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Identification of Maintainers and Restorer for WA CMS Lines in WBPH Tolerant Genotypes in Rice (*Oryza sativa* L.)

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ABSTRACT

The experiment was conducted with three CMS lines i.e., IR 68887 A, IR 79159 A and APMS 6 A which were crossed with eleven WBPH tolerant diverse male parents in line x tester fashion in order to estimate restorers/maintainer nature. Total 33 hybrids were subjected to pollen study, and observed that, out of 33 hybrids, 6 hybrids have more than 80 per cent pollen fertility, whereas 27 hybrids showed the pollen fertility ranging from 51.1 to 80 percent and they were considered as partial restorers. The identified restorers and maintainers could be utilized for development of new WBPH tolerant rice hybrids and CMS lines in future.

Key words: *Rice, Restorer, Pollen fertility.*

INTRODUCTION

Rice (*Oryza sativa* L.*)* is the staple food crop of more than half of world's population and the world population particularly that of the rice consuming countries increasing at a faster rate. By the year 2025, about 785 million tonnes of paddy which is 70 per cent more than the current production will be needed to meet the growing demand[5].

Cytoplasmic male sterility (CMS) is maternally inherited and is associated with a specific mitochondrial gene whose expression impairs the production of viable pollen without affecting the plant. Since restorer of fertility (Rf) genes in the nucleus function to suppress the CMS phenotype, nuclear restoration allows commercial exploitation of the CMS system for the production of high yielding and heterotic seeds and eliminates the need for intensive labour and extensive hand emasculation. The practical importance of CMS system in breeding is highly dependent on the presence of a restorer of fertility (Rf) gene. Hence, a study was undertaken to identify maintainers with higher adaptability of restorers.

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MATERIAL AND METHODS

The three CMS lines *i.e*., IR 68887 A, IR 79159 A and APMS 6 A which were crossed with eleven WBPH tolerant diverse male parents (received from IRRI, Philippines and maintained by MRRS, Nawagam, Gujarat) in line x tester fashion to developed 33 hybrids at Main Rice Research Station, Anand Agricultural University, Nawagam (Gujarat) during *kharif*-2014. Twenty days old seedlings were transplanted in the main field. A spacing of 20 x 20 cm was maintained in a single row of 1.8m length consisting of 10 plants. Single seedling per hill was transplanted. Recommended package of practices were adopted. Pollen studies were carried out to assess fertility/ sterility status of F1 hybrids. For this purpose, 15-20 spikelets from the just emerged panicles of three randomly selected plants were collected in a vial containing 70% ethanol. All the anthers from at least six spikelet's were taken out with forceps and placed on a glass slide with a drop of 1 per cent iodine potassium iodide (I-KI) stain.

$$\text{Pollen fertility (\%)} = \frac{\text{No. of fertile pollen grains (Stained and round)}}{\text{Total no. of pollen grains in microscopic field (Fertile and sterile)}} \times 100$$

Experimental procedure

The crosses were made using three CMS lines (IR 68887A, IR 79159 A and APMS 6 A) and eleven diverse male parents to produce F1S at MRRS, Nawagam during kharif 2014. These hybrids were evaluated for pollen fertility. Pollen fertility of the plants were classified as fully fertile (FF), Semi fertile (SF), Semi Sterile (SS) and Complete Sterile (CS) for estimation of pollen fertility. Anthers of three randomly selected spikelets representing lower, middle and top position of the panicles from at least 6 spikelets were taken out with the help of a forcep and placed in 1 % Iodine Potassium Iodide (IKI) solution. These were gently crushed by using a needle to release the pollen grains. After removing the debris, a cover slip is placed and the slide is ready for observation. Scan the entire slide and take the pollen sterility count in three random Fields. The pollen grains are classified based on their shape, size, and extent of staining and examined under light microscope. The pollen grains were classified as sterile or fertile on their staining behavior and shape. Restorer (>80 %), partial restorer (50.1-80%), partial maintainer (1.1-50 %) and maintainer (0-1%)[8].

