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RESEARCH ARTICLE

VALIDATION OF DNA MARKERS LINKED WITH FERTILITY RESTORER GENES OF WILD ABORTIVE CYTOPLASM IN RICE (*Oryza sativa* L.)

[1, 2]Nirmala, R., [1, 3]Gnanesh, A. U., [1]Kamalnath Reddy, K. R., and [1, 4,*]Shashidhar, H. E.

[1]Barwale Foundation, # 3-6-666, Street No. 10, Himayath Nagar, Hyderabad, Andhra Pradesh, India
[2]Directorate General of Agriculture and Livestock Research, Ministry of Agriculture, Muscat, Sultanate of Oman
[3]College of Agriculture, Dharwad, Karnataka, India
[4]Department of Biotechnology, University of Agriculture, GKVK, Bangalore, India

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ABSTRACT

Cytoplasmic male sterility-fertility restoration (CMS/*Rf*) system based three line hybrid rice breeding is popular in rice production worldwide. The conventional method of restore line identification among rice germplasm pool is time consuming and labor intensive. Molecular mapping of fertility restorer genes (*Rf*s) for various CMS sources in rice have yielded several closely linked DNA markers that can be used in identifying restorer lines. In order to utilize this available information effectively in marker assisted restorer line identification, validation of reported *Rf* gene linked DNA markers was carried out in this study. A total of eleven DNA markers spread across 5 chromosomes (Chr. 1, 4, 7, 10 and 12) reported to be closely linked with five *Rf* genes of wild abortive CMS source (WA-cms) were chosen. These markers were screened among thirty rice genotypes involving seventeen WA-cms (A-line) and thirteen restorer lines (R-line). The genotypic data set was generated based on the specific PCR product size. The restoration ability of the rice lines used in this study was estimated by test cross procedure involving two cms lines IR58025A & IR68888A; and their F_1s were studied for the percentage pollen and spikelet fertility. The marker-trait association analysis was carried out using Crop Stat and "TASSEL" (Trait Analysis by aSSociation, Evolutions and Linkage) software programs. Analysis of Variance (ANOVA) results indicated seven markers were significant. Amongst, four markers namely RM490/*Rf*3, RM6100/*Rf*4, RM311/*Rf*5 and RM258/*Rf*6 had higher phenotypic variance (R^2 value) compared to the rests and therefore these marker/gene combinations can be utilized in marker assisted identification of restorer lines of WA-cms. The beneficial alleles for each of the significant markers such as 95bp &105bp alleles of RM490/*Rf*3, 164bp allele of RM6100/*Rf*4, 179bp allele of RM311-*Rf*5, 187bp allele of RM258-*Rf*6 were identified for the gain in trait.

INTRODUCTION

Cytoplasmic male sterility (CMS) is a common phenomenon in higher plants characterized by sterile, non functional pollen grains due to the alterations in mitochondrial genome. And, the sterility can be restored by crossing CMS lines with the restorer lines (R-line) containing fertility restorer genes (*Rf*) in it. This CMS-*Rf* system has been widely used in hybrid seed production in self pollinating crops like rice, maize and several vegetable crops. In rice, wild abortive CMS (WA-cms) source has been utilized extensively in commercial hybrid rice seed production duo to its stability, excellent out crossing potential and the availability of broader genetic base for the restorer lines (Virmani and Kumar, 2004). Restorer line plays an important role in successful hybrid rice development. They are detected conventionally through test cross procedure by crossing rice germplasm lines (male parents) with the sterile CMS lines (female parents) and the F_1s are evaluated for the pollen and spikelet fertility. This system of restorer line identification is time consuming and labor intensive. Studies on genetic inheritance of *Rf* genes of WA-cms indicated single gene control (Shen *et al*., 1996, Yao *et al*., 1997), two linked genes (Li and Zhu 1986), two independent genes (Virmani *et al*., 1986; Raj and Virmani 1988; Teng and Shen 1994; Bharaj *et al*., 1995, Tan *et al*., 1998, Jing *et al*., 2001, Namatzadeh and Kiani 2010) and four genes (Zhu *et al*., 1996). Attempts on DNA marker based linkage mapping analysis revealed chromosomal location of several *Rf* gene loci: *Rf*3 on Chromosome 1 (Yao *et al*., 1997, Zhang *et al*., 1997, Zhuang *et al*., 2000, He *et al*., 2002, Ahmadikhah *et al*., 2007, Sattari *et al*., 2007), *Rf*4 on Chromosome 10 (Yao *et al*., 1997, Tan *et al*., 1998, Jing *et al*., 2001, Zhang *et al*., 2002, Ahmadikhah *et al*., 2007, Sattari *et al*., 2007, Sheeba *et al*., 2009, Agdaca *et al*., 2010) *Rf*4 on Chromosome 7 (Bazrkar *et al*., 2008), *Rf*5 on chromosome 10 (Jing *et al*., 2001, Ahmadikhah *et al*., 2007), *Rf*6 on Chromosome 10 (Bazrkar *et al*., 2008) and *Rf*7 on chromosome 12 (Bazrkar *et al*., 2008). These studies have shown several DNA markers closely linked with specific *Rf* genes that are useful in marker assisted identification of those genes in the rice germplasm and further use in breeding program. In order to utilize this information in practical breeding practices, the reported markers need to be validated.

