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Identifying Restorers and Maintainers through Pollen and Spikelet Fertility Studies on Hybrid Rice (*Oryza sativa* L.)

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ABSTRACT: A study was carried out to identify restorers and maintainers for use as parental lines in a hybridization programme. Twenty elite rice genotypes were crossed in a Line x Tester pattern with four CMS lines (CMS 23A, CMS 59A, CMS 64A and JMS 13A) in a Randomized block design with two replications to examine their maintainer or restorer response during *Kharif*-2018. Out of the 20 male genotypes studied for pollen and spikelet fertility analysis, 8 lines (MTU 1153, RNR 26015, RNR 28355, JGL 25960, MTU 1010, IET 27253, RNR 26085 and JAYA) were identified as restorers for the majority of CMS lines used, 6 lines as partial restores and 6 lines as partial maintainers. There are no maintainers identified in any of the lines. In heterosis breeding programmes, it has been suggested that stable germplasm with restorer behaviour be used, while those with maintainer behaviour be used to create new CMS lines through conversion. The newly discovered restorers in this study could be used as pollen parents in future hybrid rice breeding programme to develop promising rice hybrids with improved grain and cooking quality to combat malnutrition and suitable for local climatic conditions.

Keywords: *Oryza sativa,* Testcrosses, CMS lines, Pollen fertility, Spikelet fertility, Restorers.

Introduction

The most significant food crop in the world is rice (*Oryza sativa* L.), whose consumption has continuously climbed from 474 million tonnes in 2015 to 504 million tonnes in 2020 and is projected to rise by around 650 million tonnes by 2050**.** Rice is known as the "grain of life" because it is the most important basic food in the world, providing more than 80 percent of the calories for nearly 2 billion people. Not only is it a fundamental requirement for life but also the most important grain in the human diet, providing 15% of the protein and 21% of the calories consumed globally by people per capita, in addition to being Asia's main source of carbohydrates (Veeresha *et al.,* 2015). To fulfil the needs of a growing population while remaining self-sufficient, world's rice production level must be increased to 852 million tonnes by 2035. This is a challenging endeavour given the rise in the yield potential of high yielding cultivars and the declining natural resources. As the People's Republic of China has amply demonstrated, Rice hybrids are one of the feasibly and economically viable and easily adoptable genetic variations for increasing rice production. As a result of breeders' ongoing efforts, rice breeding programmes have shifted to hybrid rice development, demonstrating the hybrid rice technology's ability to boost output and productivity.

A major development in rice improvement has been the introduction of hybrid rice cultivars using a male sterility and fertility restoration mechanism. A trustworthy mechanism for restoring fertility and preventing male sterility in the cytoplasm is necessary for commercial heterosis to exist in rice. Cytoplasmic genetic male sterile lines that have been presented by different sources might not be well suited to a particular target region. For hybrid vigour to be used successfully in rice, locally produced cytoplasmic genetic male sterility and restorer lines are essential (Kumar *et al.,* 1996). Identification of regionally appropriate maintainers and restorers that demonstrate full sterility and consistently high degrees of restoration of CMS lines would be tremendous utility in commercial hybrid if high combining ability is paired with restorative ability.

Prior research (Parimala *et al.* 2019; Upendi *et al.* 2017) looked at rice test crosses to detect restorative and maintainer reactions and observed variable degrees of pollen and spikelet fertility %. The initial stage in three line heterosis breeding is to set up a test cross nursery to find restorers and maintainers (Priyanka *et al.,* 2016). Male sterility would be a suitable strategy for commercialising heterosis in rice. It would be suitable to utilise a male sterility mechanism. Given the preceding, the current study sought to find the most efficient fertility restorers and maintainers.

MATERIALS AND METHODS

Materials for the current study include 20 different male fertile genotypes, four CMS lines (CMS 23A, CMS 59A, and CMS 64A) and crosses produced using a L×T fashion. To achieve synchronous flowering and adequate crossed seed, parental lines were sown in stages. During *Rabi* 2017-18, using a 20 × 15 cm spacing, 28-day-old seedlings of CMS lines and male viable genotypes were transplanted in a crossing block at the Rice Research Centre in Rajendranagar, Hyderabad.

A suggested set of procedures and need-based plant defence strategies were put into operation in order to grow a healthy crop. Newly developed panicles on male sterile lines were removed and potted into mud-filled plastic buckets before being brought to the crossing room. The panicles' leaf sheaths were gently cut off. Additionally, the panicle's top and bottom florets were cut off. Florets that were scheduled to open the next day used for crossing. Each floret had its top third chopped off the day before and it was then wrapped in butter paper bags. Pollen from male parents was gathered at anthesis the next morning and sprinkled on CMS line panicles that were bagged and labelled. The crossed seeds were gathered once the seeds had fully developed. To assess the restorer / maintainer reaction, 80 test crosses that were created during *Rabi* 2017-18 were transplanted in 4 metre rows with a 20 x 15 cm spacing in *Kharif* 2018.

