The Ecoscan 10(1&2): 293-297, 2016 (Supplement on Rice)
AN INTERNATIONAL QUARTERLY JOURNAL OF ENVIRONMENTAL SCIENCES
www.theecoscan.com

SCREENING OF RICE VARIETIES OF KERALA FOR THE PRESENCE OF RESTORING FERTILITY (RF) LOCI USING SSR MARKERS

RAJIB DAS*, V. G. JAYALEKSHMY, LEENAKUMARY S AND RAMALING HUNDEKAR
Department of Plant Breeding and Genetics, College of Agriculture, Vellayani - 695 522
e-mail: rajeeb25@gmail.com

ABSTRACT

Molecular marker technology assists screening a large number of cultivars and breeding lines with a short period avoiding conventional test crossing. In the present investigation 13 SSR primers used to screen 21 Kerala rice varieties for the presence of R*f* gene. 5 Primers namely RM1, RM3233, RM3873, RM315 and RM443 specific to Rf3 gene having product size ranging from 111bp to 215 bp and 4 primers namely RM171, RM6100, RM228 and RM216 having product size ranging from 144 bp to 328 bp specific to Rf4 gene was found to be present in rice varieties, Remya, Jayathi, Neeraja, Aruna, Swarnaprabha, Manupriya, Annapurna, Jyoti, PTB10, Bharathy, Kanakom, PTB-32, Aiswarya, Pavizham, PTB9, Varsha, Uma and Mattatriveni. Lines Remya, PTB9 and Neeraja had four (RF3, RF4, RF6 and RF7) gene in common and varieties PTB-9, Pavizham had three gene viz., RF4, RF5, RF6. Marker RM7003 (101bp) is linked to a restorer gene Rf7, which is present in variety Remya ,PTB-9, Mattatriveni ,Neeraja and Hraswa. Among identified varieties Remya, Jayathi, Swarnaprabha, Uma, Jyothi, Bharathy, Kanakom, Pavizham, Mattatriveni and Neeraja are high yielding rice varieties of Kerala and also have restorer genes, hence they can be used in hybrid breeding programmes as restorer after field evaluation.

KEY WORDS
Hybrid seed
Restoring fertility gene
Restorer

Received : 20.10.2015
Revised : 16.11.2015
Accepted : 14.01.2016

***Corresponding author**

INTRODUCTION

After the Chinese experience of almost three decades, hybrid rice appears to be another genetic option which can bring in a mini revolution in irrigated areas of few selected countries for yet another enhancement of yield potential. Hybrid rice contribute more than 50 % of total rice production in highest rice producing country *i.e.* China. Thus, it is much important technology proved to be exploited successfully to increase production (Chauhan *et al*., 2015). Hybrid rice technology is considered as one of the promising, practical and sustainable option to break the yield ceiling witnessed in rice. Rice hybrids are cultivated in more than 50% of rice area in China (about 15 Mha out of a total of 30 Mha of rice area) and the technology is being adopted in India, Vietnam , Philippines, Indonesia, Mayanmar and Bangladesh. During 2006, hybrids were cultivated in India in around 1 million hectare and it is estimated by 2010 and 2020 hybrid will be cultivated in more than 3 and 10 million hectare, respectively (Sheeba *et al.,* 2009).

Hybrid rice technology particularly utilizing the Cytoplasm Genic Male Sterility (CMS) has now been widely adopted across several countries in Asia and USA. Hybrid rice technology is the most viable option for sustaining the global production (Alahgholipour *et al*., 2007). The most desirable requirements in hybrid rice breeding programme are highly heterotic parents for developing high yielding hybrids and a restorer with 100% restoring ability, so it is always desirable to develop and identify an efficient restorer for hybrid breeding.

Anandakumar and Subramaniam (1992) 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 multiple genes namely *Rf3, Rf4, Rf5, Rf6 and Rf7* (Yao *et al*., 1997; Zhang *et al.,* 1997; Ahmadikhah and Karlov 2006; Ahmadikhah and Alavi 2009, Bazrkar *et al.,* 2008). 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. Hence employment of molecular markers is the best strategy for discriminating maintainers from restorer lines. 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 (Ahmadikhah and Alavi, 2009).

Keeping in view the above facts the present investigation was conducted to screen rice varieties of Kerala for the presence of restoring fertility (Rf) loci using different gene specific SSR markers.

MATERIALS AND METHODS

Plant materials

In the present investigation 21 rice varieties of Kerala along with 2 reported restorers were used to screened for the presence of fertility restorer genes by SSR

markers. Genotypes were collected from two different research stations namely Moncombu and Pattambi, Kerala Agricultural University, Thrissur (Table 1).