In addition, While validating the markers that are tightly linked with the specific gene of interest in a germplasm, it is obvious that multiple alleles of those marker loci are detected and each allele contributes differentially to the trait and association mapping studies helps to get this information. Association mapping also known as linkage disequilibrium mapping is a powerful high resolution mapping tool useful for dissecting complex traits (Zhou and Stephens 2012). It has advantages over conventional linkage mapping studies such as comprehensive mapping resolution with genome wide scanning, faster identification of potential alleles and QTLs. Successful application of unbiased association mapping approaches in human

Corresponding author: Shashidhar, H. E., Department of Biotechnology, College of Agriculture, Univ. Agril. Sciences, Karnataka, India

genetics had encouraged plant breeders to adopt such tool in crop breeding program (Abdurakhmonov and Abdukarimov, 2008, Ibrokhim *et al*., 2008) and made great advances in several plant species including wheat (Breseghello and Sorrells 2006), rice (Agrama *et al*., 2007, Agrama and Yan, 2009, Jia *et al*., 2012), sweet sorghum (Shehzad *et al*., 2009), maize (Wisser *et al*., 2011), tomato (Mazzucato *et al*., 2008), barley (Cockram *et al*., 2010) and arabidopsis (Brachi *et al*., 2010). Studies on association mapping of straight head disorder in rice, Agrama and Yan (2009) reported three alleles at each of three associated loci (RM490-allele 87bp, RM143-allele 105bp and RM277-allele 122bp) and two alleles at another locus (RM263-alleles 182bp and 183bp) had contributed significantly to straight head resistance than other counterparts. The study reported in this paper was undertaken with the objectives of (i) validation of reported DNA markers associated with specific *Rf* gene(s) of WA-cms and (ii) identification of beneficial alleles of validated markers responsible for the trait gain using association mapping analysis.

MATERIALS AND METHOD

Plant materials and DNA extraction

A set of thirty rice germplasm accessions comprising seventeen wild abortive-cytoplasmic male sterile lines and thirteen restorer lines were used in this study (Table 1). All the rice lines were grown in the field at Maharajpet farm, Barwale Foundation experimental station, Hyderabad, India and the genomic DNA was extracted from four-week old rice seedlings using modified Dellaporta method (Dellaporta *et al*., 1983).

Table 1. List of plant materials used for validation of reported *Rf* gene linked marker

Sl. No	Accession No	Cytoplasm	Source*
1	IR58025A	Sterile	IRRI, Philippines
2	IR68888A	Sterile	IRRI, Philippines
3	IR68897A	Sterile	IRRI, Philippines
4	IR68902A	Sterile	IRRI, Philippines
5	IR69624A	Sterile	IRRI, Philippines
6	IR72078A	Sterile	IRRI, Philippines
7	IR72080A	Sterile	IRRI, Philippines
8	IR72081A	Sterile	IRRI, Philippines
9	IR73321A	Sterile	IRRI, Philippines
10	IR75596A	Sterile	IRRI, Philippines
11	IR79128A	Sterile	IRRI, Philippines
12	IR79156A	Sterile	IRRI, Philippines
13	IR80151A	Sterile	IRRI, Philippines
14	IR80156A	Sterile	IRRI, Philippines
15	IR80555A	Sterile	IRRI, Philippines
16	IR80559A	Sterile	IRRI, Philippines
17	IR80561A	Sterile	IRRI, Philippines
18	KMR3	Fertile	UAS, Mandya, India
19	PRR78	Sterile	IARI, New Delhi, India
20	BR-827-35	Fertile	KKV, Karjat, India
21	NDR-3026	Fertile	NDUAT, Faizabad, India
22	IR40750	Fertile	IRRI, Philliphines
23	C-20R	Fertile	BF, Hyderabad, India
24	UPRI-92-133	Fertile	BF, Hyderabad, India
25	Suraksha	Fertile	BF, Hyderabad, India
26	Prasanna	Fertile	BF, Hyderabad, India
27	Kavya	Fertile	BF, Hyderabad, India
28	IR50	Fertile	BF, Hyderabad, India
29	Swarna	Fertile	BF, Hyderabad, India
30	Jaya	Fertile	BF, Hyderabad, India

