



ORIGINAL ARTICLE

Molecular Profiling of *Rf3* and *Rf4* Genes, Fertility Restoration, and Combining Ability for Yield Traits of Climate Resilient Advanced Breeding Lines of Rice (*Oryza Sativa* L.)

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ABSTRACT

Hybrid rice technology is very useful to increase rice production to feed the growing population of the world. Identification of hybrid rice parental lines with high general combining ability (GCA) is crucial for developing specific hybrids with more fertility restoration ability and higher yield. We screened a total of 97 genotypes, which include stress-tolerant (abiotic/biotic) advanced breeding lines, a few popular released rice varieties, known restorer KMR3 (presence of *Rf3* and *Rf4* genes), and known maintainer IR58025B (absence of *Rf3* and *Rf4* genes), using the reported functional markers RMS-SF21-5 and RMS-PPR9-1 to *Rf3* and *Rf4*, respectively. Among these genotypes, 21 (21.65%) carried *Rf3Rf3/Rf4Rf4*, 38 (39.18%) carried *rf3rf3/Rf4Rf4*, 13 (13.40%) carried *Rf3Rf3/rf4rf4*, and the remaining 25 (25.77%) carried *rf3rf3/rf4rf4* allelic combinations. The identified 21 lines possessing *Rf3Rf3/Rf4Rf4*, 25 lines carried *rf3rf3/rf4rf4*, six lines carried *Rf3Rf3/rf4rf4*, and four lines carried *Rf4Rf4/rf3rf3* gene combinations were crossed with two CMS lines IR 58025A and APMS 6A to estimate high fertility and maintainer ability. All the 112 F₁s were evaluated for spikelet fertility. *Rf3Rf3/rf4rf4* genotypes mostly behaved as partial restorers. In contrast, *rf3rf3/Rf4Rf4* genotypes were partial or effective restorers. However, *Rf3Rf3/Rf4Rf4* genotypes showed better fertility restoration than the genotypes containing *Rf3Rf3/rf4rf4* or *rf3rf3/Rf4Rf4* individually, whereas *rf3rf3/rf4rf4* genotypes were found to be partial maintainers. Eleven restorer lines were found to have good GCA, and crossing these lines with CMS lines showed significant positive heterosis over mid parent and better parent for grain yield. Twelve crosses/hybrids were also observed that showed significant positive standard heterosis over KRH2 for grain yield. The identified stress-tolerant parental lines with high GCA and fertility restoration alleles will be used for the development of abiotic (submergence and/or drought) stress-tolerant high-yielding hybrids.

1 | INTRODUCTION

Paddy grain is a source of food for more than half of the world population (7.8 billion people) (Worldmeters 2020; UN 2020). To feed the fast-growing population, India needs to produce ~125 million tons of rice by 2030 (Pranathi et al. 2016). The

worldwide environmental change and growing population further lead to drought stress in different parts of the world, especially Asia (Khush 2005). The development of stress-tolerant (abiotic/biotic) high-yielding inherited rice varieties, hybrid rice parental lines, and hybrid rice varieties remains the key priority for almost all the rice-growing countries.

Among the abiotic stresses, drought and submergence are important stresses that reduce rice yield in rainfed environments of India (Muthuvijayaragavan and Murugan 2019; Sonaji and Lal 2015). The main aim of many breeding programmes is resource constraint, low productivity and yield stagnation, enhancing productivity in rice. Hybrid rice technology has been found to be one of the possible and practical options to increase rice yield (Yuan and Peng 2005). Hybrid rice is reported to show a yield advantage of 15–20% over the best commercial inbred rice varieties. The exploitation of hybrid vigour is one of the sustainable, proven, and promising technologies for increasing productivity (Virmani 1996; Virmani and Shinjo 1988; Virmani et al. 2003). In China, hybrid rice varieties have shown a 20–30% yield advantage over conventionally inbred modern rice varieties (Lin and Yuan 1980; Ma and Yuan 2002). At present, hybrid rice is grown on ~3.5 million hectares in India (ICAR-IIRR annual progress report 2020). Two decades of continuous hard work have seen the commercial introduction of 124 hybrids across India (Directorate of rice development, Patna 2022).

Hybrid rice released in India is mainly based on the wild abortive (WA) cytoplasmic male sterility (CMS), because of its stability, easy seed production and availability of a wide range of restorer lines (R lines). In the seed production system, the CMS line is maintained by A × B (A is CMS line, B is maintainer line) cross in the field through natural outcrossing and hybrid seed is produced by A × R (R is the restorer line) hybridization (Virmani et al. 2003). Fertility restoration of the WA system is governed by fertility restoring (*Rf*) genes, which are naturally existing in the rice gene pool. As many as 17 *Rf* genes are reported in rice compatible for various CMS systems, and all except *rf17* are dominant genes. Two genes, *Rf3* mapped on the short arm of chromosome 1 (Zhang et al. 1997) and *Rf4* located on the long arm chromosome 10 (Yao et al. 1997) are known as the most potential fertility restoration genes for the WA system (Alavi et al. 2009; Balaji et al. 2012). Conventional test cross methods with selected WA-CMS lines and evaluating the F₁ progenies for spikelet fertility have been largely used by plant breeders for identification of restorer and maintainer lines from rice genotypes. This method is tedious, time-consuming, costly and has led to identification of a limited number of maintainer and restorer lines using a large number of rice genotypes. Genotype with progenies showing > 70% and 0% spikelet fertility is considered as restorers and maintainer respectively (Govinda Raj and Virmani 1988). The use of *Rf* genes linked molecular markers to identify potential restorers could therefore increase the efficiency of selection of traits, save time and reduce the phenotypic complications of a trait. The functional marker RMS-SF21–5 and RMS-PPR9-1 were reported to be linked with *Rf3* and *Rf4* genes (Pranathi et al. 2016). The efficiency of functional marker RMS-SF21–5 and RMS-PPR9-1 was reported at 85–92% with different rice genotypes (Revathi et al. 2013). Tightly linked molecular markers with different restorer genes were used for screening of drought tolerant advanced breeding lines of rice (Singh et al. 2022).

Line x tester analysis provides a systematic method for the selection of suitable parents and crosses for hybrid development. The proper screening and selection of suitable germplasm lead

to the exploitation of heterosis for obtaining higher grain yield (Hossain et al. 2009). Combining ability (CA) analysis is another powerful approach accessible for the assessment of the combining ability effects that help in selecting the desired parents and crosses for the exploitation of heterosis (Sarker et al. 2002; Muhammad et al. 2007; Dalvi and Patel 2009).

