



Genetic Evaluation of Promising Restorer Lines in Rice (*Oryza sativa* L.)

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ABSTRACT

Background: Hybrid rice reduces the yield gap and helps in realising and nutritional and food demands of the global humanity. This investigation aims to study genetic components of inheritance and identification of heterotic combination of parents for realising promising rice hybrids.

Methods: The study took place at the Regional Agricultural Research Station, Maruteru, focusing on 30 experimental hybrid varieties derived from the crossing of three CMS lines with ten distinct restorers, utilising Line×Tester method of breeding strategy during the Kharif-2022. Following this, conduct of the resulting 30 hybrids with respective parents and a hybrid Check (HRI-174), were evaluated, using a randomized block design during the *Rabi* season of 2022-23.

Result: The analysis of combining ability in CMS lines, restorers and line × tester combinations revealed the predominance of non-additive genetic effects on traits correlated with yield. A detailed examination of individual restorers' performance, heterosis and specific combining abilities identified several promising heterotic combinations for future assessment and commercial exploitation. Significantly, promising hybrid combinations with potential for future research and commercial utilization were identified as following, APMS 17A × MTU 2055, APMS 17A × RGL 5613, APMS 15A × MTU 1213, APMS 15A × RGL 5613 and APMS 15A × MTU 2055. This endeavour in identification of promising heterotic rice hybrids poised for potential commercial cultivation, ultimately contributing to the advancement of hybrid rice breeding practices.

Key words: Combining ability, Fertility restorer genes, Grain yield, Heterosis, Rice.

INTRODUCTION

Rice stands as a crucial staple cereal globally, feeding over half of the world's population (Duppala *et al.*, 2023), with a global cultivation area of 167.6 million hectares, rice yields approximately 527.6 million metric tonnes, averaging 5.2 t ha⁻¹ in production (Childs and LeBeau, 2024). In India, paddy covers 46.3 million hectares, yielding a production of 129.5 million tonnes and an average productivity of 2798 kg ha⁻¹ during 2021-22. In Andhra Pradesh, rice cultivation spans 2.6 million hectares, yielding around 13.1 million tonnes with a productivity of approximately 5130 kg ha⁻¹ (Reddy *et al.*, 2024). Rice crop production faces a significant challenge because of abiotic and biotic stress leading to substantial yield losses on a global scale each year, abiotic stresses contribute to yield reductions ranging from 30 to 60 per cent annually (Manojkumar *et al.*, 2023). Hybrid rice technology is recognized as a promising approach to significantly enhance rice grain yields by harnessing heterosis and minimizing losses through utilization of resistant parental lines against key pests and diseases. This technology has promising role for achieving significantly increased yields compared to traditional high-yielding varieties (HYVs) by leveraging the genetic principle of heterosis or hybrid vigor (Virmani, 1994). The introduction of the first rice hybrids (APHR 1 and APHR 2) in India, 1994, developed at the ARS, Maruteru andhra Pradesh, signalled a significant change in the significance and adoption of hybrid rice technology (Singh *et al.*, 2016).

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The key to successful and sustainable hybrid rice breeding lies in the identification of suitable restorers that exhibit high yield and excellent grain quality while also displaying high degree of resistance to significant pests and diseases of rice (Huang *et al.*, 2011). Combining ability studies play a critical role in selecting optimal parent lines and specific hybrid combinations. Line × Tester analysis serves as a valuable tool for evaluating general combining ability (gca) of parents and selecting crosses with high specific combining ability (sca) to maximize heterosis (Yuan and Peng, 2005). In light of these critical requirements, the present investigation was initiated to identify restorers

carrying major fertility-restoring genes and excelling in both grain yield and quality, while also exhibiting resistance to major pests and diseases.