Table 1: Classification of pollen based on sterility/fertility

Category	Appearance	Classification
Unstained withered sterile (UWS)		Sterile
Unstained spherical sterile (USS)		Sterile
Stained round (light) sterile (SRS)		Sterile
Stained round fertile (SRF)		Fertile

Table 2: Identification of restorer gene from the hybrids in rice

Sr. No.	Hybrids	Pollen fertility %	Remarks
1.	IR 68887 A X MILYANG 54 GAYABYEO)	63.85	Partial restorer
2.	IR 68887 A X MILYANG 55	68.55	Partial restorer
3.	IR 68887 A X MILYANG 63	82.55	Restorer
4.	IR 68887 A X POKKALI	68.55	Partial restorer
5.	IR 68887 A X IR77186-148-3-4-3	72.55	Partial restorer
6.	IR 68887 A X IR 04N 106	85.55	Restorer
7.	IR 68887 A X IR 05N412	62.55	Partial restorer
8.	IR 68887 A X IR 07A 166	78.55	Partial restorer
9.	IR 68887 A X IR 09A 104	83.55	Restorer
10.	IR 68887 A X IR 09N 534	70.63	Partial restorer
11.	IR 68887 A X IR 10 A 117	74.63	Partial restorer
12.	IR 79159 A X MILYANG 54 (GAYABYEO)	74.83	Partial restorer
13.	IR 79159 A X MILYANG 55	70.56	Partial restorer
14.	IR 79159 A X MILYANG 63	80.36	Restorer
15.	IR 79159 A X POKKALI	76.36	Partial restorer
16.	IR 79159 A X IR77186-148-3-4-3	70.56	Partial restorer
17.	IR 79159 A X IR 04N 106	78.36	Partial restorer
18.	IR 79159 A X IR 05N412	72.56	Partial restorer
19.	IR 79159 A X IR 07A 166	70.46	Partial restorer
20.	IR 79159 A X IR 09A 104	78.36	Partial restorer
21.	IR 79159 A X IR 09N 534	75.19	Partial restorer
22.	IR 79159 X IR 10 A 117	73.49	Partial restorer
23.	APMS 6 A X MILYANG 54 (GAYABYEO)	68.49	Partial restorer
24.	APMS 6 A X MILYANG 55	78.49	Partial restorer
25.	APMS 6 A X MILYANG 63	78.29	Partial restorer
26.	APMS 6 A X POKKALI	74.49	Partial restorer
27.	APMS 6 A X IR77186-148-3-4-3	72.39	Partial restorer
28.	APMS 6 A X IR 04N 106	78.29	Partial restorer
29.	APMS 6 A X IR 05N412	68.49	Partial restorer
30.	APMS 6 A X IR 07A 166	82.49	Restorer
31.	APMS 6 A X IR 09A 104	81.29	Restorer
32.	APMS 6 A X IR 09N 534	78.79	Partial restorer
33.	APMS 6 A X IR 10 A 117	72.61	Partial restorer

Table 3: Genotype identified as effective restorer

CGMS used	Effective Restorers	Pollen fertility %
IR 68887 A	MILYANG 63	82.55
	IR 04N 106	85.55
	IR 09A 104	83.55
IR 79159 A	MILYANG 63	80.36
APMS 6 A	IR 07A 166	82.49
	IR 09A 104	81.29

RESULTS AND DISCUSSION

The main step in the exploration in the hybrid is to identify maintainers and restorers. The result showed that (Table 2) IR 68887 A X MILYANG 63 (82.55 %), IR 68887 A X IR 04N 106 (82.55 %), IR 68887 A X IR 09A 104 (83.55 %), IR 79159 A X MILYANG 63 (80.36 %), APMS 6 A X IR 07A 166 (82.49 %) and APMS 6A X IR 09A 104 (81.29 %).

The present study revealed a lot of variation for the presence of fertility restorer gene in rice genotypes which used as males on

all three CMS lines. On the basis of pollen study, it was observed that, out of 33 hybrids, 6 hybrids have more than 80 per cent pollen fertility, whereas 27 hybrids showed the pollen fertility ranging from 51.1 to 80 percent and they were considered as partial restorers. The hybrids were classified into different fertility-sterility groups as suggested by Virmani *et al.*[8] in rice. The results are match with the findings of Hemareddy *et al.*[3], Gannamani *et al.*[2], Joshi *et al.*[4], Murugan and Ganesan[6] and Das *et al*[1].

The results shows that (Table 3) the pollen parent MILYANG 63, IR 04N 106, IR 09A 104 is restorer of IR 68887A, the pollen parent MILYANG 63is restorer of IR 79159A and the pollen parent IR 07A 166and IR 09A 104 is restorer of APMS 6 A.

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