The present study is undertaken with the objectives to identify potential maintainers, restorers of the WA system through molecular profiling of abiotic stress tolerant genotypes for presence/absence of *Rf3* and *Rf4* genes, and validation of maintainer and restorer ability of lines identified with presence of *Rf3* and *Rf4* genes through test crossing with WA based CMS lines IR 58025A and APMS 6A.

2 | Materials and Methods

2.1 | Genetic Materials

The material for the present study included two wild abortive cytoplasmic male sterile (CMS) lines, IR58025A and APMS6A, and 97 genotypes consisting of abiotic stress tolerant (submergence and or drought stress tolerance), released inbred rice varieties with known maintainer IR58025B and known restorer KMR3 (Table S1).

2.2 | Molecular Screening for Fertility Restoration Genes, *Rf3* and *Rf4*

The molecular profiling of *Rf3* and *Rf4* genes was conducted during *Wet season* 2016. The young and healthy leaf samples collected from 97 genotypes were used to isolate total genomic DNA using IRRI protocol (TPS buffer) and were finally diluted in 100 μL TE buffer. The DNA quantification was done on 0.8% agarose gel electrophoresis. The functional marker RMS-SF21-5 and RMS-PPR9-1 for *Rf3* and *Rf4* genes (Pranathi et al. 2016), respectively, were used for genotyping (Table S2).

polymerase chain reaction (PCR) was carried out using 15–20 ng of template DNA, 0.05 mM of dNTPs (Eppendorf, United States), 5 pM of each forward and reverse primer, 0.5 units of Taq DNA polymerase (Bangalore Genei, India) and 1X PCR reaction buffer (Bangalore Genei, India) in a total volume of 10 μL. PCR was carried out using a Thermal Cycler (Eppendorf AG, Hamburg, Germany) with initial denaturation at 94 °C for 5 min followed by 35 cycles of PCR amplification under the following parameters: 40 s at 94 °C, 45 s at 55 °C and 45 s at 72 °C, followed by a final extension at 72 °C for 10 min. PCR amplified products along with a 100-bp DNA ladder (BR Biochem Life Sciences, New Delhi, India) were resolved on a 4% agarose gel in 1× TAE buffer at 110 V. Following staining with ethidium bromide and tracked using 6× loading dye, the gels were visualised under UV light in a gel documentation system.

Data were scored based on amplicon size in restorer, KMR3 (*Rf3Rf3Rf4Rf4*; positive control), and maintainer, IR 58025B (*rf3rf3rf4rf4*; negative control) lines. The genotypes were classified as *Rf3Rf3Rf4Rf4* (*Rf3* and *Rf4* genes), *Rf3Rf3rf4rf4* (*Rf3* gene), *rf3rf3Rf4Rf4* (*Rf4* gene) and *rf3rf3rf4rf4* (non-restorer).

2.3 | Testcross With CMS Lines

All genotypes possessing allelic combinations (*rf3rf3rf4rf4*) and (*Rf3Rf3Rf4Rf4*) were used in the test cross study, but only representative genotypes possessing (*Rf3Rf3rf4rf4*) and (*rf3rf3Rf4Rf4*) were used for the test cross study. Two CMS lines, IR 58025A and APMS 6A, were used for the generation of F₁s during the *Wet season 2016*, and all the resulted 112 F₁s were grown again for verification of the male fertility status to identify restorer and maintainer lines during the dry season 2016–2017 at the International Rice Research Institute-South Asia Hub (IRRI-SAH), Patancheru (78°16' longitudes, 17° 32' latitudes and 540m above sea level). Twenty-one-day-old seedlings were transplanted, and recommended standard agronomic practices were followed for rice. For each F₁s, the average numbers of filled grains taking three panicles per plant were recorded on randomly chosen five plants. The unfilled spikelets were considered sterile, and spikelets filled with grain were considered fertile. Spikelet fertility was calculated in percentage using the following formula:

$$\text{Spikelet fertility (SF) \%} = \frac{\text{Number of filled spikelets in the panicle}}{\text{Total number of spikelets in the panicle}} \times 100$$

Based on spikelet fertility (%), the genotypes used in test crossing were classified as effective restorers (>70% spikelet fertility), partial restorers (30 to <70% spikelet fertility), partial maintainers (1 to <30% spikelet fertility) and effective maintainers (0% spikelet fertility) (Govinda Raj and Virmani 1988).

2.4 | Agronomic Performance and Combining Ability Study

The identified restorer line along with MTU1010 and IR64 was grown under irrigated control following a randomised complete block design (RCBD) with two replications for agronomic performance. The experimental materials used for the present study consisted of F₁ hybrids of 44 crosses developed by crossing 22 lines of rice (Table S3). During the *Dry season 2017*, total of 44 F₁'s (hybrids) were generated using two testers and 22 lines, and their evaluation along with parental lines (22 identified restorer and 2 maintainer lines) was done in RBD with two replications in *Sali* season 2017 at International Rice Research Institute-South Asia Hub (IRRI-SAH), Hyderabad, India. In each replication, entries (F₁'s and its parents) were grown in a single row of 3m length with a spacing of 20 cm × 15 cm transplanted as a single seedling/hill. The data was recorded on five random competitive plants in each replication for plant height, number of productive tillers, panicle length, and percentage spikelet fertility studied, except days to 50% flowering and grain yield which were recorded on a plot basis. The details of fertiliser application, weed management, harvesting, drying, and yield calculation at 14% grain moisture content are presented below:

2.4.1 | Fertiliser Application

For transplanted rice, fertiliser recommendation per hectare is 120-60-40-5: N-P-K-Zn. The base nitrogen dose is split into three

equal applications—about 1/3rd as basal, 1/3rd at tillering and 1/3rd at panicle initiation. The potash dose is split into 2 equal applications—about 1/2 as basal and 1/2 at panicle initiation. The application of 5 kg Zn or 20 kg ZnSO₄ per hectare is recommended in *Dry* season to *Dry* season/alternate season.

2.4.2 | Weed Management

For weed management, ploughing and harrowing in fallow were undertaken at least 10–14 days apart or after rain. Good land levelling was maintained to reduce weed growth because most weeds have trouble germinating under water. Quality seed was used, which was free of weed seeds. Permanent water was applied early so that weeds could not germinate under water. Pre-emergence (PE) herbicide pretilachlor 50% EC (1500 mL/ha) was sprinkled by splash method in 3–5 cm standing water in the field, preferably within 2–3 days of transplanting to control narrow leaf and some broad leaf herbicides. Manual weeding

was done before fertiliser application. The first manual weeding was done 25–30 days after transplanting. If required, the second manual weeding was done 45–50 days after transplanting to further reduce weed intensity in the experimental field.

