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Short Communication

Efficiency of molecular markers in identifying fertility restoration trait of WA-CMS system in rice

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Abstract

To assess the efficiency of different molecular markers which are linked to fertility restorer genes *Rf3* and *Rf4* of WA-CMS system, 103 breeding lines with no information on fertility restoration were screened with the help of molecular markers linked to major fertility genes *Rf3* and *Rf4.* The breeding lines were crossed with APMS6A and the F_1s were evaluated for pollen and spikelet fertility to identify restorers and maintainers. The SSR primer RM6100 linked to *Rf4* gene of chromosome 10 and RM10313 linked to *Rf3* gene of chromosome 1 showed eighty five and eight one percentage efficiency respectively in identifying restorer lines. Therefore, these markers are useful tool for evaluating large number of breeding lines to know about their fertility restoration in a short period of time without generating and evaluating large number of test crosses. The potential restorers may be identified with hundred percentage efficiency based on molecular screening itself, if candidate genes based markers are developed and validated for both *Rf4* and *Rf3* genes.

Key words: Fertility restoration, *Rf4*, *Rf3*, molecular markers, WA-CMS

In hybrid rice seed production using CGMS system, the combination of a CMS line, maintainer line and restorer line carrying the restorer gene (*Rf*) to restore fertility is indispensable for the development of hybrids [1]. The most widely used CMS in rice is based on wild abortive (WA) cytoplasm derived from *Oryza sativa* f. sp. *spontanea* [2, 3]. WA based CMS lines are highly stable and also their pollen sterility is complete [4]. Fertility restoration of WA-CMS is extensively investigated trait. All the studies have consistently demonstrated that two dominant independent loci controlling fertility restoration of WA-CMS system [3, 5, 6]. Molecular marker studies have also been employed to determine the chromosomal location of *Rf* genes of WA-CMS system. Zhang *et al*. [7, 8] with the help of molecular markers designated the loci restoring the fertility as *Rf3* & *Rf4* and mapped *Rf3* on chromosome 1 and *Rf4* on chromosome 10 using NIL as a mapping population. Currently with the availability of rice genome sequence, highly robust, co-dominant, cost effective, and highly polymorphic PCR based SSR (Simple Sequence Repeats) markers linked to *Rf* genes have been reported by many investigators.

RM6100 linked to *Rf4* gene has been mapped at a distance of 6-7cM on chromosome 10 in restorer lines PRR 78 R, IR 40750 and MTU9992 [9]. RM6100 linked to *Rf4* gene and RM10313 linked to *Rf3* could differentiate restorer and maintainer lines with 75 and 80% efficiency, when they were used together restorer lines could be identified with 95% efficiency [10]. While validating molecular markers linked to fertility restoration trait of WA-CMS, reported that RM6100 linked to *Rf4* gene at a distance of 1.2 cM with the selection accuracy of 94.87% in identifying restorers [11]. Alavi *et al*. [12] mapped *Rf3* locus linked to SSR markers RM1, RM3233 and RM3873 on the short arm

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of chromosome 1 and showed that the primers RM1 and RM3873 when used together; their efficiency in MAS is 99.2%. In the present investigation, we made an attempt to confirm the efficiency of previously reported markers linked to *Rf* genes namely, RM6100, RM10313, RM3233, RM 3873 and RM1 in identifying fertility restoration trait by evaluating 103 testcrosses for pollen and spikelet fertility traits.

Total genomic DNA was isolated from young leaves by Mini-preparation method [13]. PCR reactions was carried out using 50 ng/µl of template DNA, containing 2.5 mM of each dNTP, 0.25 µM of each forward and reverse primer, 1 U of Taq DNA polymerase, 1X PCR reaction buffer in a total volume of 10 µl in thermal cycler (Eppendorf, USA). The amplified PCR products along with 100 bp molecular marker (Bangalore Genie, India) were separated on a 3.0% Seakem® LE agarose gel (Lonza, USA), stained with ethidium bromide and documented using Gel documentation system (Alpha Innotech, USA). Based on the banding pattern gels were scored for presence and absence of bands as restorers and non-restores.The sequence of primers and their amplification product sizes are presented in (Table 1). The breeding lines were crossed with a CMS line APMS6A to produce F_1 hybrid seeds and the F_1 progeny was raised during next season to study their pollen and spikelet fertility status. The pollen and spikelet fertility was estimated according to Virmani *et al*. [14].