MATERIALS AND METHODS

The study was performed at RARS, Maruteru, located at 26°38'N latitude, 81°44'E longitude and 5 meters above mean sea level. The site features a semi-humid climate with black alluvial clayey soils, falling within the Godavari zone of Andhra Pradesh. The experimental material comprised of three male sterile lines (APMS 15A, APMS 17A and APMS 18A) and ten restorer lines (RGL 5613, MTU 2716, MTU 1224, RM 409-26-1-1-1, MTU 2055, MTU 2347-158-3-1-1, RM 67-60-1-1-1, UTR 76, MTU 2846-34-1-1 and MTU 1213). During the Kharif season of 2022, a set of 30 rice hybrids were created through crosses between male sterile lines and restorers, employing a Line × Tester mating design and adhering to standard hybridization procedures. The study also examined quality traits of the CMS lines and restorers, including hulling, milling, head rice recovery, Grain L/B ratio, Alkali Spreading Value (ASV) and Gelatinization temperature, during *Kharif*, 2022 from a random grain sample and were not analysed statistically.

The restorers are evaluated for BPH, BLB and sheath blight pests and diseases during *Kharif*, 2022 and for blast during *Rabi*, 2022-23 in artificial screening nurseries of the Entomology and Plant pathology Departments of RARS, Maruteru and the scores were given as per the Standard Evaluation Scale (SES) outlined by IRRI (2013). During the *Rabi* 2022-23, an evaluation of 30 experimental rice hybrids took place, including their parent lines and the hybrid check variety, HRI-174. The experiment was performed in two replication following randomized block design at RARS, Maruteru. Notably, observations for yield, yield attributes and quality traits were specifically recorded for the maintainer (B) lines associated with the respective male sterile lines. The collected data on grain yield and yield-related traits underwent standard statistical analyses, following the procedures outlined by (Panse and Sukhatme, 1967). The observations were analysed statistically using the software Window Stat Version 8.5 and the R software.

RESULTS AND DISCUSSION

The outcome of ANOVA, heterosis, general and specific combining effects are presented and discussed here under.

Table 1: Mean performance of parents for yield and yield associated traits.

Parents	Days to 50 per cent flowering	Ear bearing tillers per plant	Panicle length (cm)	Spikelet fertility (%)	1000-grain weight (g)	Grain density	Grain yield per plant (g)
Lines							
APMS15A	99.00	7.75	25.95	82.55	17.05	13.50	26.00
APMS17A	98.50	8.60	25.30	84.65	13.00	17.10	21.70
APMS 18A	102.00	8.90	26.15	79.35	12.90	14.05	27.00
Minimum	98.50	7.75	25.30	79.35	12.90	13.50	21.70
Maximum	102.00	8.90	26.15	84.65	17.05	17.10	27.00
Mean	99.83	8.41	25.80	82.18	14.31	14.88	24.90
Restorers							
RTCNP 2	98.00	8.20	25.40	85.05	19.90	9.55	19.50
RTCNP 23	99.00	10.25	28.90	91.00	17.90	10.80	31.00
RTCNP 33	102.00	10.50	22.90	89.30	15.30	9.55	19.00
RTCNP 37	99.00	9.80	21.15	92.45	17.60	10.85	38.00
RTCNP 38	99.00	8.00	25.15	88.05	13.35	9.50	37.00
RTCNP 66	96.50	8.50	25.85	88.55	21.00	10.15	28.60
RTCNP 73	97.50	10.35	21.90	90.50	16.75	9.00	36.50
RTCNP 90	98.50	9.85	26.95	88.10	14.00	12.15	19.50
RTCNP 120	97.00	9.85	26.25	88.55	17.15	10.35	38.50
RTCNP 150	96.00	7.90	24.60	87.90	17.25	7.60	24.00
Minimum	96.00	7.90	21.15	85.05	13.35	7.60	19.00
Maximum	102.00	10.50	28.90	92.45	21.00	12.15	38.50
Mean	98.25	9.32	24.90	88.94	17.02	9.95	29.16
HRI-174 (Check)	93.50	8.25	27.45	92.05	24.80	14.45	31.50
SEd	2.45	0.39	1.17	3.42	0.75	0.43	3.79
CD (0.05)	4.95	0.78	2.37	6.90	1.51	0.86	7.64

SEd: Standard error of deviation; CD: Critical difference.

ANOVA and Mean performance of yield and quality traits

The analysis of variance (ANOVA) conducted on 44 genotypes (3 male sterile lines, 10 restorers, 30 hybrids and one check) for yield and yield-related characters were differing significantly among the treatments towards yield and related attributes, indicating sufficient variability for effective selection. The result obtained was in line with the earlier studies (Ratnam *et al.*, 2023). The results of mean performance of CMS lines and restorers towards yield and yield-related attributes is showed in the Table 1.