2.4.3 | Harvesting

The harvesting was done at 20–25% grain moisture level or when 80–85% of the grains are straw-coloured, and the grains in the lower part of the panicle are in the hard dough stage. This condition was observed about 30 days after flowering.

2.4.4 | Drying

Drying is the process of reducing grain moisture content. It is the most critical operation after harvesting, and delays in drying or incomplete drying will reduce grain/seed quality (quality loss) and quantity (physical loss). Drying was done under sunlight. Sun drying is a process wherein grains are spread under the sun, on mats and pavements. The grain was dried within 24 h of harvesting. During sun drying, the thickness of the grain layer was kept at 3–5 cm. On hot days, when temperatures go above 42 °C, the grain was covered during midday to prevent overheating, and it was also covered immediately if it started raining.

2.4.5 | Yield Calculation at 14% Moisture Content

Moisture content is usually referred to the wet basis, meaning the total weight of the grain including the water. In research, moisture content referred to the dry matter of the grain. The following formula was used for conversion of moisture content at 14%:

$$Mf = Mi \times \frac{100 - MCi}{100 - MCF}$$

TABLE 1 | Molecular profiling of Rf3 and Rf4 genes and study of fertility restoration and maintainer ability of climate resilient advanced breeding lines along with known checks.

S. no.	Genotype	Molecular based restorer gene	% SF ± SE with IR 58025A	% SF ± SE with APMS 6A	Phenotype based restorer or maintainer
1	IR 58025B	<i>rf3rf3rf4rf4</i>	0 ± 0.00	0 ± 0.00	M
2	IR 68897B	<i>rf3rf3rf4rf4</i>	0 ± 0.00	0 ± 0.00	M
3	IR 99784-11-2-1-1	<i>rf3rf3rf4rf4</i>	4 ± 0.15	0.98 ± 0.10	PM
4	IR 71604-4-1-4-4-4-2-2-2R	<i>Rf4Rf4</i>	71 ± 1.20	71 ± 1.24	R
5	IR 60819-34-2R	<i>Rf4Rf4</i>	74 ± 1.40	74 ± 1.38	R
6	KMR 3R	<i>Rf3Rf3</i> and <i>Rf4Rf4</i>	86 ± 1.48	86 ± 1.58	R
7	RPHR 1005	<i>Rf3Rf3</i> and <i>Rf4Rf4</i>	79 ± 0.36	79 ± 0.40	R
8	IR 99784-11-8-1-2	<i>rf3rf3rf4rf4</i>	12 ± 1.26	12 ± 1.30	PM
9	IR04N106	<i>Rf4Rf4</i>	73 ± 2.24	71 ± 2.26	R
10	RPHR-GPA-175	<i>Rf3Rf3</i> and <i>Rf4Rf4</i>	84 ± 2.20	80 ± 2.22	R
11	RPHR-GPA-200	<i>Rf3Rf3</i>	47 ± 3.15	44 ± 3.20	PR
12	RPHR-GPA-202	<i>Rf3Rf3</i>	54 ± 1.75	53 ± 1.70	PR
13	RPHR-GPA-208	<i>Rf3Rf3</i>	61 ± 1.35	63 ± 1.37	PR
14	RPHR-GPA-209	<i>Rf3Rf3</i>	67 ± 1.35	61 ± 1.39	PR
15	IR 87707-445-B-B	<i>Rf3Rf3</i> and <i>Rf4Rf4</i>	85 ± 1.61	73 ± 1.65	R
16	IR 83876-B	<i>rf3rf3rf4rf4</i>	13 ± 0.51	21 ± 0.41	PM
17	IR 93376-B-B-130	<i>Rf3Rf3</i> and <i>Rf4Rf4</i>	76 ± 1.35	79 ± 1.38	R
18	CR Dhan 203	<i>rf3rf3rf4rf4</i>	9 ± 1.45	7 ± 1.35	PM
19	IR 83927-B-B-279	<i>rf3rf3rf4rf4</i>	13 ± 2.35	15 ± 2.30	PM
20	IR 74371-70-1-1	<i>rf3rf3rf4rf4</i>	12 ± 0.82	2412 ± 0.78	PM
21	IR 55423-01	<i>rf3rf3rf4rf4</i>	10 ± 1.10	13 ± 1.15	PM
22	Swarna Shreya	<i>rf3rf3rf4rf4</i>	17 ± 2.39	15 ± 2.41	PM
23	DRR Dhan 46	<i>rf3rf3rf4rf4</i>	16 ± 2.00	18 ± 2.15	PM
24	RCPR-A-5-1	<i>rf3rf3rf4rf4</i>	12 ± 2.70	17 ± 2.67	PM
25	IR 88964-11-2-2-4	<i>Rf3Rf3</i> and <i>Rf4Rf4</i>	82 ± 1.32	80 ± 1.30	R
26	IR 87759-5-2-1-3-1	<i>rf3rf3rf4rf4</i>	10 ± 1.16	14 ± 1.10	PM
27	IR 92521-24-5-1-3	<i>rf3rf3rf4rf4</i>	12 ± 1.86	14 ± 1.85	PM
28	IR 87759-5-2-1-3-2	<i>Rf3Rf3</i> and <i>Rf4Rf4</i>	77 ± 2.46	82 ± 2.40	R
29	IR 87761-39-2-3-2	<i>rf3rf3rf4rf4</i>	24 ± 3.26	16 ± 3.30	PM
30	IR 96322-34-202-B-2-1-2	<i>Rf4Rf4</i>	76 ± 3.41	76 ± 3.35	R
31	IR 96322-34-223-B-1-1-1	<i>Rf3Rf3</i> and <i>Rf4Rf4</i>	73 ± 1.46	73 ± 1.50	R
32	IR 102774-31-21-2-4-7	<i>Rf3Rf3</i> and <i>Rf4Rf4</i>	70 ± 1.36	72 ± 1.30	R
33	TDK 1	<i>Rf3Rf3</i> and <i>Rf4Rf4</i>	70 ± 2.36	72 ± 2.42	R
34	IR 99734:1-33-69-1-12-LSM-1	<i>Rf3Rf3</i> and <i>Rf4Rf4</i>	7370 ± 1.46	70 ± 1.50	R
35	IR 99734:1-33-69-1-9-LSM-1	<i>Rf3Rf3</i> and <i>Rf4Rf4</i>	70 ± 0.88	70 ± 0.78	R
36	IR 99734:1-33-69-1-7	<i>Rf3Rf3</i> and <i>Rf4Rf4</i>	72 ± 1.40	72 ± 1.48	R
37	BPT 5204	<i>rf3rf3rf4rf4</i>	10 ± 1.26	15 ± 1.22	PM
38	IR 99734:1-33-69-1-39-6	<i>Rf3Rf3</i> and <i>Rf4Rf4</i>	78 ± 2.36	72 ± 2.38	R