A total 103 breeding lines without prior information about fertility restoration status along with

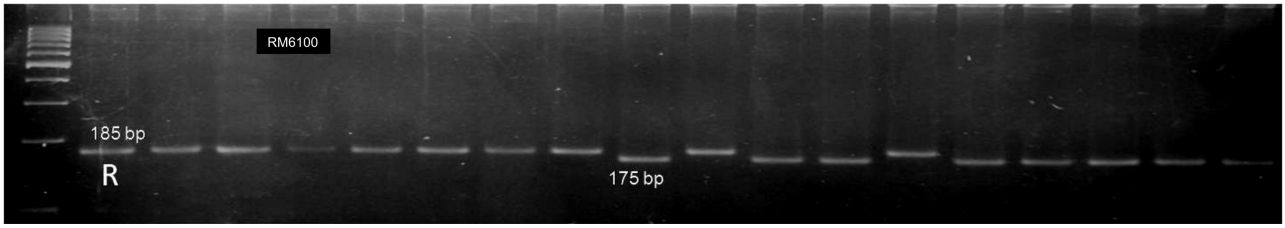

Fig. 1. Amplification pattern of *Rf4* linked marker

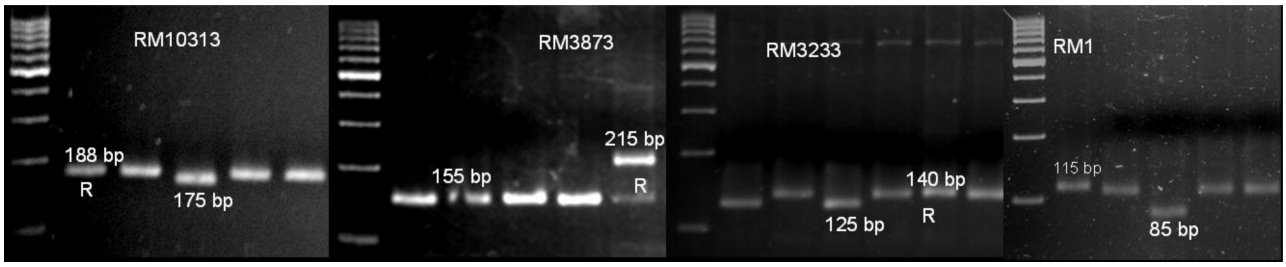

Fig. 2. Amplification pattern of *Rf3* linked markers

Table 1. Primer sequences of *Rf4* & *Rf3* linked markers

Molecular marker/primer	Sequence (5' to 3')	Gene linked	Chromosomal location	Amplification product size in restorer (base pair)	Amplification product size in non-restorer (base pair)
RM6100	TTCCCTGCAAGATTCTAGCTACACC TGTTCGTCGACCAAGAACTCAGG	*Rf4*	10	185	175
RM1	GCGAAAACACAATGCAAAAA GCGTTGGTTGGACCTGAC	*Rf3*	1	115	85
RM3873	GCTATAGACGCCTCCTCCTTATCC AAAGCTAGCTAGGACCGACATGC	*Rf3*	1	210	155
RM3233	GAAATTCGAAATGGAGGGAGAGC GGTGAGTAAACAGTGGTGGTGAGC	*Rf3*	1	140	125
RM10313	ACTTACACAAGGCCGGGAAAGG TGGTAGTGGTAACTCTACCGATGG	*Rf3*	1	188	175

few known restorers were screened with five SSR primers namely RM6100 linked to fertility restorer gene *Rf4* located on chromosome 10, RM 1, RM3233, RM3873 and RM10315 linked to fertility restorer gene *Rf3* located on chromosome 1. The lines were scored as restorers based on the presence of restorer specific allele band. The amplification pattern of SSR markers linked to *Rf4* and *Rf3* genes were shown in the Figs. 1 & 2. Of the 103 lines screened 78, 77, 81, 96 and 98 lines were identified as restorers by PCR analysis of SSR primers RM6100, RM10313, RM3233, RM3873 and RM1 respectively. Identification of effective restorers and maintainers are the initial steps in three line heterosis breeding. The pollen fertility and spikelet fertility traits are important criteria at test cross nursery stage for identifying restorer and maintainer lines. Based on pollen fertility seventy seven lines were identified as restorers (>75 %) and 26 lines as non restorers. With respect to spikelet fertility sixty five lines were identified as restorers, thirty eight lines as non restorers. Twenty restorer lines identified to carry both *Rf4* & *Rf3* genes with pollen and spikelet fertility more than 80% (Table 2) and these lines can be immediately utilized in hybrid rice breeding programme.

