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Marker Assisted Development of Effective Fertility Restorers Suitable for Use in Temperate Three-line Hybrids

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Identification of restorers and maintainers from genetically diverse materials is a prerequisite for hybrid rice development. Generally restorers and maintainers are identified by evaluation of test cross hybrids for pollen and spikelet fertility. Phenotypic evaluation is a cumbersome, labour intensive and time consuming method. Also, in our previous studies the number of germplasm lines carrying traits for restoration seemed to be limited. Therefore, present study aimed to transfer *Rf-4* gene from Basmati to one of our promising temperate background by employing Marker assisted backcross breeding. A genotype G-8 was crossed to elite WA-restorer line PRR-78 and were derived at least four lines carrying gene *Rf-4* in BC_1F_2 with the help of gene linked marker RM6100. These lines were phenotyped for pollen and spikelet fertility restoration after crossing these in Line x tester fashion along with nine other lines with CMS lines SKAU-7A, SKAU-11A and IR68888A. Out of 13 lines two of the lines viz., PRR-78, SKAU-K-R-3-6-11 and SKAU-K-R-3-6-16 showed complete restoration coupled with high degree of heterosis for grain yield. The lines could be useful in development of three-line hybrids suitable for temperate high altitude areas beyond 1600 to 2000 m amsl.

Key words: Rice, Hybrids, CMS, Restorer, Marker.

Over the next 30 years, the production of staple cereal grains including wheat, maize, and rice must to be doubled to keep pace with global population and income growth (Spindel, 2015). Rice is a staple food for more than half of the world's population. More than 90% of rice is produced and consumed in Asia. Increasing rice production would play a key role in efforts to secure the world food supply. Hybrid rice is being perceived as a practically feasible and readily adoptable genetic option to increase the rice production, as has been amply demonstrated in People's Republic of China. Exploitation of hybrid vigour through development and commercialization of hybrids is a practical approach for increasing productivity (Zhang 2008; Yuan and Peng, 2005). Approximately, 2.5 m ha area is grown under hybrid rice in India with 69 hybrids released from both public and private sector (Tiwari *et al*., 2014). Three line system comprising A-, B- and R-lines is indispensible for hybrid seed production in rice. Cytoplasmic male sterile (CMS) lines and nucleus controlled fertility restorers combine seed producing traits along with required heterosis. This requires identification of better combiners among the available germplasm/ breeding lines, which are to be further classified as

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maintainer of male sterility or restorer of male fertility in test cross nursery. The process requires raising of F_1s and subsequently, the screening based on pollen and spikelet sterility to infer the male parent to be restorer or maintainer which is tedious and involves extra season. Precise transfer of fertility restorer (*Rf*-) gene(s) in desirable background would solve the problem and help us to develop a male line (R) with good restoration ability in a background with high combining ability.

Kashmir region of India has purely temperate ecology where rice is grown under irrigation waters sourcing out from melting Himalyan glaciers. Most of the rice hybrids released from private/ public sectors don't mature or depict low yield heterosis. Also, restorers are lacking in our germplasm collections. Thus, present study was taken with the objective of transferring *Rf-4* gene in elite rice germplasm line for development of high heterotic F_1s for temperate highlands.

MATERIALS AND METHODS

The seed materials comprised 13 lines which were crossed with three WA based CMS lines in L x T fashion two of which viz., SKAU-7A and SKAU-11A are temperate CMS lines having low temperature tolerance previously developed at our centre (Shikari *et al*., 2010). The test cross nursery was raised and observations were recorded on agronomical traits of interest and also on pollen and spikelet fertility. Direct F_1s from each cross were planted under two replicates in 3m rows flanked by corresponding parents. Recommended package of practices were adopted and data was recorded for plant height (PH), effective tillers per plant (NT), panicle length (PL), number of grains per panicle (GP), 1000-seed weight (SW) and grain yield per plant (GY). The combining ability analysis was performed following Sharma (2006).