(Continues)

TABLE 1 | (Continued)

S. no.	Genotype	Molecular based restorer gene	% SF ± SE with IR 58025A	% SF ± SE with APMS 6A	Phenotype based restorer or maintainer
39	IR 99734:1-33-69-1-12-4	<i>Rf3Rf3</i> and <i>Rf4Rf4</i>	72 ± 1.34	72 ± 1.36	R
40	IR 99734:1-33-69-1-12-9	<i>Rf3Rf3</i> and <i>Rf4Rf4</i>	74 ± 2.99	73 ± 2.75	R
41	IR 102796-14-77-2-1-2	<i>Rf3Rf3</i> and <i>Rf4Rf4</i>	78 ± 2.99	72 ± 2.79	R
42	IR 102783:2-70-91-2-1-2	<i>Rf3Rf3</i> and <i>Rf4Rf4</i>	73 ± 2.96	79 ± 2.90	R
43	IR 102784:2-42-47-2-1-4	<i>Rf3Rf3</i> and <i>Rf4Rf4</i>	72 ± 1.26	75 ± 1.30	R
44	IR 106529-20-40-3-1-B	<i>Rf3Rf3</i> and <i>Rf4Rf4</i>	73 ± 1.60	75 ± 1.65	R
45	IR 106523-23-8-1-2-B	<i>rf3rf3rf4rf4</i>	14 ± 1.70	6 ± 1.60	PM
46	IR 99784-226-237-1-5-1-1	<i>rf3rf3rf4rf4</i>	2 ± 1.50	11 ± 1.40	PM
47	IR 99784-226-335-1-6	<i>Rf3Rf3</i>	7 ± 0.50	11 ± 0.70	PM
48	IR 99784-11-8-1-8	<i>rf3rf3rf4rf4</i>	5 ± 1.40	7 ± 1.60	PM
49	IR83383-B-B (RP5333-12-2-1)	<i>Rf3Rf3</i> and <i>Rf4Rf4</i>	87 ± 1.30	80 ± 1.50	R
50	IR 102604-4-B-2-1-B	<i>rf3rf3rf4rf4</i>	8 ± 0.80	6 ± 0.90	PM
51	IR 102612-31-B-1-1	<i>rf3rf3rf4rf4</i>	12 ± 3.80	7 ± 3.60	PM
52	IR 90257-B-65-B-B	<i>rf3rf3rf4rf4</i>	19 ± 4.20	17 ± 4.50	PM
53	IR 99784-188-202-1-1	<i>rf3rf3rf4rf4</i>	17 ± 2.25	27 ± 2.40	PM
54	IR 99784-255-91-1-5	<i>rf3rf3rf4rf4</i>	7 ± 3.20	7 ± 3.35	PM
55	IR 99784-255-29-1-2	<i>rf3rf3rf4rf4</i>	4 ± 1.20	1 ± 1.30	PM
56	MTU 1010	<i>Rf3Rf3</i>	56 ± 1.00	55 ± 1.30	PR

Abbreviations: %SF, percentage spikelet fertility; M, maintainer; PM, partial maintainer; PR, partial restorer; R, restorer; SE, standard error.

where M_i = initial weight in gram or kilogram; M_f = final weight in gram or kilogram; M_{Ci} = initial moisture content (%) and M_{Cf} = final moisture content (%).

2.5 | Statistical Analyses

The agronomic performance was analysed using the software CropStat v 7.2 (IRRI), PB Tools. The statistical analysis for the analysis of variance (ANOVA) as per Panse and Sukhatme 1985 and combining ability (line × tester) analysis as per Kempthorne 1957 was carried out using TNAU STAT software. The magnitude of heterosis was estimated in relation to mid parent and better parent as per the standard method.

3 | Results

3.1 | Distribution of Fertility Restorer *Rf3* and *Rf4* Genes in Rice Genotype

The alleles of the functional marker were identified based on the amplicon size present in the test lines, 'KMR 3' for the restorer allele and 'IR 58025B' for the non-restorer/maintainer allele. The functional marker RMS-SF21-5 for the *Rf3* gene was observed to be 172 bp for the fertility restoring (FR) allele and 127 bp for the non-restorer allele (NR) allele. The functional marker RMS-PPR9-1 for the *Rf4* gene was observed

to be 114 bp for the fertility restoring (FR) allele and 159 bp for the non-restorer allele (NR) allele (Figure S1). Out of the 99 genotypes, 21 (21.65%) had dominant functional alleles of both *Rf3* and *Rf4* (*Rf3Rf3Rf4Rf4*), 13 (13.40%) carried only *Rf3* dominant functional allele (*Rf3Rf3rf4rf4*), 38 (39.18%) had only *Rf4*-specific dominant functional allele (*rf3rf3Rf4Rf4*), and 25 (25.77%) had recessive alleles of both genes (*rf3rf3rf4rf4*) (Table S4). However, two of the genotypes IR 94391-131-358-19-B-1-1-1 and IR 102777-18-64-1-2-6 were either *Rf3Rf3rf4rf4* or *rf3rf3Rf4Rf4* amplified. On the basis of this screening, 21 genotypes possessing the dominant alleles of both genes and 25 genotypes possessing recessive alleles of both genes were considered as putative restorers and maintainers, respectively. The rest of the genotypes possessing either dominant or recessive alleles of the loci were considered as either putative partial restorers or maintainers. This result revealed that the dominant allele of *Rf3Rf3rf4rf4* (13.40%) in our genotype collection was relatively less common than that of the *rf3rf3Rf4Rf4* gene (39.18%).

3.2 | Fertility Restoration Ability of Genotypes With Different *Rf* Gene Combinations

The identified 21 genotypes possessing *Rf3Rf3/Rf4Rf4*, 25 genotypes possessing *rf3rf3/rf4rf4*, 6 genotypes possessing *Rf3Rf3/rf4rf4* and 4 genotypes possessing *rf3rf3/Rf4Rf4* gene combinations were crossed with two CMS lines IR 58025A and APMS6A

to estimate their fertility restoration and maintainer ability. All the genotypes possessing *Rf3Rf3/Rf4Rf4* showed > 70% spikelet fertility with both the CMS lines, namely, IR 58025A and APMS6A. The genotypes possessing *Rf3Rf3/Rf4Rf4* allele(s) showed perfect restoration with both the CMS lines and can be utilised as restorers. This result showed that the presence of dominant functional alleles of both the genes ensures higher fertility restoration. The spikelet fertility of genotypes possessing *Rf3Rf3/rf4rf4* and *rf3rf3/Rf4Rf4* genes ranged from 70.13% (IR 99734:1-33-69-1-9-LSM-1) to 87.05% (IR83383-B-B) (RP5333-12-2-1) with IR 58025A and 70.30% (IR 99734:1-33-69-1-12-LSM-1) to 81.90% (IR 87759-5-2-1-3-2) with APMS6A. Out of six genotypes possessing *Rf3Rf3/rf4rf4* alleles, five genotypes were found to be good restorers while one genotype was found to be a partial maintainer with both CMS lines (IR 58025A and APMS 6A). The range of spikelet fertility of partial restorers in the identified genotypes possessing *Rf3Rf3/rf4rf4* allele is 46.60% (RPHR-GPA-200) to 66.55% (RPHR-GPA-209) with IR 58025A and 43.57% (RPHR-GPA-200) to 60.85% (RPHR-GPA-209) with APMS 6A.