* BF: Barwale Foundation; DRR: Directorate of Rice Research; IARI: Indian Agricultural Research Institute; IRRI: International Rice Research Institute; KKV: Konkan Krishi Vidyapeeth; NDUAT: Narendra Dev University of Agricultural Sciences and Technology; UAS: University of Agricultural Sciences

Phenotyping

Sterility level of CMS lines was assessed by calculating the percentage pollen and spikelet fertility during flowering and harvesting stage of the plants respectively (Yui *et al*., 2003). The fertility restoration ability of the R-lines was estimated by test crossing (Virmani *et al*., 1997) using two CMS lines (female parents) namely IR58025A and IR68888A; Followed with the evaluation of percentage pollen and spikelet fertility among F_1 progenies. To estimate the mean value of percentage pollen fertility, 10-15 spikelets were collected; anthers were removed, crushed, stained with 2% I_2KI solution and visualized under microscope. The unstained, irregular pollen grains were recorded as sterile and completely stained, round pollen grains as fertile. Finally, the percentage pollen sterile/fertility were calculated based on the number of sterile/fertile pollens over the total number of pollen grains analyzed. To estimate the mean value on percentage spikelet fertility, three panicles per plant were bagged with selfing covers during flowering stage; at harvesting stage, bagged panicles were collected, sun dried and spikelets were counted for the number of filled and unfilled grains. The percentage spikelet sterility/fertility was calculated by considering the number unfilled/filled grains over total number of spikelet per panicle.

Genotyping

Molecular markers reported for five fertility restorer (*Rf*) genes of WA-cms was studied. Based on the centiMorgan (cM) distance between the gene and the marker, closely linked markers were identified and used for polymerase chain reaction (PCR) analysis (Table 2 & 3). PCR reaction was carried out in 15µl reaction volume containing 10-15ng genomic DNA, 1X PCR buffer (10mM Tris-Cl [p^H8.4]; 50mM KCl), 200µM dNTPs, 5 pM of each forward and reverse primers, 0.5U *Taq* polymerase enzyme. Amplification was carried out in MJ research thermal cycler with the program of initial denaturation at 95°C for 5 minutes, cyclic denaturation at 94ºC for 8 seconds, primer annealing at 55°C for 5 seconds and the primer extension at 72ºC for 40 seconds. The cycle was repeated 30 times and ended with the final extension at 72ºC for 7 minutes. The amplified PCR products were resolved in 5% polyacrylamide gel electrophoresis, followed with silver staining procedure. The genotypic dataset was generated based on the PCR amplification profile by scoring presence (+) and absence (-) of specific allele with specific base pair (bp) size for all the samples.

Table 2. List of *Rf* gene linked DNA markers used for validation

Chr	*Rf* gene	DNA marker ID	Genetic Distance (cM)	Reference
1	R*f*3	RM490	2.8cM	Sattari *et al.,* 2007
1	*Rf*3	RM1	5.6 cM	Ahmadikhah *et al.,* 2007
1	R*f*3	RM443	4.4cM	Bazrkar *et al.,* 2008
10	*Rf*4	RM228	3.4cM	Jing *et al.,* 2001
10	*Rf*4	RM6100	1.2cM	Sheeba *et al.,* 2009
10	*Rf*4	RM171	1.5cM	Agdaca *et al.,* 2010
10	*Rf*4	RM1108	4.6cM	Agdaca *et al.,* 2010
7	*Rf*4	RM6344	-	Bazrkar *et al.,* 2008
10	*Rf*5	RM311	2.9cM	Ahmadikhah et al., 2007
10	*Rf*6	RM258	4.4cM	Bazrkar *et al.,* 2008
12	*Rf*7	RM7003	13.3cM	Bazrkar *et al.,* 2008

Association analysis

The statistical significance of marker-trait association was evaluated by considering the phenotypic and genotypic datasets of the rice lines using Crops Stat software program version 7.2 (www.irri.org). Data set of significant marker-trait association were further used for association mapping analysis using "TASSEL" (Trait analysis by Association, Evolutions and Linkage) - the software program version 3.0 (Bradbury *et al*., 2007). Best linear unbiased estimates of alleles and their phenotypic variance (marker R^2) were recorded.