On examination of the results studied on quality traits of the CMS lines and restorers (Table 2) revealed, The Alkali Spreading Value (ASV), when applied to whole

kernels (milled rice), serves as a metric for assessing the gelatinization characteristics of rice. The ASV is, in essence, indirectly linked to the gelatinization temperature, providing a valuable indicator of rice's cooking and textural properties. The CMS lines, APMS 17A and the restorers, MTU 2716, MTU 2347-158-3-1-1, RM 67-60-1-1-1 and MTU 1213 had recorded low to intermediate ASV (<5) and intermediate Gelatinization temperature, desirable from the consumer view point for cooked rice, indicating their potential in the improvement of quality rice hybrids, preferred by the millers and consumers, important stakeholders of the rice crop. The 10 restorers were also phenotyped for BLB, blast, sheath blight and BPH resistance in addition to the existence of fertility restorer genes, *Rf3* and *Rf4* in additional

Table 2: Mean performance of parents for grain quality traits.

Lines/Restorers	Quality parameters						
	Hulling (%)	Milling (%)	Head rice recovery (%)	L/B Ratio	Grain Type	Glume colour	Alkali Spreading value (ASV)
CMS lines							
APMS15B	77.78	69.65	55.01	2.62	MS	SG	4.00
APMS17B	75.58	68.22	49.89	2.85	MS	SG	2.65
APMS18B	73.49	63.92	44.77	2.87	MS	SG	2.30
Restorers							
RGL 5613	71.91	62.05	51.84	3.10	LS	SG	2.50
MTU 2716	78.00	71.00	64.00	3.50	MS	SG	2.50
MTU 1224	75.46	68.98	55.75	2.87	MS	SG	2.65
RM 409-26-1-1-1	75.88	66.55	51.04	2.21	MB	SG	2.40
MTU 2055	78.23	68.15	57.63	2.15	LS	SG	2.60
MTU 2347-158-3-1-1	77.47	69.86	63.03	2.72	MB	SG	5.05
RM 67-60-1-1-1	79.33	70.65	67.00	2.61	MS	SG	7.00
UTR 76	79.11	70.25	61.85	3.00	LS	SG	2.30
MTU 2846-34-1-1	75.33	69.85	69.29	2.62	MS	SG	2.40
MTU 1213	75.22	65.13	44.77	2.68	MS	SG	4.25

MS- Medium slender, LS- Long Slender, MB- Medium Bold, SG- Straw glume, L- Low; I- Intermediate and H- High.

Table 3: Characterization of restorers for disease, pest and presence of fertility restorer and other genes.

Restorers	Disease and pests score				Fertility restorer genes		Any other genes
	Bacterial leaf blight	Leaf blast	Sheath blight	Brown Plant Hopper	Rf3	Rf4	
RGL 5613	7	3	5	9	[+]	[+]	Bph32
MTU 2716	7	5	7	7	[+]	[+]	-
MTU 1224	7	4	7	7	-	-	-
RM 409-26-1-1-1	7	5	7	9	-	-	-
MTU 2055	7	6	7	5	-	-	-
MTU 2347-158-3-1-1	7	4	7	9	-	-	-
RM 67-60-1-1-1	7	2	7	7	-	-	-
UTR 76	7	2	5	5	-	-	-
MTU 2846-34-1-1	5	2	5	7	-	-	-
MTU 1213	9	4	5	7	-	-	-

1-3 : Resistant, 4-5 : Moderately Tolerant, 6-7 : Moderately susceptible; 9-Highly susceptible

to other genes for resistance and quality, based on 1k Rica testing. The results are conferred in Table 3. Research of the results disclosed that the restorer, MTU 2055 had recorded moderate tolerance to BPH, while, MTU 1224, RM 409-26-1-1-1, MTU 2347-158-3-1-1 and RM 67-60-1-1-1 had recorded resistance to moderately resistance reaction for leaf blast, similar multiple gene effects in rice were studied (Manojkumar *et al.*, 2023), indicating the scope of these restorers in the development of resistant hybrids for the key pests and diseases of rice crop. Predominance of non-additive genetic effects, such as dominance and epistasis, significantly influence breeding decisions and guide future research, hybrids generation is opted instead of sections. These effects can enhance hybrid vigor, leading to offspring with superior traits compared to their parents, making the selection of complementary parent lines crucial for optimizing yield, resilience and quality (Sari *et al.*, 2019).