Similarly, four genotypes possessing *rf3rf3Rf4Rf4* alleles showed >70% spikelet fertility and performed as a restorer with both CMS lines (IR 58025A and APMS 6A). The range of spikelet fertility of the restorer in identified genotypes ranged from 71.00% (IR 71604-4-1-4-4-2-2-2R) to 75.58% (RPHR-GPA-209) with IR 58025A and 70.81% (IR 71604-4-1-4-4-2-2-2R) to 75.75% (IR 96322-34-202-B-2-1-2) with APMS 6A. All the genotypes possessing *rf3rf3/rf4rf4* showed <30% spikelet fertility, but none showed 0% spikelet fertility with IR 58025A and APMS 6A, except for the known maintainer IR 58025B and APMS 6B. The spikelet fertility of the genotype possessing non-restorer (*rf3* and *rf4*) genes ranged from 3.58% (IR 99784-11-2-1-1) to 23.62% (IR 87761-39-2-3-2) with IR 58025A and 0.98% (IR 99784-11-2-1-1) to 26.97% (IR 99784-188-202-1-1) with APMS 6A. It was clearly observed that the genotypes possessing *Rf3Rf3/Rf4Rf4* genes showed better fertility restoration than the genotypes containing *Rf3Rf3/rf4rf4* or *rf3rf3Rf4Rf4* individually. The genotypes possessing *rf3rf3Rf4Rf4* showed better fertility restoration than the genotypes containing *Rf3Rf3/rf4rf4*. The genotype not possessing *Rf3* and *Rf4* genes was observed as non-restorer with both the CMS lines (Table 1).

3.3 | Agronomic Performance of Identified Restorer Lines

The performance of restorer lines for yield and yield-related traits was mentioned in Table S5. The DFF of all the restorer lines is significantly higher than variety IR64 (86 days) and it ranged from 90 days (IR 102783:2-70-91-2-1-2) to known restorer KMR 3R (114 days). The PH of restorer lines was significantly lower than IR64 (104 cm) and it ranged from 71 cm (RPHR-GPA-175) to known restorer KMR 3R (95 cm). The number of productive tillers (PN) of 10 restorer lines is statistically similar to IR64. The statistically lower number of productive tillers was observed in the 12 restorer lines as compared to IR64. The variation among the genotypes for PL showed two genotypes with significantly longer panicle length, 14 genotypes statistically similar, and six genotypes significantly lower panicle length as compared to IR64 (24 cm). % Spikelet fertility (SF) ranged from 71% (RPHR 1005) to 86% (KMR 3R; IR 99734:1-33-69-1-7; IR 99734:1-33-69-1-12-9 and IR83383-B-B) (RP5333-12-2-1). The variation among the genotypes for % spikelet fertility showed 11 genotypes with similar %SF and 11 genotypes with lower %SF as compared to IR64 (86%). For grain yield (kg/ha) seven genotypes yielded higher, nine genotypes yielded similar and six genotypes yielded lower as compared to IR64 (5548 kg/ha). The seven genotypes having significantly higher grain yield showed 11% (IR 99734:1-33-69-1-39-6) to 25% (IR 87759-5-2-1-3 and IR83383-B-B) (RP5333-12-2-1) yield advantage over IR64.

3.4 | Combining Ability Study for Yield and Yield-Related Traits

The ANOVA for combining ability for yield and yield components is presented in Table 2. The significant difference in DFF, PH, PN, PL, SF, and GY was observed for crosses and lines. The significant difference in PL and %SF was observed for replication and testers, but for the rest of the traits, it is non-significant. The significant difference in PH, PN, PL, %SF and GY was observed for line × tester (L×T), but DFF is non-significant. The general combining ability (GCA) of parents (lines and testers) for yield and yield components is mentioned in Table 3. Eleven lines were identified to have good combining

TABLE 2 | Analysis of variance (ANOVA) for combining ability for yield and yield components.

Source of variation	MSS						
	DF	DFF	PH	PN	PL	SF	GY
REPLN	1	3.68ns	0.56ns	1.92ns	7.10*	92.05**	200550.01ns
CROSS	43	10.72**	172.10**	9.78**	5.63**	70.88**	4936280.40**
LINE(c)	21	19.81**	190.17**	7.99**	4.67**	98.35**	5636855.58**
TEST(c)	1	0.41ns	13.92ns	7.10ns	7.10*	28.41**	39355.92ns
LXT (c)	21	2.12ns	161.56**	11.70**	6.53**	45.43**	4468892.11**
ERROR	43	1.57	2.51	0.94	0.33	0.32	38213.36
TOTAL	87						

Abbreviations: %SF, percentage spikelet fertility; DFF, days to 50% flowering; DF, degree of freedom; GY, grain yield (kg/ha); MSS, mean sum of squares; ns, non-significant; PH, plant height (cm); PL, panicle length (cm); PN, no. of panicles.

*, **Significant difference at 5% and 1% probability level, respectively.

TABLE 3 | General combining ability (GCA) effect of parents (lines and testers) for yield and yield components.