RESULTS AND DISCUSSION

Trait evaluation of hybrid rice parental lines

The percentage pollen and spikelet fertility data was recorded for the rice lines under study. The data on percentage pollen fertility among

Table 3. Primer sequence of selected DNA markers used for validation

Rf gene	RM marker ID	Forward sequence (5'-3')	Reverse sequence (5'-3')
*Rf*3	RM1	GCGAAAACACAATGCAAAAA	GCGTTGGTTGGACCTGAC
*Rf*3	RM490	ATCTGCACACTGCAAACACC	AGCAAGCAGTGCTTTCAGAG
*Rf*3	RM443	GATGGTTTTCATCGGCTACG	AGTCCCAGAATGTCGTTTCG
*Rf*4	RM228	CTGGCCATTAGTCCTTGG	GCTTGCGGCTCTGCTTAC
*Rf*4	RM6100	TCCTCTACCAGTACCGCACC	GCTGGATCACAGATCATTGC
*Rf*4	RM171	AACGCGAGGACACGTACTTAC	ACGAGATACGTACGCCTTTG
*Rf*4	RM1108	GCTCGCGAATCAATCCAC	CTGGATCCTGGACAGACGAG
*Rf*4	RM6344	ACACGCCATGGATGATGAC	TGGCATCATCACTTCCTCAC
*Rf*5	RM311	TGGTAGTATAGGTACTAAACAT	TCCTATACACATACAAACATAC
*Rf*6	RM258	TGCTGTATGTAGCTCGCACC	TGGCCTTTAAAGCTGTCGC
*Rf*7	RM7003	GGCAGACATACAGCTTATAGGC	TGCAAATGAACCCCTCTAGC

Table 4. Spikelet fertility data on F_1 hybrids

Cross F_1 hybrid	% spikelet fertility (Mean±SE)	Cross	% spikelet fertility (Mean±SE)
IR58025A/KMR3	98.33±0.8	IR58025A/Suraksa	95.7±0.3
IR68888A/KMR3	97.66±0.5	IR68888A/Suraksa	81.3±1.3
IR58025A/PRR78	97.66±0.3	IR58025A/ Prasanna	97.3±0.3
IR68888A/PRR78	95.66±0.3	IR68888A/Prasanna	89.7±0.3
IR58025A/BR827-35	93.33±0.3	IR58025A/Kavya	93.7±0.3
IR68888A/ BR827-35	92.0±0	IR68888A/Kavya	83.7±0.7
IR58025A/NDR3026	96.33±0.3	IR58025A/IR50	95.7±0.3
IR68888A/ NDR3026	93.66±0.3	IR68888A/IR50	90.7±0.7
IR58025A/IR40750	96.33±0.3	IR58025A/Swarna	94.7±0.3
IR68888A/IR40750	93.66±0.3	IR68888A/Swarna	90.0±1.15
IR58025A/C20R	96.7±0.7	IR58025A/Jaya	97.7±0.3
IR68888A/C20R	90.7±0.3	IR68888A/Jaya	86.0±1.0
IR58025A/UPRI92-133	95.7±0.3		
IR68888A/UPRI92-133	90.7±0.3		

Table 5. Details of different marker alleles identified among the rice germplasm used in this study

RM Marker	Size of the allele	# alleles detected
RM1	111bp, 115bp	2
RM490	95bp, 100bp, 105bp	3
RM443	115bp, 117bp	2
RM6100	148bp, 160, 164bp	3
RM1108	137bp, 140bp, 145bp	3
RM171	322bp, 328bp	2
RM228	138bp, 140bp, 142bp, 144bp, 146bp, 148bp, 150bp, 162bp, 165bp	9
RM6344	108bp, 118bp, 138bp	3
RM311	177bp, 179bp	2
RM258	187bp, 191bp, 195bp, 200bp	4
RM7003	108bp, 110bp, 112bp	3

seventeen CMS-lines was ranged between 0 to 3 percent with the mean value of 1.64 percent; the percentage spikelet fertility was recorded as zero for all the CMS-lines under study. Ahmedikhah and Karlov (2006) suggested the pollen fertility of less than 5 percent can be considered as completely sterile as they do not produce fertile seed. Accordingly, all the CMS lines used in this study were found to be completely sterile. CMS line IR58025A showed 100% pollen and spikelet sterility. It confirms the previous reports on sterility maintenance of IR58025A, so that most of the released commercial rice hybrids were derived from this single CMS source (Virmani *et al*., 1997). Analysis of variance results indicated no significant difference between the pollen and spikelet fertility as observed earlier (Ahmedikhah *et al*., 2007). Pollen fertility test of F_1 hybrids showed 100 percent fertile pollen and the spikelet fertility was above 90 percent. Therefore, their paternal lines were classified as putative restorer lines (Table 4).

