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Marker assisted selection for two major fertility restorer genes in promising Warangal rice genotypes

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Abstract
Pollen fertility is restored by nuclear-encoded genes called fertility restorer (*Rf*) gene. For developing high yielding heterotic hybrids, the first step is to identify restorers that can efficiently restore the fertility of CMS lines. As the fertility restoration of CMS-WA lines is principally controlled by two independent and dominant nuclear fertility restoring genes, *Rf3* and *Rf4*. In the present study, we made an attempt for genotyping of 25 rice genotypes for the presence of *Rf3* and *Rf4*genes by using functional markers. Out of 25 rice genotypes, we observed that six genotypes namely RR 32, RR 49, WGL 616, WGL 639, WGL-674& WGL 1068 were observed to be double positives for *Rf3* and *Rf4*genes and hence these five genotypes may be used as male parents or restorers in CMS based hybrid rice breeding programs.

Keywords: MAS, fertility restorer genes, rice

Introduction
Rice (*Oryza sativa* L.) is a staple food for more than half of the world's population. Hybrid rice have clearly shown a standard heterosis of 15–20% in commercial cultivation mainly in the indica genotypes. The magnitude of heterosis depends on the choice of appropriate parental lines. Rice being self-pollinated crop, use of male sterility system is a prerequisite for commercial exploitation of heterosis in rice. The WA cytoplasm is the most widely used since it is a most stable system and the pollen sterility is almost nearly complete. Pollen abortion in WA-CMS is sporophytic, forming typical abortive pollen. CGMS system/Three-line system has been widely used for developing rice hybrids. This system involves a CMS or 'A' line, a maintainer or 'B' line and a restorer or 'R'line. Cytoplasmic male sterility (CMS) is a maternally inherited trait that results in inability of the plant to produce fertile pollen. Pollen fertility is restored by nuclear-encoded genes called fertility restorer (*Rf*) gene. For developing high yielding heterotic hybrids, the first step is to identify restorers that can efficiently restore the fertility of CMS lines. Earlier investigations confirmed that fertility restoration is governed by two independent dominant nuclear genes with one gene being stronger in action than the other.

Different studies also indicated different types of gene interaction like recessive epistasis, semi-epistasis, epistasis with incomplete dominance, epistasis with complete dominance or no interaction. Huang *et al.* (1986) [4], Anandakumar and Subramaniam (1992) [3] reported that a major dominant gene controls fertility restoration of WA-cytoplasm. However most of the genetic studies of fertility restoration for the WA-CMS system have suggested that fertility restoration is governed by two genes namely *Rf4* and *Rf3* have been mapped to chromosomes 10 and 1 respectively. The use of molecular markers linked to *Rf* genes can enhance the selection efficiency, save time and avoid the complications associated with phenotype-based screening. The genetic linkage analysis indicated that the SSR markers RM6100 reported by Singh *et al*. (2005) [8], on the long arm of chromosome 10, linked with the *Rf4* gene at distance of 1.2 cM and RM10313 reported by Neeraja (2009) [5], on the short arm chromosome 1, linked with *Rf3* gene at a distance of 4.2 Cm have been utilized to screen one hundred breeding lines for the identification of restorers. Considering the importance of fertility restorer genes, the present study was aimed at molecular confirmation of two major fertility restorer genes i.e. *Rf3*&*Rf4* in the 25 rice genotypes by using functional markers.

Material and Methods
Plant Material
The present study was conducted at Regional Agricultural Research Station, Warangal,

Professor Jayashankar Telangana State Agricultural University (PJTSAU) during *Kharif*, 2016 under field conditions in Vertisols, with an objective of molecular confirmation of Restores lines for presence of two major fertility restorer genes i.e. *Rf3* and *Rf4* through functional markers (Table 2). The list of rice genotypes used in the present investigation were given in (Table 1).

Table 1: Details of rice genotypes and their genotyping results by using functional markers for the present investigation.

S. No.	Name of the rice genotype	*Rf3* gene: RF3-10 marker	*Rf4* gene: RF4-14 marker
Positive control	KMR-3R	RR	RR
Negative control	25B	rr	rr
1.	RR3	rr	RR
2.	RR4	rr	RR
3.	RR 13	rr	RR
4.	RR15	rr	RR
5.	RR17	rr	RR
6.	RR18	rr	RR
7.	RR 23	rr	RR
8.	RR 25	rr	rr
9.	RR32	RR	RR
10.	RR49	RR	RR
11.	RR50	rr	RR
12.	RR55	rr	rr
13.	RR65	rr	rr
14.	WGL-347	rr	RR
15.	WGL-616	RR	RR
16.	WGL-639	RR	RR
17.	WGL-674	RR	RR
18.	WGL-676	rr	RR
19.	WGL-705	rr	RR
20.	WGL-501	rr	RR
21.	WGL-810	rr	RR
22.	WGL-1058	rr	RR
23.	WGL-1068	RR	RR
24.	MTU 1156	RR	rr
25.	MTU-320-20	RR	rr

Table 2: Details of markers used in present study

S. No.	Nameof the Marker	5'-3'	Sequence	TM
1	Rf4-14 (F)		GCAATGCTTGTATTCAGCAAA	55 ºC
2.	Rf4-14 (R)		TCCAGCTGTAAATCCGTCAA	55 ºC
3.	Rf3-10 (F)		TCACCTCTTCCTGCTTCGAC	55 ºC
4.	Rf3-10 (R)		CTCCACCAGTGCAGGTTTTT	55 ºC

Marker assisted selection for fertility restorer genes

DNA was isolated from the all the 25rice genotypes along with checks by following the protocol of Zheng *et al*., (1995) [11]. The PCR based SSR marker Rf3-10 andRf4-14were used to identify the allelic status with respect to two major fertility restorer genes i.e. R*f3* and *Rf4*. PCR was performed using 1 U of Taq DNA polymerase (Fermentas, Lithuania) and 1x PCR buffer (Genei, India) in 10-µl reaction volume with a thermal profile of 94 ºC for 5 min (initial denaturation), followed by 35 cycles of denaturation at 94 ºC for 30 s, annealing at 55 ºC for 30 s, extension at 72 ºC for 1 min and a final extension of 7 min at 72 ºC. The amplified product of *Rf3*and*Rf4* were electrophoretic ally resolved on a 1.2% Seakem LE® agarose gel (Lonza, USA) containing 0.5 mg/ml of ethidium bromide in 0.5x TBE buffer and visualized under UV.