Screening for pollen and spikelet fertility

Pollen fertility and seed-setting rate were used as the main criteria for the evaluation of fertile and sterile plants. Pollen grain fertility was recorded after anthers were collected from spikelets two days before anthesis and later anthers were fixed in acetic acid-alcohol (1:3) solution (Sarial and Singh, 2000). Three undehised spikelets were randomly selected from different positions on the panicle. The anthers from each spikelet were smeared in a drop of 1% I2-KI solution on a glass slide separately and observed under compound microscope. Plants were classified into different fertility-sterility groups based on proportion of stained-round pollen grains (No. of fertile grains x 100/total number observed). Accordingly, the plants were classified into the following classes: Restorers (R): Plants showing more than 80% pollen fertility; Partial restorers (PR): 21 to 80% pollen fertility; Partially sterile (PS): 1 to 20% pollen fertility and maintainers (M): <1% pollen fertility. The panicles that emerged from the primary tiller were bagged before anthesis and the number of filled grains and chaffs in the panicle were counted at the time of maturity. The ratio of filled grains to the total number of spikelets was expressed as seed setting rate (He *et al*., 2006).

Marker analysis

Genomic DNA of all the accessions was extracted from fresh, healthy and young leaf tissue from twenty day-old seedlings following Cetyl-Tri Methyl Ammonium Bromide method (Murray and Thompson, 1980). The DNA was purified by adding RNase (10 g/100ml) to the sample at the rate of 1µ l/100 ml of crude DNA. DNA quantification was done using 0.8% Agarose gel and the final concentration was adjusted to ~50 ng/ml. The samples were subjected to Polymerase Chain Reaction using microsatellite primer RM6100. PCR reaction mixture contained 50 ng of DNA, 10x PCR buffer (10 mM Tris, pH 8.4, 50 mM KCl, 1.8 mM MgCl2), 0.05 mM dNTPs (MBI, Fermentas, Lithuania, USA), 5 pmol each of forward and reverse primer and 0.5 U of Taq DNA polymerase (Sigma-Aldrich Co., India) in a reaction volume of 10 µl. Polymerase chain reaction (PCR) was performed in a thermal cycler (TaKara, Otsu, Shiga, Japan). Initial denaturation was performed at 94°C for 5 minutes followed by 35 PCR cycles with following thermal conditions: denaturation at 94°C, annealing at 55°C and extension at 72°C; afterwards a final extension for 7 min at 72°C was provided. The amplified products were resolved on 3.5% agarose gel containing 0.5 g/ml ethidium bromide and were visualized in UV trans-illuminator and documented in gel documentation system (Bio-Rad Laboratories Inc., Hercules, CA).

RESULTS AND DISCUSSION

Marker Assisted Incorporation of Rf-4

A medium slender line G-8 was crossed as female to PRR-78 (a fine grain donor) carrying gene *Rf-4* for fertility restoration (Singh *et al*., 2012, Singh *et al*., 2014) in order to transfer the gene through marker assisted backcrossing in G-8 (Fig.1). Polymorphism survey was carried out with the markers flanking *Rf-4* on chromosome 10 and a marker namely RM6100 was found to be polymorphic between parents. F_1s developed were conformed for heterozygosity and single F_1 plant SKAU-K-R-3 was backcrossed to recurrent parent G-8 to generate 15 BC_1F_1 plants. The foreground analysis was carried out using RM6100 which helped us to recover seven heterozygous : eight homozygous plants showing 1:1 ratio (χ^2=1.25) (Fig. 2). Phenotypically, best looking plant SKAU-K-R-3-6 was selfed to raise BC_1F_2. One hundred fourteen BC_1F_2 plants were raised in next generation. Based on grain type akin to RP, 20 best plants were tagged and harvested. Leaf sample was collected and they were subjected to foreground analysis using RM6100. Four out of 20 plants named SKAU-K-R-3-6-4, SKAU-K-R-3-6-7, SKAU-K-R-3-6-11 and SKAU-K-R-3-6-16 were found homozygous for restorer specific allele and each of them were crossed to CMS lines SKAU-7A, SKAU-11A and IR68888A to generate test cross F_1s.