Screening of *Rf* gene linked DNA markers

A total of eleven DNA markers reported to be linked with five different *Rf* genes of WA-cms were screened among thirty rice genotypes. Genotypic data showed the number of alleles identified per marker was variable. Four markers namely RM1, RM443, RM171 and RM311 had two alleles each and the rest of the seven markers had multiple alleles. Amongst the multi allelic markers, RM490, RM6100, RM1108, RM6344 and RM7003 had three alleles; markers RM258 and RM228 had four and nine alleles respectively (Table 5). This result indicated the reported *Rf* gene linked DNA markers were polymorphic. Since the DNA markers used in this study were simple sequence repeats (SSR), it is often expected that multiple alleles are detected while using such markers in germplasm screening (McCouch *et al*., 2002). Along with the phenotypic data on percent spikelet fertility, single marker-trait association analysis was carried out using Cropstat software program version7.0. Analysis of Variance (ANOVA) results showed seven out of eleven markers screened were found to be significantly associated with the trait under study at various level of statistical significance (Table 6). Among, the markers RM490, RM443 and RM1 linked with *Rf*3 gene located on short arm of chr.1 with the map distance of 2.8cM, 4.4cM and 5.6cM (Ahmadikhah *et al*., 2007, Sattari *et al*, 2007) investigated, marker RM490 was found to be highly significant association at p value <0.001. The probability value of markers RM443 and RM1 were 0.145 and 0.170 respectively, which indicated the marker-trait association for these two markers was insignificant. Similarly,

investigation of four markers RM6100, RM1108, RM171 and RM228 located on Chr.10 with the map

Table 6. F-stat values of Analysis of variance (ANOVA) for marker validation

S. No	*Rf* gene	RM Marker ID	F -ratio	F-probability
1	*Rf*3	RM1	1.88	0.170
2	*Rf*3	RM490	24.56	0.000***
3	*Rf*3	RM443	2.20	0.145
4	*Rf*4	RM6100	35.05	0.000***
5	*Rf*4	RM1108	4.48	0.021*
6	*Rf*4	RM171	6.48	0.005**
7	*Rf*4	RM228	1.82	0.159
8	*Rf*4	RM6344	3.78	0.022*
9	*Rf*5	RM311	3.79	0.022*
10	*Rf*6	RM258	2.77	0.041*
11	*Rf*7	RM7003	0.77	0.556

*** Markers are highly significant at P value <0.001; ** Markers are highly significant at P value <0.01;* Markers are significant at P value <0.05

distance of 1.2cM, 1.5cM, 4.6cM and 3.4cM respectively from *Rf*4 gene, except RM228, rests were showed statistical significance at p value < 0.001, 0.01 and 0.01 respectively. Bazrkar *et al* (2008) mapped *Rf*4 gene on chromosome 7 with the linked marker RM6344, was found to be significant at p value <0.05 in this study. Markers RM311 and RM258 linked with *Rf*5 and *Rf*6 respectively and located on chromosome 10 were also significant at p value <0.05. The results suggested lesser the distance between the marker and the gene, higher the marker-trait association (Ngangkham *et al*., 2010, Balajisuresh *et al*., 2012). Fine mapping followed by positional cloning of a specific *Rf* gene helps in developing tightly linked DNA markers for marker assisted trait identification as well as molecular characterization of the gene (Ngangkham *et al*., 2010; Balajisuresh 2012). Marker-Trait association mapping analysis of the data set using "Trait Analysis by aSSociation, Evolutions and Linkage (TASSEL)" software program revealed the phenotypic variance (R^2) (prediction of the proportion of phenotypic variance contributed by the markers) of four significant markers such as RM490/*Rf*3, RM6100/*Rf*4, *Rf*311/*Rf*5 and RM258/*Rf*6) had contributed higher proportion to the phenotypic variation compared to the rests with the percentage phenotypic contribution of 99%, 99%, 36% and 26% respectively (Table 7).