Estimation of pollen fertility. Pollen fertility was assessed at the blooming stage by gathering 5–10 spikelets in a vial of 20% ethanol from 5 randomly chosen plants from each entry. Forceps were used to extract the anthers from the spikelets, which were then put on a glass slide with a 2 % iodine potassium iodide solution. To release the pollen grains, gently crushing the anthers with a needle. To determine the pollen fertility %, the slides were cleaned of dirt, covered with a cover slip and viewed under a microscope.

$$\text{Pollen fertility (\%)} = \frac{\text{Number of stained pollen grains}}{\text{Total number of pollen grains}} \times 100$$

Estimation of spikelet fertility. From five randomly chosen plants in each test, three panicles were cross-tagged and the panicles were harvested and threshed once they reached maturity. Using the following formula, each panicle's full and chaffy grains were counted individually in order to calculate spikelet fecundity.

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

Classification of pollen parents. Based on the pollen and their spikelet fertility percentages, the pollen parents were divided into four groups.

Class	Pollen fertility (%)	Spikelet fertility (%)
Maintainer (M)	0-1	0
Partial Maintainer (PM)	1.1-50	0.1-50
Partial Restorer (PR)	50.1-80	50.1-75
Restorer (R)	>80	>75

RESULTS AND DISCUSSIONS

The first step in three-line hybrid rice breeding is to establish a test cross nursery to identify restorers and maintainers. Restorers can help to develop good hybrids by serving as parental lines. The current study's findings indicated that the genotypes' response to fertility restoration depends on their ancestry.

The pollen fertility percentage in crosses with CMS 23A ranged from 5.1 percent (IET 27258) to 96.05 percent (RNR 28363) and the spikelet fertility percentage ranged from 10.4 percent (CMS 23A × IET 27260) to 93.61 percent (CMS 23A × MTU 1010). With CMS 23A, nine lines displayed greater than 80% pollen fertility and ten lines displayed more than 75% spikelet fertility.

The pollen fertility percentage of hybrids with CMS 59A ranged from 12 percent (WGL 1054) to 92 percent (WGL 1063) and the spikelet fertility percentage ranged from 15.8 percent (IET 27260 x CMS 59A) to 93.09 percent (IET 27253 × CMS 59A). With CMS 59A, 7 lines demonstrated more than 80% pollen fertility and 10 lines demonstrated spikelet fertility of greater than 75%.

The pollen fertility percentage in crosses with CMS 64A ranged from 8.5 percent (WGL 1054) to 95.05 percent (RNR 28363) and the spikelet fertility percentage ranged from 10.5 percent (WGL 1054 × CMS 64A) to 83.23 percent (RNR 28355 × CMS 64A). With CMS 64A, 6 lines demonstrated pollen fertility of greater than 80% and 6 lines demonstrated more than 75% spikelet fertility.

Spikelet fertility ranged from 10.2 percent (IET 26132 × JMS 13A) to 84 (MTU 1010 × JMS 13A) percent in hybrids with MS 13A, while pollen fertility ranged from 12 percent (WGL 1054 × JMS 13A) to 90 percent (WGL 1063 × JMS 13A). With JMS 13A, six lines demonstrated more than 80% pollen fertility and seven lines demonstrated more than 75% spikelet fertility. According to the results above, (Awad-Allah 2020; Pankaj Kumar *et al.,* (2015) indicated that the genotypes' responses to fertility restoration depend on the genetic background of CMS lines.

The pooled analysis reveals, the pollen fertility percentage ranged from 10.62 % (WGL 1054) to 88.07% (WGL 1063) and spikelet fertility percentage ranged from 16.05 % (WGL 1054) to 86.49 % (MTU 1010) with varying fertility restoration according to male parent (Mirzababapour *et al.,* 2021). Eight lines that are considered restorers and have spikelet fertility rates of more than 75% include MTU 1153, RNR 26015, RNR 28355, JGL 25960, MTU 1010, IET 27253, RNR 26085 and JAYA. These results are according to (Singh *et al.,* 2022; Parimala *et al.,* 2019).

Eight lines from this research (MTU 1153, RNR 26015, RNR 28355, JGL 25960, MTU 1010, IET 27253, RNR 26085 and JAYA) have been identified as restorers for the majority of CMS lines employed, with more than 75% average spikelet fertility, out of the 20 male genotypes evaluated for pollen and fertility analysis. However, 6 lines had average spikelet fertility ranging from 50.1 to 75 percent and were classified as partial restores, whereas hybrids with 6 lines had average spikelet fertility between 0.1 and 50 % and were classified as partial maintainers. These results were supported by (Singh *et al.,* 2022; Ramesh *et al.,* 2018). There are no maintainers identified in the lines studied.