Test cross nursery

Besides the lines developed from marker assisted backcrossing in above step, nine other lines including donor PRR-78 were crossed to CMS lines SKAU-7A, SKAU-11A and IR68888A to produce F_1s. The test cross nursery was laid in next year at MRCFC, Khudwani for evaluation of potential fertility restorers. Based on pollen and spikelet fertility, the lines were classified into effective restorers (R), partial restorers (PR), partial restorers (PM), and maintainers (M). The lines PRR-78, SKAU-K-R-3-6-11 and SKAU-K-R-3-6-16, Pusa Sugandh-3, Pusa Sugandh-5 were recorded to show pollen and spikelet fertility of more than 80% and were regarded as restorers (Table 1.) On the other hand the local cultivars Jhelum, Mushk

Table 1. Classification of potential restorers and maintainer lines in test cross nursery (SES-IRRI, 1996)

S. No.	Line	SKAU-7A	SKAU-11A	Pollen/ Spikelet fertility	Status	Allele size (bp)
1	PRR-78	97.94	98.49	PF	R	175
		92.44	95.28	SF		
2	SKAU-K-R-3-6-4	72.50	75.87	PF	PR	160
		74.66	71.44	SF		
3	SKAU-K-R-3-6-7	73.24	71.25	PF	PR	160
		62.15	63.48	SF		
4	SKAU-K-R-3-6-11	96.58	97.28	PF	R	175
		85.77	86.33	SF		
5	SKAU-K-R-3-6-16	99.54	95.45	PF	R	175
		89.00	90.68	SF		
6	SKAU-382	65.25	69.85	PF	PR	150
		55.87	53.22	SF		
7	SKAU-389	70.23	67.18	PF	PR	175
		62.14	60.10	SF		
8	China-988	14.21	11.12	PF	PM	150
		9.25	14.09	SF		
9	Pusa Sugandh-3	89.46	92.34	PF	R	175
		83.65	86.11	SF		
10	Pusa Sugandh-5	91.84	93.55	PF	R	175
		85.41	84.18	SF		
11	Jhelum	0.53	0.24	PF	M	160
		0.00	0.00	SF		
12	Mushk Budji	0.84	0.64	PF	M	160
		0.22	0.00	SF		
13	Shalimar Rice-1	0.21	0.00	PF	M	160
		0.00	0.00	SF		

Budji and Shalimar Rice-1 were found to be effective maintainers. These lines can be used to develop new CMS lines in diverse backgrounds with the primary objective of gaining more heterosis. PRR-78 is the known restorer line of popular Basmati hybrid PRH-10 (Singh *et al*., 2011) and the lines SKAU-K-R-3-6-11 and SKAU-K-R-3-6-16 presently developed using PRR-78 in the local genetic background. SKAU-K-R-3-6-4, SKAU-K-R-3-6-7, SKAU-382 and SKAU-389 were

Table 2. Line x Tester analysis for yield and its components using a set of three CMS lines

Source of variation	d.f.	NT	PH	GP	PL	SW	GY
Entries	54	73.50**	252.91**	1949.41**	12.82**	5.58**	590.84**
Parents	15	37.05**	438.77**	1385.61**	11.24**	4.92**	538.41**
Males	12	8.12	789.15**	721.28**	7.98*	0.36	25.76*
Females	2	39.11**	383.45**	993.44**	10.80**	6.45**	213.93**
Males x Females	1	76.40**	235.86**	6243.73**	21.73**	0.29	4484.05**
Hybrids	38	49.43**	161.91**	1508.09**	13.41**	6.03**	468.77**
Females in hybrid	12	67.44**	155.54**	8789.00**	21.17**	4.51*	838.28**
Males in hybrid	2	15.00**	50.00**	106.63**	10.45**	13.07**	183.63**
Males x Females in hybrid	24	40.21**	100.00**	4148.00**	2.56	3.05	500.00**
Parent x Hybrid	1	1241.47**	3193.25**	24520.28**	122.40**	22.30	6463.96**
Error	108	0.34	15.93	21.21	0.76	0.17	9.73
% T contribution		2.61	8.15	0.32	3.54	9.86	1.78
% L contribution		43.41	30.57	60.30	50.24	23.82	56.90
% LXT contribution		19.88	27.19	5.29	12.12	32.23	7.23
δ^2 gca		0.03	0.11	8.57	0.68	0.29	0.27
δ^2 sca		13.39	3.77	16.62	0.60	0.96	0.38
δ^2_A		0.06	0.22	17.13	1.36	0.59	0.55
δ^2_D		13.39	3.77	16.62	0.60	0.96	0.38
$\delta^2_A/ä^2_D$		0.00	0.06	1.03	2.27	0.61	1.44
δ_D/δ_A		15.24	4.12	0.99	0.66	1.28	0.83
Predictability ratio		0.39	0.43	0.82	0.87	0.37	0.18