Genotype	DFF		PH (cm)		PN		PL (cm)		% SF		GY (kg/ha)	
	Mean	GCA	Mean	GCA	Mean	GCA	Mean	GCA	Mean	GCA	Mean	GCA
KMR 3R	113.50	3.48**	95.50	15.94**	10.5	-0.1ns	25.5	1.81**	85.5	0.84**	5000	1777.03**
RPHR 1005	108.50	0.73ns	82.50	3.44**	7	0.4ns	19.5	-0.69*	71	-0.41ns	3920	-660.22**
RPHR-GPA-175	109.50	1.73**	71.50	2.69**	7	0.65ns	22.5	-0.19ns	73	0.34ns	4015.5	-709.47**
IR 87707-445-B-B	95.50	0.48ns	80.50	-1.31ns	8.5	-1.35**	22.5	-2.19**	80	3.09**	4519	-842.72**
IR 93376-B-B-130	98.50	2.48**	94.50	11.94**	8.5	-0.35ns	22.5	0.56ns	80.5	-1.91**	4799	1370.03**
IR 88964-11-2-2-4	94.50	3.48**	86.50	2.19**	8.5	0.65ns	26.5	0.56ns	80.5	-4.16**	5020.5	90.78ns
IR 87759-5-2-1-3	99.50	-0.02ns	80.50	-5.31**	8.5	0.15ns	24.5	-0.94**	82.5	-3.91**	6909.5	-2122.47**
IR 96322-34-223-B-1-1-1	109.50	2.48**	72.50	1.44ns	7.5	-3.1**	21.5	1.31**	83.5	-0.66*	5739.5	444.28**
IR 96322-34-202-B-2-1-2	110.50	1.73**	76.50	-9.56**	7.5	-1.1*	20.5	-1.44**	85	-1.66**	5220	-1421.72**
IR 102774-31-21-2-4-7	95.50	2.98**	88.50	-6.81**	6.5	2.15**	23.5	-1.44**	85.5	-7.41**	6546.5	552.28**
TDK 1	109.50	1.48*	80.50	-0.06ns	7.5	1.65**	23.5	0.56ns	81.5	7.84**	5315	750.78**
IR 99734:1-33-69-1-12-LSM-1	94.50	-2.27**	89.50	11.19**	9.5	1.15*	22.5	-0.19ns	80.5	6.59**	5755.5	1543.03**
IR 99734:1-33-69-1-9-LSM-1	95.50	-3.77**	79.50	-5.06**	8.5	-1.1*	22.5	-0.44ns	81.5	10.34**	5503	237.78*
IR 99734:1-33-69-1-7	96.50	-2.52**	91.50	-5.31**	8.5	-1.1*	22.5	-0.69*	85.5	3.59**	6207	-674.72**
IR 99734:1-33-69-1-39-6	94.50	-2.27**	85.50	-0.06ns	7.5	0.4ns	22.5	0.56ns	82.5	5.59**	6182.5	720.78**
IR 99734:1-33-69-1-12-4	91.50	-0.52ns	76.50	-5.81**	10.5	0.9ns	24.5	0.81**	84	-4.16**	6556	355.53**
IR 99734:1-33-69-1-12-9	96.50	-2.02**	89.50	5.44**	7.5	-1.1*	23.5	-0.19ns	85.5	-3.91**	6591	113.28ns
IR 102796-14-77-2-1-2	94.50	-2.02**	84.00	-10.06**	7.5	2.9**	23.5	0.31ns	80.5	-4.66**	5488.5	-1763.22**
IR 102783:2-70-91-2-1-2	89.50	-1.52*	84.50	2.69**	7.5	-1.35**	23.5	1.31**	82	-0.66*	5235.5	732.03**
IR 102784:2-42-47-2-1-4	91.50	-0.27ns	73.50	-7.56**	6.5	0.9ns	25.5	0.06ns	84.5	0.34ns	5471.5	-993.47**
IR 106529-20-40-3-1-B	90.50	-1.27*	83.50	-0.31ns	7.5	-1.85**	23.5	-1.19**	83	-9.16**	5450.5	-1620.72**
IR8383-B-B (RP5333-12-2-1)	99.50	-2.52**	91.50	0.19ns	9.5	0.65ns	24.5	1.81**	85.5	4.09**	6930.5	2121.03**
CD (0.05)	1.78		2.25		1.38		0.82		0.81		277.84	
SE (gi)		0.63		0.79		0.49		0.29		0.28		97.74
SE (gi-gi)		0.88		1.12		0.69		0.41		0.40		138.23

(Continues)

TABLE 3 | (Continued)

Genotype	DFP		PH (cm)		PN		PL (cm)		% SF		GY (kg/ha)	
	Mean	GCA	Mean	GCA	Mean	GCA	Mean	GCA	Mean	GCA	Mean	GCA
Parent lines												
Genotype	DFP		PH (cm)		PN		PL (cm)		% SF		GY (kg/ha)	
Testers	Mean	GCA	Mean	GCA	Mean	GCA	Mean	GCA	Mean	GCA	Mean	GCA
APMS 6B	96.5	-0.07ns	82	0.4ns	14.5	-0.28ns	23.5	0.28**	75	-0.57**	4800	21.15ns
IR 58025B	107.5	0.07ns	76	-0.4ns	7	0.28ns	23.5	-0.28**	80	0.57**	3531.5	-21.15ns
CD (0.05)	0.54		0.68		0.42		0.25		0.24		83.77	
SE (gi)		0.19		0.24		0.15		0.09		0.09		29.47
SE (gi-gi)		1.25		0.34		0.21		0.12		0.12		41.68

Abbreviations: %SF, percentage spikelet fertility; DFP, days to 50% flowering; GCA, general combining ability; GY (kg/ha), grain yield (kg/ha); PH (cm), plant height (cm); PL (cm), panicle length (cm); PN, no. of panicles; SE (gi), standard error for general combining ability; SE (gi-gi), standard error of difference for general combining ability.
 *, **Significant difference at 5% and 1% probability level, respectively.

ability for grain yield, eight for percentage spikelet fertility, five for panicle length and three for the number of productive tillers. KMR 3, IR 93376-B-B-130, IR 96322-34-223-B-1-1-1, IR 102774-31-21-2-4-7, TDK 1, IR 99734:1-33-69-1-12-LSM-1, IR 99734:1-33-69-1-9-LSM-1, IR 99734:1-33-69-1-39-6, IR 99734:1-33-69-1-12-4, IR 102783:2-70-91-2-1-2 and IR83383-B-B (RP5333-12-2-1) are the lines identified to possess better general combining ability (Table 4). These lines also performed better in the hybrid combination. The restorer line of all selected hybrids also showed good general combining ability for grain yield, except one restorer line, IR 88964-11-2-2-4.

The specific combining ability (SCA) effects of hybrids for yield and yield components were presented in Table S6. The best hybrid combination on the basis of grain yield (t/ha), mid parent heterosis and standard heterosis over KRH2 were presented in Table 5. The 12 hybrid/crosses that showed good specific combining ability and standard heterosis for grain yield over KRH2 are IR 58025A x IR 102774-31-21-2-4-7; IR 58025A x IR83383-B-B (RP5333-12-2-1); APMS 6A x IR 99734:1-33-69-1-12-LSM-1; APMS 6A x KMR 3R; IR 58025A x IR 93376-B-B-130; APMS 6A x IR 88964-11-2-2-4; APMS 6A x IR 99734:1-33-69-1-9-LSM-1; IR 58025A x IR 99734:1-33-69-1-39-6; IR 58025A x TDK 1, APMS 6A x IR 96322-34-223-B-1-1-1, APMS 6A x IR 102783:2-70-91-2-1-2 and IR 58025A x IR 99734:1-33-69-1-12-4.