Table 7. Analysis results on phenotypic variance (marker R^2) of significant markers and their beneficial alleles using TASSEL software program

Rf gene	Linked marker	Significant markers	Marker R^2	Beneficial allele(s)
*Rf*3	RM490	RM490	99%	95bp=105bp
*Rf*4	RM6100	RM6100	99%	164bp
*Rf*4	RM1108	RM1108	36%	140bp
*Rf*4	RM171	RM171	18%	322bp
*Rf*4	RM6344	RM6344	16%	108bp>118bp
*Rf*5	RM311	RM311	38%	179bp
*Rf*6	RM258	RM258	26%	187bp>195bp>191bp

The results indicated that these marker/gene combinations can be considered as tightly linked markers for major *Rf* genes of WA cms. The highest phenotypic variance of the markers RM490/*Rf*3 and RM6100/*Rf*4 revealed these two *Rf* genes are dominant in nature. This result is in accordance with the previous reports (Gholizadehghara *et al*., 2012; Revathy *et al*., 2013) and Yuan *et al* (2002) suggested that *Rf*3 and *Rf*4 genes can work either independently or dependently in different restorer lines. In addition, the beneficial alleles for each of the significant markers such as 95bp 95bp &105bp alleles of RM490/*Rf*3, 164bp allele of RM6100/*Rf*4, 179bp allele of RM311-*Rf*5, 187bp allele/RM258-*Rf*6 were identified for the gain in trait (Table 8). The results revealed the specific marker-allele combinations are greatly useful in marker assisted identification of restorers of WA-cms in a rice germplasm pool. Amongst the significant markers, except marker RM171, rest of them showed more than two alleles with an example: six markers namely RM1, RM490, RM6100, RM1108, RM6344 & RM311 identified 3 alleles; RM258 identified 4 alleles. It indicated the genetic diversity for the *Rf* genes

Table 8. Allelic estimates of the different alleles of significant markers linked with different *Rf* genes derived from TASSEL software analysis

Marker	# observations	Allele (bp)	Allele estimate
RM490	9	95:95	85.17974
RM490	4	105:105	86.68529
RM490	17	100:100	0
RM6100	11	160:160	-0.40455
RM6100	17	148:148	-85.9853
RM6100	2	164:164	0
RM1108	9	140:140	56.4817
RM1108	4	137:137	2.270588
RM1108	17	145:145	0
RM6344	16	108:108	55.275
RM6344	13	118:118	20.76154
RM6344	1	138:138	0
RM171	12	322:322	35.73333
RM171	18	328:328	0
RM311	11	179:179	46.66424
RM311	4	179:177	-25.9517
RM311	15	177:177	0
RM258	4	195:195	26.95
RM258	20	191:191	33.48
RM258	5	187:187	87.38
RM258	1	200:200	0

of WA-cms in indica varieties. Raj and Virmani (1988) suggested appropriate combinations of two genes can restore the fertility of WA-cms in its F_1 hybrids completely. The results obtained in this work would be useful in marker assisted identification of various *Rf* genes of WA-cms among rice germplasm as well as in back cross breeding program to develop near isogenic lines with multiple *Rf* genes towards the development of superior restorer lines.

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REFERENCES

Abdurakhmonov IY, Abdukarimov A (2008). Application of association mapping to understanding the genetic diversity of plant germplasm resources. Intl. J. Plant Genom. 574927.

Agdaca CD, Mendioro MS, Nas TMS, Singh RK (2010). WA-CMS fertility restorer gene restores fertility of a cytoplasmic male sterile line of rice (*Oryza sativa* L.) developed from a mutagenized source. Philipp. Agric. Scientist. 93 (2): 149-155.

Agrama HA, Eizenga GC, Yan W (2007). Association mapping of yield and its components in rice cultivars. Mol. Breed. 19: 341–356.

Agrama HA, Yan W (2009). Association mapping of straight head disorder induced by arsenic in Oryza sativa. Plant Breeding 128: 551–558.

Ahmadikhah A, Karlov GI (2006). Molecular mapping of the fertility–restoration gene *Rf*4 for WA-cytoplasmic male sterility in rice. Plant Breed. 125(4): 67-71.

Ahmadikhah A, Karlov GI, Nematzadeh G, Ghasemi Bezdi K (2007). Inheritance of the fertility restoration and genotyping of rice lines at the restoring fertility (*Rf*) loci using molecular markers Intl. J. Plant Prod. 1: 13-21.