Table 3. Estimates of general combining ability effects of the lines (gi) and testers (gj)

	Plant height	Panicle length	Grains per panicle	Number of effective tillers per plant	1000-Seed weight	Grain yield per plant
Lines (gi)						
PRR-78	0.60	0.85**	-10.40**	2.12**	0.34*	-0.87
SKAU-K-R-3-6-4	3.71**	1.62**	0.60	5.12**	1.74**	15.47**
SKAU-K-R-3-6-7	1.04	1.62**	29.60**	-2.55**	0.24	-3.20**
SKAU-K-R-3-6-11	2.68*	-1.92**	-0.73	-1.08**	0.01	17.47**
SKAU-K-R-3-6-16	6.08**	1.12**	23.27**	0.59*	-1.03**	4.47**
SKAU-382	-4.29**	-2.45**	-34.73**	-3.51**	-0.39**	-14.53**
SKAU-389	-8.96**	-2.45**	-13.73**	-5.35**	-0.63**	-18.20**
China-988	-8.62**	-2.08**	-31.73**	-2.08**	-1.46**	-8.87**
Pusa Sugandh-3	0.55	1.02**	-13.52**	1.84**	0.44*	-1.08**
Pusa Sugandh-5	3.41*	1.94**	0.78	4.45**	1.71**	17.71**
Jhelum	4.09**	0.83**	12.05**	1.26**	0.52**	-4.43**
Mushk Budji	3.05*	2.51**	37.18**	4.60**	0.91**	15.18**
Shalimar Rice-1	0.96	1.46**	38.48**	-2.22**	0.20	-3.04**
Testers (gj)						
SKAU-7A	-3.09**	-0.51**	-0.93	1.04**	-0.71**	0.66
SKAU-11A	2.57**	0.56**	1.93**	-0.32**	0.30**	1.81**
IR68888A	0.33	-0.07	-0.99	-0.62**	0.35**	-2.29**

Table 4. Estimates of specific combining ability effects (Sij) of the crosses generated between lines and testers

