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Genetics of pollen fertility restoration trait in rice (*Oryza sativa* L.) for wild abortive cytoplasmic male sterility system

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Abstract

Two crosses in F_2 generation *viz.,* APMS 8A × RM 141-91-11-2-1-1 and APMS 6A × RM 71-42-2-1-1 used to study the genetics of fertility restoration, revealed that one major gene governs the fertility restoration, with dominant monogenic gene action. The crosses APMS 8A × RM 141-91-11-2-1-1 and APMS 6A × RM 71-42-2-1-1 segregated in the ratio of 3:1 in F_2 generation, indicating the presence of single independently segregating dominant gene governing the fertility restoration in both the restorers under study.

Keywords: Genetics, Fertility restoration, Gene action, Rice, BSA

Introduction

Rice (*Oryza sativa* L. 2n = 2x = 24, Family: *Gramineae*) is the most important cereal food crop of our country and occupies about 24 per cent of gross cropped area of the country. In India, rice is cultivated over an area of 43.79 m ha with a production of 112.91 m tonnes of milled rice and productivity of 3520 kg ha^{-1}. In Andhra Pradesh area, production and productivity of rice are 22.08 lakh ha, 123.52 lakh tones of milled rice and 5593 kg ha^{-1}, respectively (Statistical Abstracts of AP, 2019). Among the various approaches to improve the yield threshold of rice, exploitation of hybrid vigor is considered to be the most feasible and readily practicable. China pioneered hybrid rice research in 1970's and demonstrated 20-30% yield advantage over conventional varieties. The hybrids grown in India, China, Vietnam, Bangladesh and other countries are based on *indica* rice sources which on an average show a standard heterosis of 15-20% in commercial cultivation. It has been demonstrated very clearly on large scale that hybrids give 15-20% increased yield over the highest yielding varieties under the similar growing conditions by using 'Wild Abortive' (WA) cytoplasmic male sterility (Hossain *et al.,* 2010). High yield potential of CMS derived F_1 hybrids depends upon their high pollen and spikelet fertility which is determined by the number and mode of action of restorer genes present in the restorer parent.

The commercial use of cytoplasmic genetic male sterility (CGMS) is possible only when effective restorers are identified. The identification of restorers for different cytoplasmic male sterile sources will increase cytoplasmic diversification and will help in the development of hybrids with greater adaptability and reduced vulnerability to different pests and diseases. The fertility of CMS system can be restored by nuclear restorer genes, which need to be identified before implementing the production of hybrid rice. Fertility restorer alleles (*Rf*s) are always tightly evolved with cytoplasmic male sterility (CMS) during plant evolution. Fertility restoration is reported to be controlled by two major genes *Rf3* and *Rf4* which are on chromosomes 1 and 10 respectively (Zhang *et al.*, 1997). To study the inheritance of fertility restoration the main three indexes (percentage of fertile pollen, bagged seed setting and opening seed-setting) are often used as the criteria to evaluate fertility restoration (Li *et al.,* 2007). The present investigation was undertaken to understand the genetics of fertility restoration of CMS lines of 'WA' cytoplasm by using two F_2 crosses.

Materials and Methods

Experimental material of the present investigation comprised of two segregating populations in F_2 generation derived from test crosses APMS 8A × RM 141-91-11-2-1-1 and APMS 6A × RM 71-42-2-1-1. The pollen fertility and spikelet fertility of these restorers are 92.41 % & 90.12 % in APMS 8A × RM 141-91-11-2-1-1 and 96.26 % & 91.28 % in APMS 6A × RM 71-42-2-1-1 in F_1 generation. Both the crosses were sown with a spacing of 20 × 15 cm in paired rows during *kharif,* 2016 at Regional Agricultural Research Station, Maruteru, West Godavari district of Andhra Pradesh. All the recommended agronomic practices were followed for a good crop stand.