Balajisuresh P, Srikanth B, Hemanthkishore V, Subhakararao I, Vemireddy L, Dharika N, Sundaram R, Ramesha M, Sambasivarao K, Viraktamath B, Neeraja C (2012). Fine mapping of *Rf*3 and *Rf*4 fertility restorer loci of WA-CMS of rice (*Oryza sativa* L.) and validation of the developed marker system for identification of restorer lines. Euphytica. 187(3): 421.

Bazrkar L, Ali AJ, Babaeian NA, Ebadi AA, Allahgholipour M, Kazemitavar K, Nematzadeh G (2008). Tagging of four fertility restorer loci for wild abortive cytoplasmic male sterility system in rice (*Oryza sativa* L.) using microsatellite markers. Euphytica 164:669–677.

Bharaj TS, Virmani SS, Khush GS (1995). Chromosomal location of fertility restoring genes for wild abortive cytoplasmic male sterility using primary trisomics in rice. Euphytica 83: 169–173.

Brachi B, Faure N, Horton M, Flahauw E, Vazquez A, Nordborg M, Bergelson J, Cuguen J, Roux F (2010). Linkage and association mapping of *Arabidopsis thaliana* flowering time in nature. PLoS Genet. 6(5): e1000940

Bradbury PJ, Zhang Z, Kroon DE, Casstevens TM, Ramdoss Y, *et al*. (2007). TASSEL: software for association mapping of complex traits in diverse samples. Bioinformatics 23: 2633–2635.

Breseghello F, Sorrells ME (2006). Association mapping of kernel size and milling quality in wheat (*Triticum aestivum* L.) cultivars. Genetics 172: 1165–1177.

Cockram J, White J, Zuluaga DL, Smith D, Comadran J, *et al*. (2010). Genome wide association mapping to candidate polymorphism resolution in the unsequenced barley genome. PNAS 107: 21611–21616.

Dellaporta RP, Wood J, Hicks JB (1983). A plant DNA mini-preparation: version II. Plant Mol. Biol. Rep. 1: 19-21.

Gholizadehghara A, Nematzadeh G, Bagheri N, Ebrahimi A, Oladi M (2012). Molecular and cytological evaluation of male sterile and restorer lines in hybrids rice. Intl. Res. J. Appl. Basic Sci. 3 (1): 183-189.

He GH, Wang WM, Liu GQ, Hou L, Xiao YH, Tang M *et al.* (2002). Mapping of two fertility restoring gene for WA cytoplasmic male mterility in Minghui63 using SSR markers. Acta Genet. Sin. 29: 798–802.

Ibrokhim Y, Abdurakhmonov, Abdukarimov A (2008). Application of association mapping to understanding the genetic diversity of plant germplasm resources. Intl. J. Plant Genomics: 1-18.

Jia L, Yan W, Zhu C, Agrama HA, Jackson A, Yeater K, Li, Huang B, Hu B, McClung A, Wu D (2012). Allelic analysis of sheath blight resistance with association mapping in rice. Plos One 7(3): e32703

Jing R, Li X, Yi P, Zhu Y (2001). Mapping fertility restoring genes of rice WA cytoplasmic male sterility using SSLP markers. Bot. Bull. Acad. Sin. 42: 167-171.

Li ZB, Zhu YG (1986). Rice male sterile cytoplasm and fertility restoration. In: Smith WH, Bostian LR, Cervantes E*,* eds*.* Hybrid Rice/Proceedings of the International Symposium on Hybrid Rice*. IRRI, Manila, 85–102.*

Mazzucato A, Papa R, Bitocchi E, Mosconi P, Nanni L, *et al*. (2008). Genetic diversity, structure and marker-trait associations in a collection of Italian tomato (*Solanum lycopersicum* L.) landraces. Theor. Appl. Genet. 116: 657–66.

McCouch SR, Teytelman L, Xu Y, Lobos KB, Clare K, Walton M, Fu B, Maghirang R, Li Z, Zing Y, Zhang Q, Kono I, Yano M, Fjellstrom R, DeClerck, G, Schneider D, Cartinhour S, Ware D, Stein L (2002). Development and mapping of 2240 new SSR markers for rice (Oryza sativa L.). DNA Res., 9, 199-207

Nematzadeh GhA and Kiani G (2010). Genetic analysis of fertility restoration genes for WA type cytoplasmic male sterility in Iranian restorer rice line DN-33-18. African J. Biotech. 9(38): 6273-6277.

Ngangkham U, Parida SK, Sandip DK, RajKumar A, Singh AK, Singh NK, Mohapatra T(2010). Genic markers for wild abortive (WA) cytoplasm based male sterility and its fertility restoration in rice. Mol. Breed. 26: 275-292.