Lines / Testers	Plant height			Panicle length			Grains per panicle		
	SKAU-7A	SKAU-11A	IR68888A	SKAU-7A	SKAU-11A	IR68888A	SKAU-7A	SKAU-11A	IR68888A
PRR-78	-0.2	-1.1	0.7	0.14	0.42	-0.06	-3.73	-3.97	-13.73**
SKAU-K-R-3-6-4	0.7	0.8	2.7	0.22	0.34	0.40	0.11	-0.24	0.27
SKAU-K-R-3-6-7	0.7	-1.3	3.71*	0.26	0.38	0.32	2.88	2.27	2.97
SKAU-K-R-3-6-11	1.6	-1.8	-0.3	-0.60	-0.22	-0.34	3.27	-2.73	-6.33*
SKAU-K-R-3-6-16	1.3	3.8	4.21*	0.14	-0.02	0.54*	2.63	2.97	6.25*
SKAU-382	0.3	-0.3	0.7	-0.66*	-0.40	-0.42	3.73	2.14	1.24
SKAU-389	-1.0	-0.6	-3.3*	-0.66*	-0.40	-0.42	-1.97	-1.77	-1.25
China-988	-2.1	-0.3	-1.3	-0.64*	-0.38	-0.24	-3.51	-2.65	-3.85
Pusa Sugandh-3	-0.2	-1.1	0.7	0.24	0.35	0.29	2.59	2.04	2.67
Pusa Sugandh-5	0.7	0.7	2.6	-0.54*	-0.20	-0.30	2.94	-2.46	-5.70
Jhelum	0.9	1.0	1.6	0.13	-0.02	0.49	2.37	2.67	5.63
Mushk Budji	2.6	2.4	0.7	-0.63*	-0.36*	-0.38	6.21*	5.12*	3.87
Shalimar Rice-1	0.7	-1.2	4.15*	-0.59*	-0.30	-0.38	-1.77	-1.59	-1.13
PRR-78	5.5**	6.12**	4.89**	-0.47*	1.24**	0.20	5.13**	7.54**	-7.87**
SKAU-K-R-3-6-4	8.12**	9.12**	4.12**	1.62**	1.24**	2.10**	12.13**	19.13**	15.13**
SKAU-K-R-3-6-7	6.12**	6.55**	2.12**	-0.28	0.67**	0.29	9.86**	9.13**	5.21**
SKAU-K-R-3-6-11	9.23**	8.52**	-0.88	-4.27**	2.00**	2.29**	21.13**	16.13**	15.13**
SKAU-K-R-3-6-16	8.23**	5.56**	-1.88	-2.56**	-0.47*	0.10	14.23**	10.13**	16.20**
SKAU-382	-3.58	-5.48	-1.48	-0.18	-0.66**	-0.28	13.86**	17.41**	8.00**
SKAU-389	-6.78	-5.58	-3.68	-0.85**	-0.56**	-0.37*	20.86**	16.32**	12.48**
China-988	0.62**	-4.48	-2.38	-0.94**	-1.99**	-1.23**	-3.87	2.10	-12.87**
Pusa Sugandh-3	1.52**	-3.88	-0.88	-0.42*	1.12**	0.18	10.92**	17.22**	13.62**
Pusa Sugandh-5	-1.23	2.56**	0.75	1.46**	1.12**	1.89**	4.62**	6.79**	7.25**
Jhelum	8.92**	6.28**	4.22**	0.78**	0.60**	0.09	8.87**	8.22**	4.69**
Mushk Budji	3.87**	3.63**	1.34*	-0.42*	1.12**	0.86**	-0.78	-8.88**	-5.28**
Shalimar Rice-1	7.56**	8.33**	3.08**	-0.25	0.60**	0.26	14.52**	19.02**	3.72

found as partial restorers. The fertility behaviour was verified by analysis with Rf-4 linked marker RM6100 and the marker profile corresponded to the phenotype for almost all the lines tested (Fig.3). Fertility restoration responds to selection since few effective genes like *Rf-3* and *Rf-4* when combined may provide effective restoration of hybrids.

The analysis of variance revealed highly significant differences among the lines, testers and lines x testers for all the characters which underlined the practicability of perusing combining ability studies (Table 2). Significant Line x tester mean squares (LxT) suggested presence of high specific combining ability (*sca*) among crosses. The mean squares due to line versus testers which accounted single degree of freedom, suggested appropriate choice of testers for most of the traits. The predictability ratio (Baker, 1978) expressed as: $2\ \delta^2gca/2\ \delta^2gca + \delta^2sca$ was greater than 0.5 for the traits GP and PL that indicated presence of high additive genetic variance. This was supported by dominance ratio (δ_D/δ_A) as well, which approximated near zero. Dominance ratio >1 along with predictability ratio <0.5 suggested over-dominance with respect to the traits NT and PH. Similar ratios were previously reported by Awan *et al.* (1986) and Pradhan *et al.* (2006) indicating the preponderance of non-additive gene action underlying the yield and contributing traits and hence, the scope for exploitation of heterosis.