Heterosis

The aim of the heterosis study was to ascertain optimal parental combinations exhibiting significant levels of effective heterosis. The parent versus hybrid comparison revealed significant divergence for all traits studied, illustrated notable levels of heterosis. Summary results of heterosis levels over mid-parent (relative heterosis), better parent (hetero beltiosis) and the standard check, HRI 174 (standard heterosis) for grain yield and yield attributes are showed in the Table 4. In this study, five crosses exhibited strong positive heterosis of more than 30 percent than the standard check, depicting their potential for commercial use. Among these, APMS 17A × RGL 5613, APMS 15A × MTU 1213, APMS 15A × RGL 5613 and APMS 17A × MTU 2055 showed significant and positive heterosis than both mid and better parents. Only one hybrid, APMS 17A × MTU 2055, exhibited significant and positive heterosis (9.69) for grain density than the check, HRI-174. However, no crosses demonstrated significant and positive standard heterosis for spikelet fertility and 1000-grain weight, in line with the findings of (Srivastava and Jaiswal, 2016) for spikelet fertility and (Premkumar *et al.*, 2017) for 1000-grain weight. In this study, the hybrids APMS 15A × RGL 5613, APMS 15A × MTU 1213, APMS 17A × RGL 5613 and APMS 17A × MTU 2055 were recognised as favourable and high-yielding heterotic hybrids, showing notable and favorable relative heterosis, hetero beltiosis and standard heterosis exceeding 25% for both grain yield per plant and days to 50% flowering.

Combining ability

Combining ability analysis serves as a valuable tool for identifying the most promising parents capable of producing heterotic hybrids for exploitation of heterosis. In this study, an evaluation of 30 hybrids derived by crossing three lines and ten restorers in a Line × Tester fashion was conducted and the findings are discussed below.

Analysis of variance

Mean squares for grain yield per plant, ear-bearing tillers per plant, 1000-grain weight and grain density were

significantly prominent when analyzing the hybrids' variation partitioned into lines, testers and line × testers. This underscores the importance of both additive and non-additive genetic effects. Particularly, non-additive genetic action played a crucial role in influencing grain yield per plant and the majority of yield components, consistent with findings from previous studies (Shanti *et al.*, 2011; Srilakshmi *et al.*, 2019; Kumar *et al.*, 2023).

General combining ability effects

The analysis of CMS lines and restorers examined their individual performance and overall combining ability (GCA) effects on grain yield and yield traits. Restorers demonstrated noteworthy and favorable GCA effects, particularly for grain yield and days to 50 percent flowering. Notably, APMS 15A showed high combining ability with to spikelet fertility percentage and 1000-grain weight, while APMS 17A exhibited superior combining ability for grain density. In contrast, APMS 18A demonstrated superior combining ability for ear-bearing tillers per plant (Table 5), suggesting its potential utilization in hybrid breeding experiments aimed at improving these traits. RGL 5613 and MTU 2055 were observed as an effective combiner for grain yield per plant, spikelet fertility percentage and 1000-grain weight. This put forward that they are suitable for hybrid breeding projects focused on generating high-yielding hybrids with greater grain size.

Specific combining ability effects

The specific combining ability (SCA) effects of the 30 hybrids were assessed for yield and yield attributes. Several hybrids demonstrated notable and beneficial SCA effects, particularly for grain yield, grain density, spikelet fertility and ear-bearing tillers per plant. Notably, hybrids APMS 17A × MTU 2055, APMS 17A × RGL 5613, APMS 15A × MTU 1213, APMS 15A × RGL 5613 and APMS 15A × MTU 2055 demonstrated dominance by its very nature, heterosis and specific combining ability. These hybrids exhibited substantially higher grain yield per plant (>41.0 g) compared to the check, HRI-174 (31.50 g), along with high standard heterosis exceeding 30 percent and significant substantial SCA effects for grain yield per plant (Table 6). Consequently, these hybrids were recognised as promising heterotic combinations (Table 7) for further evaluation and commercial exploitation as potential early-duration hybrids with good spikelet fertility and medium slender to medium bold straw glume-coloured grains.

