Oryza perennis* into rice (*O. sativa*). Euphytica, **82**: 221-225.

Dar Z. A., Lone A. A., Alaie B. A., Ali G., Gazal A. and Abidi I. 2015. Estimation of combining ability involving quality protein maize (QPM) inbreds under temperate conditions. The Bioscan. **10**(2): 863-867.

Das P., Mukherjee B., Santra C. K., Mukhopadhyay S. and Dasgupta T. 2013. Evaluation of Genotypes for fertility restoring and maintaining behaviors in rice (*Oryza Sativa* L*.).* Int. J. Sci. Tech. Res*.,* **2**(11): 228-232.

Gireesh C., Shet R. M., Jagadeesha N., Babu A. G. and, Vidyachandra B. 2010. Evaluation of new non-scented CMS lines and their maintainer lines of rice (*Oryza sativa* L.) for their agronomical and floral traits. Int. J. Plant Sci., **5**: 87-92.

Gopinath M. and Raghava R. P. 2005. Stability analysis for agronomic traits in CMS lines of rice (*Oryza sativa* L.). Oryza, **42**(4): 253-255.

GoI. 2016. Agricultural Statistics at a Glance; Area, production and yield of principal crops. Ministry of Agriculture. Government of India. (https://community.data.gov.in).

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

Ikehashi H. and Araki H. 1984. Variety screening of compatibility types revealed in F_1 fertility of distant cross in rice*.* Japanese J. Breed., **34**: 304-313.

Ingale B. V., Waghmode B. D., Sawant D. S. and Shinde D. B. 2004. Evaluation of newly developed CMS lines of rice (*Oryza sativa* L.) for their agronomic and floral traits. Indian J. Genet., **64**: 286-90.

Jaiswal H. K. and Parveen S. 2009. Identification of basmati maintainers and restorers of WA CMS lines in rice. Int. Rice. Res. Notes, **34**: 1-3.

Katara J. L., Verma R. L., Nayak D., Ngangkham U., Ray S. and Subudhi H. 2017. Frequency and fertility restoration efficiency of R*f*3 and R*f*4 genes in Indian rice. Plant Breed., **136**: 74-82.

Kumar A., Bhowmick P. K., Singh V.J., Malik M., Gup[ta A. K., Seth R., Nagarajan M., Gopala Krishnan S. and Singh A. K. 2017a. Marker assisted identification of restorer genes(s) in iso-cytoplasmic restorer lines of WA cytoplasm in rice and assessment of their fertility restoration potential across environments. Physiol. Mol. Biol. Plants, Oct., **23**(4): 891-909. DOI: 10.1007/s12298-017-0464-5.

Kumar A , Bhowmick P. K., Gopala Krishnan S. and Singh A. K. 2017b. Development and evaluation of iso-cytoplasmic rice restorer lines for different agro-morphological traits. Indian J. Genet., **77**(4): 493-500. DOI: 10.5958/0975-6906.2017.00065.7

Najeeb S., Shikari A. B and Parray G. A. 2017. Status paper on Rice in Jammu & Kashmir (www.rkmp.co.in).

Neelam S., Ramesha M. S., Reddy T. D. and Sankar A. 2009. Study of heterosis by utilizing male sterility-restoration system in rice (*Oryza sativa* L.). J. Rice Res., **2**: 110-120.

Parmar K. S., Siddiq E. A. and Swaminathan M. S. 1979. Chemical induction of male sterility in rice. Indian J. Genet., **39**: 529-541.

Rather A. G., Zargar M. A. and Sheikh F. A. 2001. Genetic divergence in rice (*Oryza sativa* L.) under temperate conditions. Indian. J. Agric. Sci., **71**(5): 344-345.

Revathi P., Pavani M., Arunkumar S., Sundaram R. M., Sravan R., Senguttuvel P., Kemparaju K. B., Hariprasad A. S., Ramesha M. S., Neeraja C. N., Rani N. S. and Viraktamath B. C. 2013. Efficiency of molecular markers in identifying fertility restoration trait of WA CMS system in rice. Indian J. Genet., **73**(1): 89-93.

Sabar M. and Akhter M. 2003. Evaluation of rice germplasm for the development of hybrid rice. Asian J. Plant. Sci., **2**: 1195-1197.

Sawanth D. S., Shetye V. N. and Shirdhankar M. M. 2003. Evaluation of some cytoplasmic male sterile lines of rice in Konkan area of Maharastra state. Ann. Agric. Res., **24**(2): 337-344.

Singh H. N., Singh S. P., Singh U. S., Singh J., Zaid N. W. and Mohanty S. 2013. Economic evaluation of Pusa Rice Hybrid 10 cultivation: a microeconomic evidence of Uttarakhand, India. Indian J. Traditional Knowledge, **12**(3): 472-477.

Singh N., Kaur L., Sodhi N. S. and Sekhon K. S. 2005. Physico-chemical, cooking and textural properties of milled rice from different Indian rice cultivars. Food Chem., **89**: 253-259.

Steel R. G. D. and Torrie J. H. 1980. Principles and procedures of statistics. A biometrical approach, 2nd Edition, McGraw-Hill Book Company, New York.

Suresh P. B., Srikanth B., Kishore V. H., Rao I. S., Vemireddy L. R., Dharika N., Sundaram R. M., Ramesha M. S., Rao K. R. S. S., Viraktamath B. C. and Neeraja C. N. 2012. Fine mapping of R*f*3 and R*f*4 fertility restorer loci of WA-CMS of rice (*Oryza sativa* L.) and validation of the developed marker system for identification of restorer lines. Euphytica, **187**: 421-43.

Vidyachandra B., Bhaskar V. and Krishnamurthy A. H. 1997. Ideal characters of parental lines. In: Hybrid rice seed production-Theory and Practices, (Eds: Vidyachandra B. and Gubbaiah) pp. 46-50.

Virmani S. S. and Edwards I. B. 1983. Current status and future prospects for breeding hybrid rice and wheat. Adv. Agron., **36**: 145-214.

Virmani S. S. 1994. Outcrossing mechanisms and hybrid seed production practices in rice. In: (Eds. Virmani S. S.). Heterosis and hybrid rice breeding. Springer, Berlin, pp. 79-109.

Virmani S. S. 1996. Hybrid rice. Adv. Agron., **57**: 377-462.

Virmani S. S., Virakamath B. C., Laral C. L., Toledo R. S., Lopez M. T. and Manalo J. O. 1997. Hybrid rice breeding manual, IRRI, Manila, Phillipines, p. 151.

Waza S. A. and Jaiswal H. K. 2016. Identification of elite grain quality restorers and maintainers for WA CMS lines of rice (*Oryza sativa* L.). SABRAO J. Breed. Genet., **48**: 145-153.

Waza S. A., Jaiswal H. K., Sravan T., Bano D. A., Priyanka K. and Umesh 2016. Heterosis for yield and quality traits in rice (*Oryza sativa* L.). J. Appl. Nat. Sci., **8**(3): 1510-1522.

Wynne J. C., Emery D. A. and Rice P. W. 1970. Combining ability estimates in *Arachis hypogea* L. II. Field performance of F_1 hybrids. Crop Sci., **10**: 713-721.