Table 5. The best heterotic combinations showing hybrid advantage for yield and contributing traits (% over standard check, *Jehlum*)

Cross Combination	Plant height	Panicle length	Grains per panicle	Number of effective tillers per plant	Grain yield per plant
SKAU-7A x SKAU-K-R-3-6-4	-5.83	5.52*	8.11**	10.72**	11.22**
SKAU-7A x SKAU-K-R-3-6-7	3.07	9.51**	7.05**	16.20**	14.63**
SKAU-7A x SKAU-K-R-3-6-11	3.89	11.75**	16.66**	20.51**	27.69**
SKAU-7A x SKAU-K-R-3-6-16	-1.02	18.36**	12.54**	16.75**	20.56**
SKAU-11A x SKAU-K-R-3-6-11	2.50	13.08**	14.62**	12.41**	24.95**
SKAU-11A x SKAU-K-R-3-6-16	2.12	20.69**	16.88**	15.11**	26.14**
SKAU-7A x Pusa Sugandh-3	-3.33	19.14**	5.78*	8.05**	20.55**

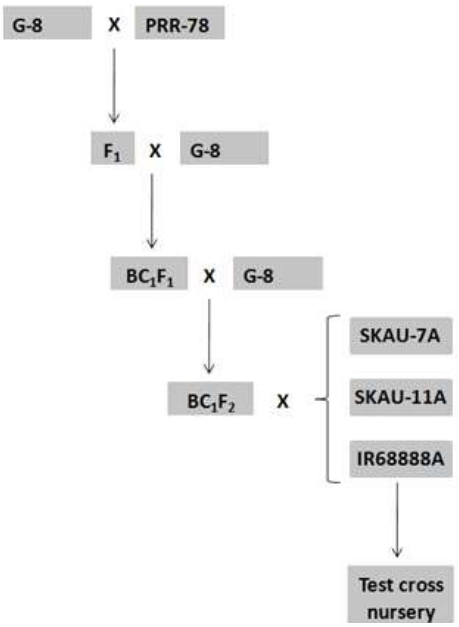
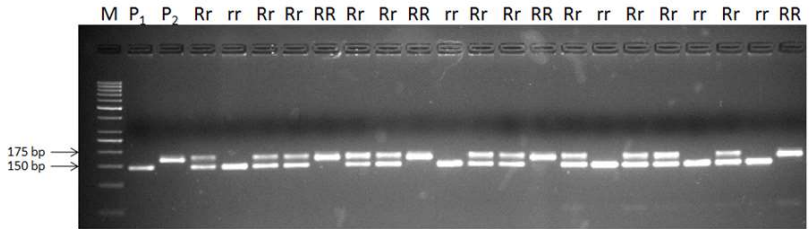


Fig. 1. Workflow of marker assisted backcross breeding for transfer of gene *Rf-4* in local cold tolerant temperate rice

The general combining ability (gca) effects (g_i) of the lines were highly significant and positive for SKAU-K-R-3-6-4, SKAU-K-R-3-6-7, SKAU-K-R-3-6-11 and SKAU-K-R-3-6-16, SKAU-382, SKAU-389, Jhelum, PRR-78 with respect to traits NT, PL and GY (Table 3). The traits showing high gca effects should be improved through pedigree breeding as suggested by Hasib *et al.* (2002). The 35 out of 39 hybrids showed significant sca effects for GY (Table 4). The genotypes PRR-78, SKAU-K-R-3-6-4, SKAU-K-R-3-6-7, SKAU-K-R-3-6-11 and SKAU-K-R-3-6-16, Jhelum, Mushk Budji and Shalimar Rice-1 had high sca effects for NT which defined its association with GY. These results revealed greater likelihood of exploiting heterosis and non-additive gene action with cross combinations involving SKAU-K-R-3-6-4, SKAU-K-R-3-6-7, SKAU-K-R-3-6-11 and SKAU-K-R-3-6-16, SKAU-382, SKAU-389 against SKAU-7A and SKAU-11A.