SCA of a cross is governed by non-additive gene action. Non-additive gene action of a trait is an important factor for the selection of a hybrid (F_1) combination. Hence, a highly significant positive SCA effect is desirable for a successful hybrid-breeding program. Mid parent and better parent, heterosis of hybrids for yield and yield components are mentioned in Table S7. Significant positive heterosis was exhibited by as many as 24 crosses over mid parent and better parent for grain yield. The earlier identified 11 crosses with good specific combining ability and its parents with good general combining ability also showed significant positive heterosis over mid parent and commercial hybrid KRH2 for grain yield. Thus, these crosses have the capability for hybrid rice production.

4 | Discussion

The success of hybrid rice or hybrid rice program is mainly dependent upon identification and use of effective restorers, maintainers and its cytoplasmic male sterile line. The selection of hybrids depends on the performance of crosses as good specific combining ability and their parents as general combining ability (Pradhan et al. 2006; Saideah et al. 2010; Selvaraj et al. 2011). Hence, proper knowledge of fertility restoration genetics is a vital requirement for an effective hybrid breeding program (Shalini et al. 2015). *rf3rf3Rf4Rf4* allele is essential either alone or in combination with *Rf3Rf3rf44f4* allele for fertility restoration to achieve enhanced grain yield in WA-CMS-based hybrids (Shidenur et al. 2020). The two major fertility restoration genes, *Rf3* and *Rf4*, were reported to restore male fertility of the WA CMS system and mapped on chromosomes 1 and 10, respectively (Ahmadikhah and Karlov 2006; Alavi et al. 2009). The functional markers RMS-SF21-5 and RMS-PPR9-1 for *Rf3* and *Rf4* genes (Pranathi et al. 2016) were used in the study for the presence of fertility restoration (*Rf3Rf3rf44f4*, *rf3rf3Rf4Rf4*)

TABLE 4 | List of identified promising lines selected as hybrid restorer with mean value of percentage spikelet fertility, grain yield (t/ha) and its GCA effects.

Line	% SF	GY (kg/ha)		Trait(s)
	Mean	Mean	GCA	
KMR3	85.5	5000	1777.03**	-
IR 93376-B-B-130	80.5	4799	1370.03**	Drought tolerance
IR 96322-34-223-B-1-1-1	83.5	5739.5	444.28**	Submergence and drought tolerance
IR 102774-31-21-2-4-7	85.5	6546.5	552.28**	Submergence and drought tolerance
TDK 1	81.5	5315	750.78**	-
IR 99734:1-33-69-1-12-LSM-1	80.5	5755.5	1543.03**	Drought tolerance
IR 99734:1-33-69-1-9-LSM-1	81.5	5503	237.78*	Drought tolerance
IR 99734:1-33-69-1-39-6	82.5	6182.5	720.78**	Submergence and drought tolerance
IR 99734:1-33-69-1-12-4	84	6556	355.53**	Submergence and drought tolerance
IR 102783:2-70-91-2-1-2	82	5235.5	732.03**	Submergence and drought tolerance
IR83383-B-B (RP5333-12-2-1)	85.5	6930.5	2121.03**	—
IR 88964-11-2-2-4	80.5	5020.5	90.78ns	Drought tolerance

Abbreviations: %SF, percentage spikelet fertility; GCA, general combining ability; GY (kg/ha), grain yield (kg/ha); ns, non-significant.

*, **Significant difference at 5% and 1% probability level, respectively.

TABLE 5 | List of best hybrid combination on the basis of grain yield (t/ha), mid parent heterosis, standard heterosis and SCA effects for percentage spikelet fertility and grain yield (t/ha).

S. no.	Hybrid	% SF		GY (kg/ha)		GY	
		Mean	SCA	Mean	SCA	Mid.Het	Std.Het over KRH2
1.	IR 58025A x IR 102774-31-21-2-4-7	76	6.18**	9647	2660.15**	91.45**	27.20**
2.	IR 58025A x IR83383-B-B (RP5333-12-2-1)	88	6.18**	9576	1019.9**	83.05**	26.27**
3.	APMS 6A x IR 99734:1-33-69-1-12-LSM-1	87	4.32**	8908	888.1**	68.78**	17.46**
4.	APMS 6A x KMR 3R	76	-1.43**	8882	628.1**	81.27**	17.11**
5.	IR 58025A x IR 93376-B-B-130	73	-2.82**	8594	788.9**	106.31**	13.32**
6.	APMS 6A x IR 88964-11-2-2-4	74	1.57**	8417	1848.85**	71.41**	10.98**
7.	APMS 6A x IR 99734:1-33-69-1-9-LSM-1	88	1.07*	8417	1701.85**	63.38**	10.98**
8.	IR 58025A x IR 99734:1-33-69-1-39-6	86	3.18**	8417	1261.15**	73.29**	10.98**
9.	IR 58025A x TDK 1	88	2.43**	8282	1096.15**	87.23**	9.20**
10.	APMS 6A x IR 96322-34-223-B-1-1-1	79	3.57**	7806	884.35**	48.12**	2.93**
11.	APMS 6A x IR 102783:2-70-91-2-1-2	79	3.57**	7806	596.6**	55.56**	2.93**
12.	IR 58025A x IR 99734:1-33-69-1-12-4	71	-2.07**	7789	998.9**	54.43**	2.70**
Check	IR 58025A x KMR 3R (KRH2)	80	-0.97*	7584	-628.1**	77.78**	—

Abbreviations: %SF, percentage spikelet fertility; GY (kg/ha), grain yield (kg/ha); Mid.Het, mid-parent heterosis; SCA, specific combining ability; Std.Het, standard heterosis.