Fertility restoration in the restorers was identified by pollen sterility study. In both the populations 100 plants were selected in each population and 15 to 20 spikelets from just emerged panicles of each plant were collected in a vial containing 70% ethanol for conducting pollen sterility studies. A glass slide was taken and a drop of 1% iodine potassium iodide (IKI) stain was taken on slide. All the anthers from at least 6 spikelets are taken out with the help of a forceps and placed in the stain. These are gently crushed by using a needle to release the pollen grains. After removing the debris, a cover slip is placed and the slide is observed for the number of fertile and sterile pollen. The entire slide is scanned under microscope and pollen sterility count is taken in 3 random fields.

The pollen grains are classified based on their shape, size, and extent of staining. Fully, dark stained pollen grains were the fertile one whereas unstained pollen grains were sterile. Plants were classified in to different fertility sterility groups as - pollen sterility per cent between 0-20 were grouped as fully fertile, 21-30 per cent as fertile, 31-70 per cent as partially fertile, 71-90 per cent partially sterile, 90-99 per cent as sterile and 100 percent pollen sterility as completely sterile plants (Virmani *et al*, 1997). The goodness of fit for various Mendelian genetic ratios in F_2 generation was tested using the Chi-square Analysis (Pearson, 1901).

Results and Discussion

In the present study, the inheritance of fertility restoration in the crosses APMS 8A × RM 141-91-11-2-1-1 and APMS 6A × RM 71-42-2-1-1, revealed F_2 segregation ratio of 3 (fertile): 1 (sterile), indicating that the restorers *viz.,* RM 141-91-11-2-1-1 and RM 71-42-2-1-1 carry single independently segregating dominant gene for fertility restoration. The Chi square test confirmed the goodness of fit of 3 fertile:1 sterile ratio in both the populations indicating that the fertility restoration in the restorer lines under study was governed by single dominant monogenic gene.

Genetics of fertility restoration by chi square analysis

Population – I (APMS 8A × RM 141-91-11-2-1-1)

Pollen sterility was studied in 100 F_2 plants in the population APMS 8A × RM 141-91-11-2-1-1. Based on the pollen sterility (%), 83 fertile plants and 17 sterile plants were observed which approximately fits in 3 (fertile): 1 (sterile) ratio, which is a typical case of dominant monogenic inheritance **(Table 1).**

Population – II (APMS 6A × RM 71-42-2-1-1)

Pollen sterility was studied in 100 F_2 plants in the population APMS 6A × RM 71-42 2-1-1. Based on the pollen sterility (%), 71 fertile plants and 29 sterile plants were observed which approximately fits in 3 (fertile): 1 (sterile) ratio, which is a typical case of dominant monogenic inheritance **(Table 2).**

Table 1 Chi-square test for goodness of fit in F_2 cross APMS 8A × RM 141-91-11-2-1-1.

S. No	Cross	Phenotype	Frequency	Observed (O)	Expected (E)	D (O-E)	D^2 $(O-E)^2$	$\chi^2=D^2/E$
1	APMS 8A x RM 141-91-11-2-1-1	Fertile	¾	83	75	8	64	0.85
		Sterile	¼	17	25	-8	64	2.56

$\chi^2=3.41$

Table 2 Chi-square test for goodness of fit in F_2 cross APMS 6A × RM 71-42-2-1-1.

S. No.	Cross	Phenotype	Frequency	Observed (O)	Expected (E)	D (O-E)	D^2 $(O-E)^2$	$\chi^2=D^2/E$
1	APMS 6A x RM 71-42-2-1-1	Fertile	¾	71	75	-4	16	0.21
		Sterile	¼	29	25	4	16	0.64

$\chi^2=0.85$

Similar findings were reported by Anandakumar and Subramaniam (1992), Gyan *et al.* (2003), Ahmadikhah *et al.* (2007) and Singh *et al.* (2015). In both the populations the calculated χ^2 value at 0.05 % probability is less than the table value of 3.841 at 1 degrees of freedom and the difference is non-significant between the observed values and expected values for the above cross. The Chi square test confirmed the goodness of fit of 3 fertile:1 sterile ratio in both the populations indicating that the fertility restoration in the restorer lines under study was governed by single dominant monogenic gene.