Raj GK, Virmani SS (1988). Genetics of fertility restoration of WA type cytoplasmic male sterility in rice. Crop Sci. 28: 787-792.

Revathi P, Pavani M, Arunkumar S, Sundaram RM, Sravan R, Senguttuvel P, Kemparaju KB, Hariprasad A S, Ramesha MS, Neeraja CN, Rani NS, Viraktamath B C (2013). Efficiency of molecular markers in identifying fertility restoration trait of WA-CMS system in rice. Indian J. Gen. Plant Breed. 73(1): 89-93.

Sattari M, Kathiresan A, Gregorio G, Hernandez JE, Nas TM, Virmani SS (2007). Development and use of a two-gene marker-aided selection system for fertility restorer genes in rice. Euphytica 153: 35–44.

Sheeba NK, Viraktamath BC, Sivaramakrishnan S, Gangashetti MG, Pawan K, Sundaram RM (2009). Validation of molecular markers linked to fertility restorer gene(s) for WA-CMS lines of rice. Euphytica 167: 217–227.

Shehzad T, Iwata H, Okuno K (2009). Genome-wide association mapping of quantitative traits in sorghum (*Sorghum bicolor* (L.) Moench) by using multiple models. Breeding Sci 59: 217–227.

Shen Y, Cai Q, Gao M, Wang X (1996). Isolation and genetic characterization of fertility restoring revertant induced from cytoplasmic male sterile rice. Euphytica 90: 17–23.

Tan XL, Vanavichit A, Amornsilpa S, Trangoonrung S (1998). Genetic analysis of rice CMS-WA fertility restoration based on QTL mapping. Theor. Appl. Genet. 96: 994–999.

Teng LS and Shen ZT (1994). Inheritance of fertility restoration for cytoplasmic male sterility in rice Rice Genet. Newsl. 11: 95-97.

Virmani SS, Kumar I (2004). Development and use of hybrid rice technology to increase rice productivity in the tropics. IRRN. 29: 10-19.

Virmani SS, Raj KG, Casal C, Dalmacio RD, Aurin PA (1986). Current knowledge of and outlook on cytoplasmic-genetic male sterility and fertility restoration in rice. In: IRRI, ed. Rice Genetics Proceedings of the International Rice Genetic Symposium. IRRI, Manila. Philipines. 633–648.

Virmani SS, Viraktamath BC, Casal CL, Toledo RS, Lopez MT, Manalo JO (1997). Hybrid Rice Manual. International Rice Research Institute, Philippines.

Wisser RJ, Kolkman JM, Patzoldt ME, Holland JB, Yu J, Krakowsky M, Nelson RJ, Balint-Kurti PJ (2011). Multivariate analysis of maize disease resistances suggests a pleiotropic genetic basis and implicates a GST gene. PNAS 108: 7339–7344.

Yao FY, Xu CG, Yu SB, Li JX, Gao YJ, Li XH, Zhang QF (1997). Mapping and genetic analysis of two fertility restorer loci in the wild-abortive cytoplasmic male sterility system of rice (*Oryza sativa* L.).Euphytica 98: 183-187.

Yuan LP (2002). *Hybrid Rice.* China Agriculture Press, Beijing

Yui R, Iketani S, Mikami T, Kubo T (2003). Antisense inhibition of mitochondrial pyruvate dehydrogenase E1α subunit in anther tapetum causes male sterility. Plant J . 34: 57– 66.

Zhang G, Bharaj TS, Ln Y, Vinnani S, Huang N (1997). Mapping of the *Rf*3 nuclear fertility restoring gene for WA cytoplasmic, male sterility in rice using RAPD and RFLP markers. Theor. Appl. Genet. 94: 27–33.

Zhang TX, Jan CC, Miller JF, Fick GN (2002). Inheritance of fertility restoration for two cytoplasmic male sterility sources of *Helianthus pauciflorus* (rigidus) Nutt. Crop Sci. 42: 1873-1875.

Zhou X, Stephens M (2012). Genome-wide efficient mixed-model analysis for associationstudies. Nat. Genet. 44: 821–824.

Zhu L, Lu C, Li P, Shen L, Xu Y, He P, Chen Y (1996). Using doubled haploid populations of rice for quantitative trait locus mapping. Rice Genet III: 631-634. IRRI, Manila, Philippines.

Zhuang JY, Fan YY, Wu JL, Xia YW, Zheng KL (2000). Mapping major and minor QTL for rice CMS-WA fertility restoration. Rice Genet. Newsl. 17: 56–58.

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