The SSR primer RM6100 linked to *Rf4* gene amplified restorer specific allele in sixty five out of seventy five lines based on pollen fertility and showed 84 % efficiency in identifying restorer lines. With respect to spikelet fertility it showed 85% efficiency. RM10313 linked to *Rf3* gene showed 81 % efficiency in comparison with pollen and spikelet fertility, Although RM1, RM3233 and RM3873 primers had a higher positive efficiency in identifying restorers, their efficiency in terms of actual pollen and spikelet fertility was less than 50%. Therefore these primers are not useful for identification of restorer lines by Marker Assisted Selection. However, the SSR primers RM6100 and RM10313 can be utilized to screen the breeding lines to identify restorers with 80 to 85%

Table 2. Molecular screening, pollen and spikelet fertility results

S.No.	Genotypes	F_1s pollen fertility %	F_1s spikelet fertility %	*Rf4*-RM6100	*Rf3*-RM10315	*Rf3*-RM-1	*Rf3*-RM 3233	*Rf3*-RM 3873
1	OR-2162-5	100	80	1	1	1	1	2
2	RP-4092-115-12-5-4	90	86	0	1	0	1	1
3	RH1531	90	81	1	1	1	1	1
4	OR 2324-8	85	89	1	1	1	1	1
5	HKR-06-47	90	82	1	1	1	1	1
6	PAU-3105-45-3-2	85	93	1	1	1	1	1
7	RAU 467-79-60	85	80	1	1	1	1	1
8	RP-4092-128-104-95-12	85	94	1	1	1	1	2
9	IR 50	80	85	1	1	1	1	1
10	IR 80905-50-1-3-2	85	81	1	1	1	0	1
11	RAMPUR MASURI	85	80	0	1	1	1	1
12	BK-50-108	85	80	2	2	1	1	1
13	CSR-9	100	91	1	1	1	0	1
14	CSR-33	80	98	2	2	1	1	1
15	CSR-38	95	82	1	1	1	1	0
16	NDRK 500013	100	89	1	1	1	0	0
17	GGV-050-02	100	98	1	1	1	2	1
18	IR06-057	100	96	1	1	1	1	1
19	APO	100	90	2	0	1	1	1
20	IR 78875-131-B-1-4	95	86	1	1	1	0	1

1-Present, 0-Absent, 2-Heterozygous

efficiency when they were used together in comparison with pollen and spikelet fertility restorer lines were identified with more than 92% efficiency.

In hybrid rice breeding, to identity the maintainer and restorer lines test cross evaluation of F_1 crosses between the unknown lines with the CMS line is a must. Therefore breeding for hybrid rice is laborious and time consuming as it takes almost two seasons or one year. Nas *et al*. [15] demonstrated for the first time use of molecular markers for restorer line identification and reported that PCR based marker RG140STS exhibited 83% efficiency in identifying putative restorers. The present study explains PCR based markers RM6100 and RM10313 exhibiting 80 to 85% efficiency in restorer identification which is in close confirmation with the earlier reports. Singh *et al*. [9] reported that usefulness of RM6100 in marker aided selection of restorer with selection accuracy of 97%. RM6100 amplified the *Rf4* linked allele in a majority of the restorers with a selection accuracy of 94.87% [11]. The higher selection accuracy may be due to the fact that these studies involved a set of less number of putative restorers only. Alavi *et al*. [12] reported that RM1 and RM3873 primers are having 89 and 74% efficiency in MAS for fertility restoration trait. However, when they are used together, their efficiency would be 99% in identification of restorers. In this study RM1 and RM 3873 showed around 90% efficiency in restorer identification whereas non-restorers also identified with higher selection accuracy in comparison with pollen and spikelet fertility. Map distance between *Rf3* gene and the molecular markers RM1, RM3233, and RM3873 is 5.6cM, 17cM and 14cM respectively. As these primers are not closely linked with *Rf* genes, they are not able to differentiate putative restorers and non-restorers. Hence, these primers are not useful in marker assisted selection of restorer lines.

Identification of candidate gene based marker for fertility restoration trait would be very useful in distinguishing restorers from non-restorers. Recently candidate gene based marker for fertility restoration trait has been reported [16]. These genic markers are based on pentatricopeptide repeat (PPR) motif containing genes on chromosome 10. But further experiment is required to validate the identified candidate PPR genes to establish its precise role in restoration of fertility of WA-CMS. The present study indicates that molecular screening for fertility restoration can be a useful tool for identifying restorers from breeding lines of unknown restoration status with 85 to 92% efficiency without making and evaluating large number of test crosses. But identified restorers based on molecular screening must be test crossed with appropriate CMS lines to confirm their fertility restoration to achieve higher level of heterosis. Thus use of molecular markers linked to *Rf* genes would save time and money besides adding accuracy in identification of restorers. If gene based markers are developed for both *Rf4* and *Rf3* genes, potential restorers may be identified based on molecular screening itself.

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