A total of 21 *Rf3* (10 markers) and *Rf4* (11 markers) gene linked markers were used for parental polymorphism among the parents of both F_2 crosses APMS 8A × RM 141-91-11-2-1-1 and APMS 6A × RM 71-42-2-1-1. Among ten *Rf3* gene linked markers, five markers *viz.*, RM 10287, RM 10305, DRRM *Rf3-5*, DRRM *Rf3-10* and RMS-SF21-1 showed polymorphism among parents in F_2 cross APMS 6A × RM 71-42-2-1-1 (**Figure 1**). Among eleven *Rf4* gene linked markers, one marker *viz.*, RM 6100 showed polymorphism between parents in F_2 cross APMS 8A × RM 141-91-11-2-1-1. Confirmation of markers linked to fertility restoration genes in the populations under study was done by bulk segregant analysis.

Sterile bulks and fertile bulks derived from F_2 population of APMS 8A and RM 141-91-11-2-1-1 did not show any polymorphism for *Rf4* gene linked marker RM 6100. In the population, APMS 6A x RM 71-42-2-1-1 polymorphic markers linked to *Rf3* genes *viz.*, RM 10287, RM 10305, DRRM *Rf3-5*, DRRM *Rf3-10* and RMS-SF21-1 showed no polymorphism between fertile and sterile bulks.

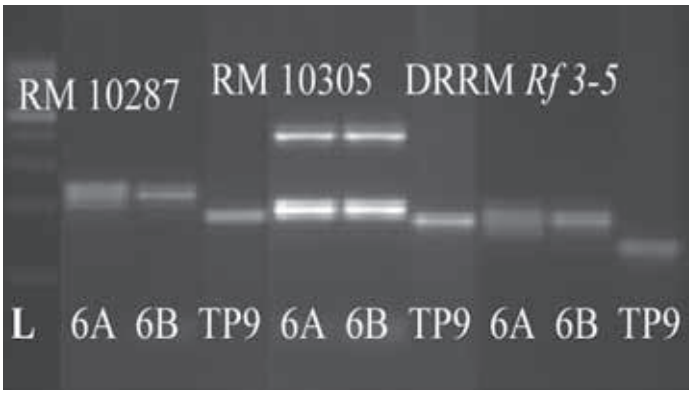

Figure 1: Parental polymorphism using *Rf3* gene linked markers RM 10287, RM 10305 and DRRM *Rf3-5*

(L: 100 bp Ladder, 6A: APMS 6A, 6B: APMS 6B, TP-9: RM 71-42-2-1-1)

The expected marker genotypic segregation ratio within the sterile extremes should be 1 (fertile band):1 (sterile band). But in the current study no such genotypic segregation ratio has been observed. No linkage has been detected between the marker loci and *Rf* gene in these populations. However, chi square analysis of phenotypic data clearly showed the marker loci to be linked strongly with *Rf* genes.

Conclusions

Two F_2 population *viz.*, APMS 8A × RM141-91-11-2-1-1 and APMS 6A × RM71-42-2-1-1 were used in the study of genetics of fertility-restoration. Confirmation of genetic studies in segregating populations was done for pollen sterility (%) in both the populations using Chi square test. Chi square values obtained were χ^2 = 3.41 (APMS 8A × RM141-91-11-2-1-1) and χ^2 = 0.85 (APMS 6A × RM71-42-2-1-1) with 5% level of significance (1 df =3.84) leading to conclusion that both the F_2 populations were segregating in the ratio

of 3 (fertile):1 (sterile), indicating single dominant gene governing the fertility restoration.

Through bulked segregant analysis it was found that no co-segregation of banding pattern of fertile parents and fertile bulks and sterile parents and sterile bulks. It clearly indicated that neither of *Rf3* or *Rf4* genes is contributing for fertility restoration and polymorphic markers used were not tightly linked to *Rf* genes in the two restorers under study. Further screening for presence of fertility restoration genes other than *Rf3* and *Rf4* has to be done to confirm the genes linked to fertility restoration in both the restorers under study.

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