DNA extraction and SSR analysis

DNA was extracted from fresh leaves by the Cetyl Trimethyl Ammonium Bromide method (Dellaporta *et al*. 1983). For SSR analysis a total of 13 SSR primer pairs (Table 2) were used for PCR amplification which spread across different chromosomes of rice. Each polymerase chain reaction was carried out in 25 ìl reaction volume containing 3ìl of DNA, 10X polymerase buffer B 2.5 ìl, $MgCl_2$ 1.25 μl, 1 ìl dNTPs, 1 ìl of each primer, 0.3 unit of Taq polymerase and 14.95 ìl sterile distilled water.. Thermal cycler (Eppendorf AG 22331, Hamburg, Germany) programme for PCR comprised 94^{0}C for 4 minutes for initial denaturation, followed by 36 cycles of 94^{0}C for 30 seconds, 55 to 65^{0}C for 45 seconds, 72^{0}C for 1 minute and ending up with 7 minutes at 72^{0}C for the final extension.The details of the primers and annealing temperatures are given in Table 2 The annealing temperature was adjusted based on the specific requirements of different primer combinations. PCR products thus obtained were fractionated by using horizontal gelelectrophoresis assembly by agarose gel. Agarose gel of 1.4% concentration for SSR primers were prepared by dissolving the calculated amount of agarose in 1 X TBE buffer (Sambrook *et al.,* 1989) and which were examined under the gel documentation instrument..

RESULTS

Thirteen microsatellite primers that were reported to be linked with fertility restoring genes in different chromosomal locations (chromosomes 1, 7, 10, and 12) (Bazrkar *et al.,* 2008, Nematzadeh A and Kiani G. 2010) were employed for polymorphism survey between 21 different rice varieties of Kerala (Table 2).

Analysis for Rf3 gene was performed by SSR markers viz., RM1, RM443, RM3233, RM3873 and RM315 which were reported to be linked to this gene (Bazrkar et al., 2008 and Ahmadikhah *et al.,* 2007). Analysis for Rf4 gene was performed by SSR markers viz., RM171, RM216, RM228, RM244, RM258

Table 1: Rice varieties used in this experiment

Searial No	Name of Varieties	Place of Development
1	PTB-9	RARS, Pattambi
2	PTB-10	RARS , Pattambi
3	PTB-32	RARS , Pattambi
4	Aiswarya	RARS, Pattambi
5	Annapoorna (PTB-35)	RARS, Pattambi
6	Jyothi (PTB-39)	RARS, Pattambi
7	Bharathy (PTB-41)	RARS , Pattambi
8	Swarnaprabha (PTB-42)	RARS , Pattambi
9	Mattatriveni (PTB-45)	RARS, Pattambi
10	Jayathi (PTB-46)	RARS , Pattambi
11	Neeraja (PTB-47)	RARS , Pattambi
12	Kanchana (PTB-50)	RARS , Pattambi
13	Aathira (PTB-51)	RARS , Pattambi
14	Varsha (PTB-56)	RARS , Pattambi
15	Kanakom (MO-11)	RARS , Moncombu
16	Karthika (MO-7)	RARS , Moncombu
17	Aruna (M0-8)	RARS , Moncombu
18	Remya (MO-10)	RARS , Moncombu
19	Aruna (MO-8)	RARS , Moncombu
20	Pavizham (MO-6)	RARS , Moncombu
21	Uma (M0-16)	RARS , Moncombu
	RESTORERS	
22	KMR-3R	DRR, Hyderabad
23	IR42266-29-3R	CRRI, Cuttack