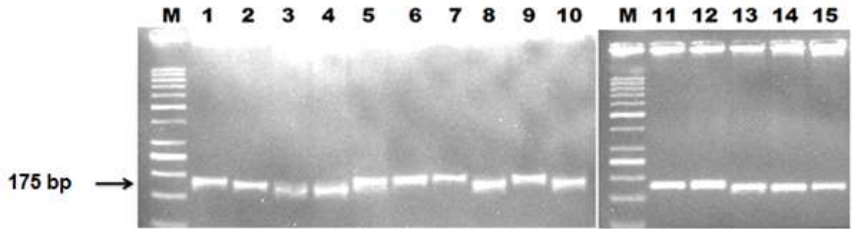
All the crosses involving SKAU-11A with

the exception of PRR-78 and China-988 showing high sca effects were in the category of high × high general combiner cross combinations. This is attributable to additive and/or additive × additive type of gene effects, which are fixable in nature (Singh *et al*., 1971). Therefore, there is high probability of obtaining good transgressive segregants in the progeny of these crosses for improvement of this trait. The lines except Mushk budji and China-988 showed high sca against SKAU-7A and belonged to high x poor combiners. These are expected to produce good segregants only if the additive genetic effects are present in the good general combiners and complementary epistatic effects in the poor combiners and they act in the same direction to maximize desirable plant attributes (Singh and Chaudhary, 1995). The non-significant sca effects as regards GY of China 988 x SKAU-11A belong to high x high combiners and as per Devraj and Nadarajan (1996) such relations depict the possibility of desirable recombinants in advance generation of inbreeding. The crosses



Lane 1: 50 bp DNA size standard (Fermentas, Lithuania, USA). Lane 2 (P_1): G-8 (Recipient line); Lane 3 (P_2): PRR-78 (Donor); Lane 4 to 23: BC_1F_2 plants homozygous dominant (RR), homozygous recessive (rr) and heterozygous (Rr) for RM6100 linked to gene *Rf-4* (1.2 cM, Singh et al, 2012)

Fig. 2. Foreground selection of BC_1F_2 population derived from G-8 x PRR-78



Lane M: 50 bp DNA size standard (Fermentas, Lithuania, USA). Lane 1-15: 1: PRR-78; 2: SKAU-K-R-3-6-4; 3: SKAU-7A; 4: SKAU-11A; 5: SKAU-K-R-3-6-7; 6: SKAU-K-R-3-6-11; 7: SKAU-K-R-3-6-16; 8: SKAU-382; 9: SKAU-389; 10: SKAU-988; 11: Pusa Sugandh-3; 12: Pusa Sugandh-5; 13: Jhelum; 14: Mushk Budji; 15: Shalimar Rice-1

Fig. 3. Amplification profile of backcross derived restorer lines along with *Rf-4* donor, CMS and potential maintainer lines

SKAU-7A x PRR-78 had high sca for GY were grouped into low x low general combiners which produced high sca effects. This is believed to be due to epistatic gene action. Similar results have been documented by Shrivastava and Shashu (1983).

Cross combinations SKAU-7A x SKAU-K-R-3-6-4, SKAU-7A x SKAU-K-R-3-6-7, SKAU-7A x SKAU-K-R-3-6-11, SKAU-7A x SKAU-K-R-3-6-16, SKAU-11A x SKAU-K-R-3-6-11, SKAU-11A x K-R-4, SKAU-7A x Pusa Sugandh-3 exhibited high degree heterosis for GY, NT, PL and GP (Table 5.). The yield heterosis ranged from 11.22 % to 26.14% for the combinations SKAU-7A x SKAU-K-R-3-6-4 and SKAU-11A x SKAU-K-R-3-6-16, respectively. Virmani *et al.* (1982) reported positive heterosis for number of GP. Positive heterosis for NT has been reported by Neelam *et al*. (2009).

CONCLUSION

With the help of marker assisted backcross breeding approach it was comparably more precise and easy to transfer restorer gene in temperate background. The restorers could not be identified in our germplasm set earlier, therefore, the derived lines SKAU-K-R-3-6-11 and SKAU-K-R-3-6-16 could be employed as effective R-lines

against temperate CMS line SKAU-7A and SKAU-11A with and average grain yield advantage of 20% or more.

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