The commercial use of identified rice hybrids comes with various challenges and important considerations (Sankar *et al.*, 2018). High costs associated with hybrid seed production, due to the complex process of maintaining genetic purity, often make seeds expensive for farmers who must purchase new seeds each season to retain hybrid vigor (Devi *et al.*, 2018). Limitations encountered in the study of rice hybrids include environmental variability, which can affect yield and trait expression across different locations, making it difficult to

Table 4: Relative heterosis, hetero beltiosis and standard heterosis for grain yield and yield component traits.

Character	Relative heterosis			Hetero beltiosis			Standard heterosis		
	Range	No. of desirable and significant heterotic hybrids	Best hybrid combination	Range	No. of desirable and significant heterotic hybrids	Best hybrid combination	Range	No. of desirable and significant heterotic hybrids	Best hybrid combination
Grain yield Per plant	-45.74 to 118.45	7	APMS 17A × RGL 5613	-54.55 to 107.37	6	APMS 17A × RGL 5613	-44.44 to 47.62	5	APMS 17A × MTU 2055
Days to 50 flowering per cent	-9.31 to 4.06	15	APMS 18A × MTU 1224	-10.29 to 4.06	15	APMS 17A × MTU 1224	-2.67 to 9.63	-	-
Ear bearing tillers per plant	-20.83 to 52.98	15	APMS 17A × RGL 5613	-25.28 to 49.42	10	APMS 17A × RGL 5613	-19.39 to 55.76	21	APMS 17A × RGL 5613
Panicle length	-6.81 to 17.35	2	APMS 18A × MTU 2055	-11.25 to 15.11	2	APMS 18A × MTU 2055	-14.57 to 9.65	1	APMS 18A × MTU 2055
Spikelet fertility	-75.93 to 8.34	2	APMS 17A × MTU 2055	-77.47 to 7.58	-	-	-71.70 to 1.63	-	-
1000-grain weight	-13.86 to 45.73	16	APMS 17A × MTU 2055	-30.48 to 43.82	5	APMS 17A × MTU 2055	-45.77 to 22.18	-	-
Grain density (grain number. cm ⁻³)	-34.49 to 19.17	6	APMS 17A × MTU 2055	-44.74 to 3.33	-	-	-47.75 to 9.69	1	APMS 17A × MTU 2055

Table 5: Characterization of parents based on *per se* performance and *gca* effects for grain yield and yield attributes in rice.

Lines/Restorers	Days to 50 percent flowering		Ear bearing tillers per plant		Panicle length (cm)		Spikelet fertility (%)		1000-grain weight (g)		Grain density		Grain yield per plant (g)	
	<i>per se</i>	<i>gca</i>	<i>per se</i>	<i>gca</i>	<i>per se</i>	<i>gca</i>	<i>per se</i>	<i>gca</i>	<i>per se</i>	<i>gca</i>	<i>per se</i>	<i>gca</i>	<i>per se</i>	<i>gca</i>
APMS 15A	H	L	L	L	H	L	H	H	H	H	L	L	H	L
APMS 17A	H	L	H	L	L	L	H	L	L	L	H	H	L	L
APMS 18A	L	L	H	H	H	L	L	L	L	L	L	L	H	L
RGL 5613	H	L	L	H	H	L	H	H	H	H	L	L	L	H
MTU 2716	L	L	H	H	H	L	H	L	H	L	L	L	H	L
MTU 1224	L	L	H	H	L	L	H	H	L	L	L	L	L	L
RM 409-26-1-1-1	L	L	H	H	L	L	H	H	H	L	H	H	H	L
MTU 2055	L	L	L	L	H	H	H	H	L	L	H	H	H	H
MTU 2347-158-3-1-1	H	L	L	L	H	L	L	L	H	L	H	H	L	L
RM 67-60-1-1-1	H	L	H	L	L	L	H	L	L	L	L	L	H	L
UTR 76	L	L	H	L	H	L	L	L	L	L	H	H	L	L
MTU 2846-34-1-1	H	L	H	L	H	L	H	H	H	L	H	H	H	L
MTU 1213	H	H	L	L	L	L	H	H	H	H	L	L	L	L

H – High (Higher than respective mean in desirable direction for *per se* performance and high if significant effect in desirable direction for *gca*); L–Low (Lower than respective mean for *per se* performance and low if non-significant effector significant in undesirable direction for *gca*).