Table 2: The sequence information of primer pairs used in this study

S.No	Marker	Linked gene	Chromosome No	Primer sequence	Annealing temp
1	RM1	RF3	1	F:GCGAAAACACAATGCAAAAAR:GCGTTGGTTGGACCTGAC	53.8
2	RM3233	RF3	1	F:GAAATTCGAAATGGAGGGAGAGCR:GGTGAGTAAACAGTGGTGGTGAGC	57
3	RM3873	RF3	1	F:GCTATAGACGCCTCCTCCTTATCCR:AAAGCTAGCTAGGACCGACATGC	62.1
4	RM315	RF3	1	F: CGGTCAAATCATCACCTGACR: CAAGGCTTGCAAGGGAAG	56
5	RM443	RF3	1	F: GGGAGTTAGGGTTTTGGAGCR: TCCAGTTTCACACTGCTTCG	50.7
6	RM6100	RF4	10	F: TCCTCTACCAGTACCGCACCR: GCTGGATCACAGATCATTGC	55.3
7	RM171	RF4	10	F: AACGCGAGGACACGTACTTACR: ACGAGATACGTACGCCTTTG	58.7
8	RM258	RF6,RF5, RF4,	10	F: TGCTGTATGTAGCTCGCACCR: TGGCCTTTAAAGCTGTCGC	56.7
9	Rm228	Rf4	10	F: CTGGCCATTAGTCCTTGGR: GCTTGCGGCTCTGCTTAC	52.8
10	RM216	RF4	10	F: GCATGGCCGATGGTAAAGR: TGTATAAAACCACACGGCCA	56.5
11	RM244	RF6	10	F: CCGACTGTTCGTCCTTATCAR: CTGCTCTCGGGTGAACGT	56.7
12	RM591	RF6	10	F: CGGTTAATGTCATCTGATTGGR: TTCGAGATCCAAGACTGACC	55.3
13	RM7003	RF7	12	F:GGCAGACATACAGCTTATAGCR:TGCAAATGAACCCCTCTAGC	56.5

Table 3: Molecular Screening of rice varieties for Restoring fertility (R*f*) gene

Primers	LINKED GENE	Allele size	Lines
RM1	RF3	113bp	Remya,Jayathi, Neeraja,Aruna
RM3233		111bp	Jayathi,Swarnaprabha,Manupriya, Annapurna,Jyothi,Neeraja, PTB10,Aruna
RM3873		215bp	Remya,Swarnaprabha ,Bharathy kanakom,PTB-32, Neeraja
RM315		133bp	Jayathi,Swarnaprabha, Manupriya, Annapoorna,Jyothi, PTB-10 Neeraja ,Aruna
RM443		124bp	PTB-9,Jayathi, Jyothi, Aiswarya, Pavizham
RM171	RF4	328bp	Jayathi,Neeraja, Aruna
RM6100		144bp	Remya, Jayathi, Swarnaprabha, Varsha, PTB-10 ,Aruna
RM228		154bp	PTB-9,Swarnaprabha,Uma
RM216		146bp	Remya, PTB-9 ,Jayathi, Swarnaprabha, Bharathy , PTB32, Neeraja,
RM244	RF6	163bp	PTB-9, Karthika, Varsha
RM591		258bp	Remya,Jayathi ,Swarnaprabha ,Manupriya,kanakom, Varsha, Mattatriveni Neeraja Hraswa , PTB-10 .
RM7003	RF7	101bp	Remya ,PTB-9, Mattatriveni ,Neeraja, Hraswa
RM258	RF4,RF5,RF6	148bp	PTB-9,Pavizham

Table 4: Important characteristics of some of the lines having R*f* gene (high yielding) selected for screening

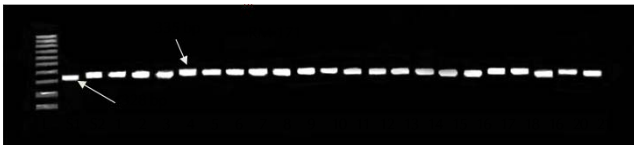
Sl No	Rice VariEties	Characteristics
1	REMYA	High yielding, grains are red long and bold, moderately tolerant to Blast and Blight, suitable for all season, Medium duration
2	JAYATHI	Dwarf high yielding ,grains are white, resistant to BPH, Green Leaf Hopper, Leaf folder and Blast.
3	UMA	High yielding, dwarf, nonlodging type.Resistant to BPH and gallmidge. Medium bold red kernelled.
4	JYOTHI	Dwraf high yieldin, grains are red medium and bold , short duration
5	BHARATHY	Grains are red long and bold, tolerant to BPH, moderately tolerant to blast, suitable to dry season
6	KANAKOM	High yielding, Medium red and bold seed,resistant to BPH, medium resistant to stem borer and resistant to rice tungro, suitable for 3 season.
7	PAVIZHAM	High yielding, Red short and bold grains, Fairly resistant to Brown plant hopper. Moderately resistant to stack burn and sheath rot and fairly resistant to sheath blight
8	MATTATRIVENI	Dwarf high yielding. Red medium and bold seeds, Tolerant to Brown plant hopper
9	NEERAJA	Semitall, high yielding and White grain, Moderately resistant to leaf folder, resistant to blast, suited to flood prone and waterlogged areas
10	ANNAPOORNA	Dwarf high yielding. Red short and bold seed,
11	ARUNA	High yielding, Grains are red long and bold, Tolerant to BPH, medium resistant to stem borer and Sheath root, short duration

and RM6100 which were reported to be linked to Rf4 gene (Huang et al., 2003).Analysis for Rf5 and Rf6 was done by RM258 and RM591 respectably which were reported to be linked to these genes (Ahmadikhah et al., 2007, Sheeba et al., 2009). The SSR markers exhibited polymorphism among the tested varieties (Fig. 1)