*, **Significant difference at 5% and 1% probability level, respectively.

and *Rf3Rf3Rf4Rf4*) alleles and non-restorer (*rf3rf3rf4rf4*) alleles. Out of the 99 rice genotypes, 21 (21.65%) had dominant functional alleles of both *Rf3* and *Rf4* (*Rf3Rf3Rf4Rf4*), 13 (13.40%) carried only the *Rf3* dominant functional allele (*Rf3Rf3rf4rf4*), 38 (39.18%) had only the *Rf4*-specific dominant functional allele (*rf3rf3Rf4Rf4*) and 25 (25.77%) had recessive alleles of both the genes (*rf3rf3rf4rf4*). On the basis of this screening, 21 genotypes possessing the dominant alleles of both the genes and 25 genotypes possessing recessive alleles of both the genes were considered as putative restorers and maintainers, respectively. The rest of the genotypes possessing either dominant or recessive alleles of the loci were considered as either putative partial restorers or maintainers. This result revealed that the dominant allele of *Rf3Rf3rf4rf4* (13.40%) in our genotype collection was relatively less common than that of *rf3rf3Rf4Rf4* gene (39.18%). Earlier, Pranathi et al. (2016) reported the screening of *Rf3* and *Rf4* genes on known 120 restorers and 44 non-restorers (with reported functional markers). Three hundred rice genotypes were screened for both *Rf3* and *Rf4* genes using molecular markers RM171, RM258, RM315 and RM443, finding 90 genotypes possessing *Rf3* gene, 65 genotypes possessing *Rf4* gene and 45 genotypes with both the genes (El-Namaky et al. 2016). Two hundred and forty-six aromatic rice genotypes were screened using gene-linked markers RM 3873 for *Rf3* and RM 6100 for *Rf4* and identified 183 restorers (Kushwaha 2015). Recently, high frequency of restorer genes, particularly *Rf3* in wild species such as *O. rufipogon* has been reported than in cultivated germplasm (Dash et al. 2018). This is in contrast to the reported predominance of *Rf4* allele in Indian cultivated germplasm (Katara et al. 2017), wherein 19% of the genotypes had *Rf3* and 63% of the genotypes carried *Rf4* gene.

The use of molecular markers reduces labour and resources required for identification of restorers and maintainers as compared to the conventional approaches. The success of fertility restoration of test hybrids was validated by crossing identified restorer lines with IR 58025A and APMS 6A for testing the efficiency of marker-based identification of restorers and non-restorers for the WA-CMS system in rice. Hence, marker-assisted screening can be of great advantage in the fast recovery of restorers from a large number of breeding lines even at the seedling stage, which can help in reducing the cost of screening a large number of genotypes.

The generation of a large number of test crosses manually, as done in the conventional approach, needs manpower, time and area, which is a tedious practice and finally increases the cost for identification of maintainers and potential restorers. All the genotypes possessing *Rf3* and *Rf4* genes showed > 70% spikelet fertility with IR 58025A and APMS 6A. All genotypes possessing both *Rf3* and *Rf4* genes (100%) showed good performance as restorer lines with both the CMS lines. Out of six genotypes possessing the *Rf3* gene, five were identified as a partial restorer and 1 (16.67) as a partial maintainer with both CMS lines (IR 58025A and APMS 6A). All the genotypes possessing *rf3rf3/rf4rf4* showed < 30% spikelet fertility, but none showed 0% spikelet fertility with IR 58025A and APMS 6A, except known maintainer IR 58025B and APMS 6B. All newly identified genotypes possessing *rf3rf3/rf4rf4* (92%) performed as partial maintainer lines, and 8% known maintainer performed as a perfect maintainer line with both the CMS lines. The presence of

dominant alleles of both *Rf3* and *Rf4* restorer genes was capable of greater fertility restoration than the presence of either *Rf3* and *Rf4*; however, the effectiveness of *Rf4* is relatively more than *Rf3*. Differential fertility restoration efficiency observed in F_1 s due to variable allelic combinations of *Rf3* and *Rf4* genes and also due to the nuclear and cytoplasmic genome interaction provides new leads for deeper insight into the molecular mechanism of fertility restoration (Upadhyay and Jaiswal 2012; Mallikarjuna et al. 2013; Shalini et al. 2015). Another reason for differential fertility restoration might be the presence of other fertility restorer gene(s) apart from *Rf3* and *Rf4* either individually or in combinations producing elaborate genetic interactions (Tan et al. 2008; Balaji et al. 2012).

The parents used in the study possessing abiotic (drought and/or submergence tolerance) traits can be effectively utilised in the development of hybrids possessing tolerance to abiotic stresses in general and drought and/or submergence in particular. Increase performance of hybrids under abiotic (drought and/or submergence tolerance) conditions with the good specific combining ability and its parent with good general combining ability shall reduce rice yield loss in rainfed areas where occurrence of drought and submergence is very frequent. This is the first of its kind of study on the identification of restorer lines highly tolerant to abiotic stresses. Most of the identified restorer lines possessing tolerance to drought or submergence are in dwarf background and therefore are free from undesirable linkages (Kumar et al. 2020) of reduced yield potential, tall height. Use of such restorer lines possessing good specific combining ability shall result in the development of hybrids with good agronomic traits, high yield potential, and good yield under drought. The top five newly identified hybrids showed better standard heterosis over KRH2 (Table 5) should be further evaluated for multi-location in the abiotic (drought and/or submergence tolerance) and non-stress condition along with suitable check for its wider acceptance.

Author Contributions

AK and VKS were involved in the design of the experiment and reviewed and edited the manuscript. RP conducted the experiment and wrote the manuscript. CV provided advanced breeding lines. PKJ was involved in statistical analysis. AKS was involved in manuscript improvement.

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Ethics Statement

The experiments complied with the current laws of the country in which they were performed.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section. **FIGURE S1:** The PCR products were resolved on 4% agarose gel. (A) Amplification pattern of RMS-SF21–5, the candidate gene-specific marker for *Rf3* (targeting in-del upstream of SF21). L: 100-bp ladder, lanes 1: +ve control (KMR3), 11: -ve control (IR 58025B), 2–10 and 12–19: selective rice genotypes. (B) Amplification pattern of RMS-PRR9-1, the candidate gene-specific marker for *Rf4* (targeting in-del within PPR9-782-M). L: 100-bp ladder, lanes 1: +ve control (KMR3), 11: -ve control (IR 58025B), 2–10 and 12–19: selective rice genotypes. **TABLE S1:** Details of the molecular markers used for screening of breeding lines for restorer gene(s) identification. **TABLE S2:** Rice genotypes used in the study. pbr70018-sup-0002-Tables.xlsx. **TABLE S3:** List of genotypes used in the study of general combining ability (GCA), specific combining ability (SCA), mid parent and better parent heterosis. pbr70018-sup-0002-Tables.xlsx. **TABLE S4:** Molecular profiling of fertility restoration genes in the promising lines along with known checks. pbr70018-sup-0002-Tables.xlsx. **TABLE S5:** Agronomic performance of restorer line. pbr70018-sup-0002-Tables.xlsx. **TABLE S6:** Specific combining ability (SCA) effects of hybrids for yield and yield components. pbr70018-sup-0002-Tables.xlsx. **TABLE S7:** Mid parent and better parent heterosis of hybrids for yield and yield components. pbr70018-sup-0002-Tables.xlsx.