Table 6: Specific combining ability of rice hybrids for yield and yield related traits.

Character	Range	Number of hybrids with desirable and significant <i>sca</i> effects		Best hybrid combinations
Grain yield per plant (g)	-9.80 to 13.06	5		APMS 15A × MTU 1213
Days to 50 per cent flowering	-3.48 to 4.85	-		-
Ear bearing tillers per plant	-1.74 to 2.06	10		APMS 17A × RGL 5613
Panicle length (cm)	-1.12 to 1.26	-		-
Spikelet fertility (%)	-13.98 to 18.59	8		APMS 15A × MTU 2716
1000-grain weight (g)	-2.10 to 2.15	5		APMS 18A × MTU 1224
Grain density	-2.32 to 2.65	10		APMS 17A × MTU 2055

Table 7: Best heterotic combinations identified for grain yield based on *per se* performance, sca effect and heterosis.

Hybrids	<i>per se</i> performance	sca effects	Characterization of parents with respect to <i>gca</i>	Relative heterosis (%)	Heterobeltiosis (%)	Standard Heterosis (%)	Desirable sca effects for other yield attributes	Grain type
APMS 17A × MTU 2055	46.50	12.30**	Low × High	58.43**	25.68**	47.62**	Spikelet fertility and grain density	Medium slender, Straw glume
APMS 17A × RGL 5613	45.00	6.90*	Low × High	118.45**	107.37**	42.86**	Ear bearing tillers per plant, and grain density	Medium slender, Straw glume
APMS 15A × MTU 1213	42.50	13.06**	Low × Low	70.00**	63.46**	34.92**	Ear bearing tillers per plant, Spikelet fertility and grain density	Medium bold, Straw glume
APMS 15A × RGL 5613	42.00	9.06**	Low × High	84.62**	61.54**	33.33**	Grain density	Medium slender, Straw glume
APMS 15A × MTU 2055	41.50	7.90**	Low × High	31.75**	12.16**	31.75**	Ear bearing tillers	Medium bold, Straw glume

assess the hybrids' true potential. The scale of field trials may be restricted due to logistical challenges and resource constraints, potentially limiting the representativeness of the results (Cui *et al.*, 2020).

Additionally, rice hybrids can perform inconsistently across different environments, which may limit their reliability in stress-prone areas. Market acceptance also hinges on factors like grain quality and consumer preferences, which need to align with local demand (Bhadru *et al.*, 2012). Genotype-environment interaction models help refine hybrids by predicting how they perform in various conditions, ensuring stable yield across diverse environments (Kesh *et al.*, 2023). Genomic selection and marker-assisted selection are key tools, allowing breeders to select hybrids with desired traits more accurately and efficiently. Additionally, advances in CRISPR and gene editing allow for targeted modifications that can enhance specific traits, like pest resistance, without compromising yield (Budhlakoti *et al.*, 2021). Together, these strategies pave the way for robust, high-yielding rice hybrids that meet market and environmental demands.

CONCLUSION

The restorer RGL 5613, known for its resistance to leaf blast and moderate tolerance to sheath blight with Bph32, Rf3 and Rf4 genes, besides with strong combining ability for grain yield per plant, spikelet fertility percentage and 1000-grain weight, holds promise for use in developing heterotic and resistant high-yielding rice hybrids. Hybrid combinations such as APMS 17A × MTU 2055, APMS 17A × RGL 5613, APMS 15A × MTU 1213, APMS 15A × RGL 5613 and APMS 15A × MTU 2055 have been recognised as potential candidates for commercial exploitation due to their promising heterotic characteristics. It is recommended to evaluate these hybrids across various locations and over multiple years to assess their performance stability.

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Conflict of interest

Authors do not have any conflict of interests to declare.

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