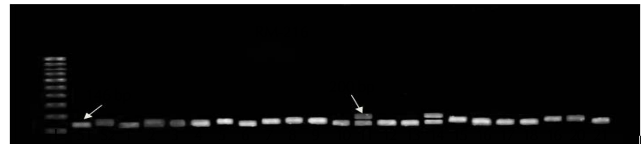
Rice varieties, Remya, Jayathi, Neeraja, Aruna, Swarnaprabha, Manupriya, Annapurna, Jyothi, PTB10, Bharathy, Kanakom, PTB-32, Aiswarya, Pavizham, PTB9, Varsha, Uma, Mattatriveni produced allele size were having common gene RF3 and RF4.Three lines *viz*., Remya, PTB9 and Neeraja had four (RF3, RF4, RF6 and RF7) gene in common and varieties PTB-9, Pavizham had three gene *viz*., RF4, RF5, RF6 (Table 3). Marker RM7003 is linked to a restorer gene Rf7(101 bp) (Bazrkar et al., 2008) which is present in variety Remya ,PTB-9, Mattatriveni ,Neeraja, Hraswa.Variety PTB-9,Pavizham had shown presence of RF5 gene. Primer RM1 has 3 alleles having the size of 113, 100 and 200 and primer RM244 has 3 d alleles having size of 163, 170 and 100. Remaining all the primers has ampolified 2 amplicons.

DISCUSSION

Hybrid breeding based on CMS/*Rf* system achieved great success worldwide (Cao and Zhan, 2014) At least 90% of the rice hybrids use the wild abortive cytoplasmic source (Yao et al, 1997). Molecular marker technology assists screening a large number of cultivars and breeding lines with in a short period and avoiding test crossing, thus saving resources and time. Bazrkar et al in the year 2008 used SSR markers linked to fertility restorer genes (R*f3* and *Rf4*) for identification of potential restorer lines for WA-CMS system, thus avoiding routine testcrossing. Sheeba *et al*. (2009) reported that the selection accuracy in a set of 21 restorer lines with RM6100 from *Rf4* was 94.4%. RM1, RM3233, RM3873, RM315, RM443and RM171, RM6100, RM228, RM216 markers were used for sreening RF3 and Rf4 gene respectively. Rice varieties *viz*., Rice varieties, Remya, Jayathi, Neeraja, Aruna, Swarnaprabha, Manupriya, Annapoorna, Jyothi, PTB10, Bharathy, Kanakom, PTB-32, Aiswarya, Pavizham, PTB9, Varsha, Uma and Mattatriveni had shown presence of markers RM1, RM3233, RM3873, RM315, RM443, RM171, RM6100, RM228 and RM216. Thsese primers has been used earlier by Bazrkar *et al.,* 2008 , Singh *et al*., 2014 and El-Namaky *et al*., 2016 for screening of rice varieties for the presence of Rf3 and Rf4 gene. Three lines *viz*., Remya, PTB9 and Neeraja found to be carrying Rf3,Rf4,Rf6 and Rf7. Rice varieties Remya ,PTB-9, Mattatriveni ,Neeraja, Hraswa were found to carry Rf7 gene .

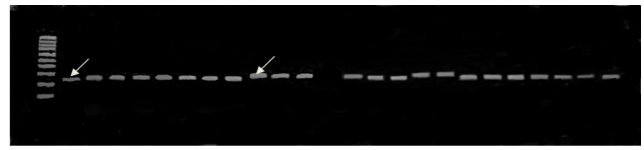


L -100bp, S1-KMR, S2-293R, 1) PTB-9, 2) Remya, 3) Jayanthi, 4) Swarnaprabha, 5) Manupriya, 6) Bharathy, 7) Uma, 8) Annapoorna, 9) Jyothi, 10) Karthika, 11) Kanakom, 12) Varsha, 13) PTB-32, 14) Kanchana, 15) Mattatriveni, 16) Neeraja, 17) Hraswa, 18) PTB-10, 19) Aruna, 20) Aiswarya, 21) Pavizham