Other researchers have reported similar findings (Mamdouh *et al.,* 2022; Samuel *et al.,* 2018; Rajendraprasad *et al.,* 2017; Shalini *et al.,* 2015; Ghosh *et al.,* 2013; Krishnalatha *et al.,* 2012). It is possible that the different nuclear cytoplasmic interactions between the testers and CMS lines, as well as the penetrance or expressivity of certain genes that varied with genotype or the presence of modifier genes, account for the genotype-specific differences in fertility restoration (Umadevi *et al.,* 2010). Contrarily, adding a lot of legitimacy to such fertility restoration investigations through the use of several CMS lines in testcrosses (Hossain *et al.,* 2010).

Table : Fertility classification of 20 rice genotypes based on pollen and spikelet fertility percent for 4 cytoplasmic males terilelines (CMS).

S. No.	Genotypes	CMSLINES								Average pollen fertility %	Average spikelet fertility %	Classified as
		CMS 23A		CMS 59A		CMS 64A		JMS13A				
		Pollen fertility%	Spikelet fertility%	Pollen fertility%	Spikelet fertility%	Pollen fertility%	Spikelet fertility%	Pollen fertility%	Spikelet fertility%			
1.	DULAR	40.5	18.5	55.2	25.3	30.2	15.8	40.5	20.8	41.6	20.1	PM
2.	MTU1153	90	84.66	86.5	82.52	86	80.44	82.5	80.55	86.25	82.04	R
3.	RNR26015	87.5	91.06	57.5	83.77	47.5	69.63	80	80.9	68.12	81.34	R
4.	WGL 1054	10	15.2	12	20.2	8.5	10.5	12	18.3	10.62	16.05	PM
5.	WGL 1063	90.3	77.34	92	71.55	80.05	65.04	90	70.99	88.07	71.23	PR
6.	RNR28353	70.5	40.8	35.1	60.8	70.2	65.2	70.23	60.15	61.50	56.73	PR
7.	RNR28355	82.5	80.58	47.5	88.88	37.5	83.23	90	82.83	64.37	83.88	R
8.	RNR28363	96.05	78.2	30.3	65.2	95.05	76.2	83	76.5	76	74.02	PR
9.	RNR28398	15.5	25.02	20.2	30.4	75.2	35.4	20.6	28.1	32.8	29.73	PM
10.	RNR28399	10.6	15.6	90.03	75.2	10.5	15.3	70.3	63.99	45.35	42.52	PR
11.	JGL 25960	82.5	80.4	85.5	87.1	82.5	79.7	43.5	78.8	73.5	81.6	R
12.	IET26132	65.2	50.2	70.1	77.19	85.2	75.2	30.2	10.2	62.67	53.19	PR
13.	NDR359	8.5	10.8	15.5	25.8	12.5	15.15	15.2	18.3	12.92	17.51	PM
14.	MTU1010	92.5	93.61	86.5	83.3	82.5	83.18	86.5	85.9	87	86.49	R
15.	IET27253	30	80.53	47.5	93.09	37.5	61.95	47.5	73.87	40.62	77.36	R
16.	IET27255	15.3	45.4	80.1	60.2	30.8	55.2	50.5	65.8	44.17	56.65	PR
17.	RNR26085	82.5	74.95	82.5	80.73	42.5	73	82.5	80.03	72.5	77.17	R
18.	IET27258	5.1	15.3	70.3	20.15	75	25.6	25.2	30.4	43.9	22.86	PM
19.	JAYA	92.5	84.3	42.5	80.73	80	75.94	40	72.06	63.75	78.25	R
20.	IET27260	8.5	10.4	10.2	15.8	12.5	18.2	12.5	20.9	10.92	16.32	PM

R = Restorer Pr = Partial Restorer PM = Partial Maintainer

CONCLUSION

In hybrid rice breeding, more emphasis should be placed on using well-known rice cultivars as parental lines in order to produce superior hybrids with improved grain quality. Despite the fact that no maintainers were discovered throughout the experiment, the restored lines that have been recognized can be employed as pollen parents to create new commercial hybrid types. Additionally, by pyramiding complementing features from multiple sources based on breeding objectives, a crossover programme can increase the genetic diversity of restorers and produce new restorers.

FUTURE SCOPE

Hybrid rice technology is likely to play a pivotal role in increasing the rice production. In spite of having great potential to enhance rice production and productivity, area expansion under hybrid rice has not increased significantly in the last few years due to major constraints like lack of parental lines with desirable specific traits and lower frequency of restorers and maintainers, poor grain and cooking quality, marginal heterosis, higher seed cost, non-involvement of public sectors in hybrid rice seed production. While considering the above stated constraints and wider scope for large scale adoption of rice hybrids, the present study was planned and carried out with an objective to identify restorers and maintainers with good grain and cooking quality. In future breeding programmes it will aid in the development of new superior hybrids with improved grain and cooking quality to combat malnutrition by using the restorer lines as pollen parents and to create strong breeding pipe line of rice crop.

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Conflict of interest. None.

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