Results and Discussion

Among the various technological options available to increase the production of rice, hybrid rice technology is the most promising (Ahmed and Siddiq 1998) [1]. Rice hybrids possess a yield advantage of 15-20% over the high-yielding inbred varieties (Yuan 1994, Normile 1999) [9, 6]. Fertility restoration of CMS-WA lines is principally controlled by two independent and dominant nuclear fertility restoring genes, *Rf3* and *Rf4* (Zhang *et al*. 2002, Sattari *et al*. 2007) [10, 7]. Similar to our study, earlier, Hari *et al* (2011) [3] while improving a stable restorer line KMR-3R for Bacterial Blight resistance, utilized *Rf3* and *Rf4*genes for confirmation of fertility restoration in F_1& subsequent backcrossing generations.

Molecular confirmation of Rf3 gene by using RF3-10 functional Marker: The 25rice genotypes along with checks, when screened for the presence of *Rf3* gene by using RF3-*10* functional marker, the expected product size in positive control (KMR-3R)is 190 bp and similar type of band was observed in8 rice genotypes i.e.RR-32,RR-49,WGL-616,WGL-639, WGL-674, MTU1156 andMTU-320-20,while the expected product size in negative control (IR580 25B)is 210 bp and similar type of band was observed in17rice genotypes i.e.RR-3,RR-4,RR-13,RR-15,RR-17,RR-18,RR-23,RR-25,RR-50,RR-55,RR-65,WGL-347,WGL-676,WGL-705,WGL-501,WGL-810 andWGL-1058.

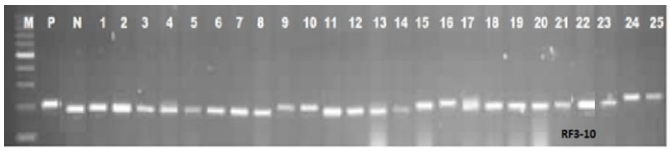

Fig 1: Molecular confirmation of Rf3 gene by using RF3-10 functional Marker

The Lane number M represents 100bp ladder, P represents positive control (KMR-3R), N represents negative control (IR 580 25B), while the numbers from 1 to 25 written on the top of gels represents list of rice genotypes used for present study and details were given in Table 1.

Molecular confirmation of Rf4 gene by using RF4-14 functional Marker

The 25rice genotypes along with checks, when screened for the presence of *Rf4* gene by using RF4-*14*functional marker, the expected product size in positive control (KMR-3R)is 700 bp and similar type of band was observed in20genotypes i.e.RR-3, RR-4, RR-13, RR-15, RR-17, RR-18, RR-23, RR-32, RR-49, RR-50, WGL-347, WGL-616,WGL-639,WGL-674, WGL-676, WGL-705, WGL 501, WGL-810, WGL-1058 and WGL-1068,while the expected product size in negative control (IR580 25B)is 780 bp and similar type of band was observed infiverice genotypes i.e. RR-25, RR-55, RR-65, MTU1156 and MTU-320-20.

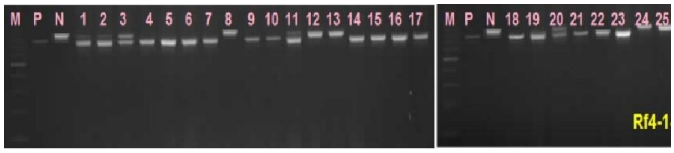

Fig 2: Molecular confirmation of Rf4 gene by using RF4-14 functional Marker

The Lane number M represents 100bp ladder, P represents positive control (KMR-3R), Nrepresents negative control (IR 580 25B), while the numbers from 1 to 25 written on the top of gels represents list of rice genotypes used for present study and details were given in Table 1.

Out of 25 rice genotypes, 3 genotypes were observed to be negative for two major fertility restorer genes (*Rf3* &*Rf4*) namely RR 25, RR 55 and RR 65, while two rice genotypes were observed to be positive for only *Rf3* gene namely MTU 1156 and MTu-320-20, while 14 rice genotypes were observed to be positive for only *Rf4* gene namely RR-3, RR-4, RR-13, RR-15, RR-17, RR-18, RR-23, RR-50, WGL-347, WGL-676, WGL-705, WGL 501, WGL-810 and WGL-1058.

Conclusion

Cultivation of the rice genotypes possessing two fertility restorer genes i.e. *Rf3* &*Rf4*would be of great advantage in Hybrid rice breeding programs. Out of 25 rice genotypes, we observed that six genotypes namely RR 32, RR 49, WGL 616, WGL 639, WGL-674& WGL 1068 were observed to be double positives for *Rf3* and *Rf4*genes and hence these five genotypes may be used as male parents or restorers in CMS based hybrid rice breeding programs.

Further Research

The rice genotypes possessing two fertility restorer genes i.e. *Rf3* &*Rf4*will be available for use as donors in Hybrid rice breeding programs.

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Conflict of interests: The authors have declared no conflicts of interests exist.

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