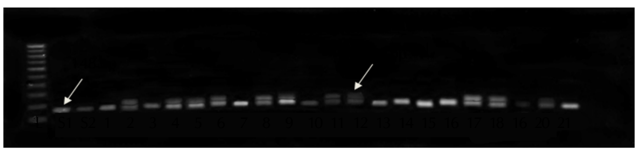


L -100bp, S1-KMR, S2-293R, 1) PTB-9, 2) Remya, 3) Jayanthi, 4) Swarnaprabha, 5) Manupriya, 6) Bharathy, 7) Uma, 8) Annapoorna, 9) Jyothi, 10) Karthika, 11) Kanakom, 12) Varsha, 13) PTB-32, 14) Kanchana, 15) Mattatriveni, 16) Neeraja, 17) Hraswa, 18) PTB-10, 19) Aruna, 20) Aiswarya, 21) Pavizham

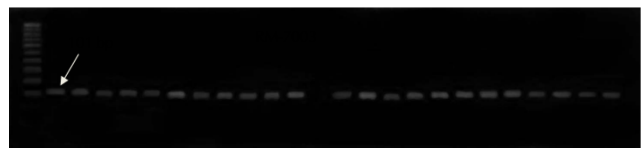
L -100bp, S1-KMR, S2-293R, 1) PTB-9, 2) Remya, 3) Jayanthi, 4) Swarnaprabha, 5) Manupriya, 6) Bharathy, 7) Uma, 8) Annapoorna, 9) Jyothi, 10) Karthika, 11) Kanakom, 12) Varsha, 13) PTB-32, 14) Kanchana, 15) Mattatriveni, 16) Neeraja, 17) Hraswa, 18) PTB-10, 19) Aruna, 20) Aiswarya, 21) Pavizham



L -100bp, S1-KMR, S2-293R, 1) PTB-9, 2) Remya, 3) Jayanthi, 4) Swarnaprabha, 5) Manupriya, 6) Bharathy, 7) Uma, 8) Annapoorna, 9) Jyothi, 10) Karthika, 11) Kanakom, 12) Varsha, 13) PTB-32, 14) Kanchana, 15) Mattatriveni, 16) Neeraja, 17) Hraswa, 18) PTB-10, 19) Aruna, 20) Aiswarya, 21) Pavizham



L -100bp, S1-KMR, S2-293R, 1) PTB-9, 2) Remya, 3) Jayanthi, 4) Swarnaprabha, 5) Manupriya, 6) Bharathy, 7) Uma, 8) Annapoorna, 9) Jyothi, 10) Karthika, 11) Kanakom, 12) Varsha, 13) PTB-32, 14) Kanchana, 15) Mattatriveni, 16) Neeraja, 17) Hraswa, 18) PTB-10, 19) Aruna, 20) Aiswarya, 21) Pavizham



L -100bp, S1-KMR, S2-293R, 1) PTB-9, 2) Remya, 3) Jayanthi, 4) Swarnaprabha, 5) Manupriya, 6) Bharathy, 7) Uma, 8) Annapoorna, 9) Jyothi, 10) Karthika, 11) Kanakom, 12) Varsha, 13) PTB-32, 14) Kanchana, 15) Mattatriveni, 16) Neeraja, 17) Hraswa, 18) PTB-10, 19) Aruna, 20) Aiswarya, 21) Pavizham

Figure 1: Molecular assay using markers for *Rf* genes in rice lines

Bazrkar *et al.,* 2008 reported marker RM 7003 linked to Rf7 gene in Rice. The results revealed that total 18 lines had *Rf4* and 45 lines had *Rf3* and *Rf4* alleles, similar work has been done by Moukoumbi *et al.*, 2016 where they have identified total 65 lines having both *Rf3* and *Rf4* alleles out of 300 lines.

Among the selected varieties some varieties (Table 4) gives higher yield in Kerala condition,which is also been reported by Rrosamma *et al.*, 2003 These high yielding semi dwarf rice varieties inspire farmers to enhance the coverage cultivable rice land under high yielding varieties and thereby boosting productivity. Its also been found these varieties are resistant to different biotic and abiotic stresses which can be used as a source of resistant. Red and bold kernelled rice is most preferred rice among Kerala farmers (Leenakumari *et al*., 1998). Rrosamma *et al*., 2003 also repoted that these varieties (Table 4) have red and bold kernelled grain.

Rice varieties Remya, Jayathi, Swarnaprabha, Uma, Jyothi, Bharathy, Kanakom, Pavizham, Mattatriveni, Annapoorna and Aruna are high yielding and have bold red kernelled seed which local farmers likes very much (Rrosamma *et al*., 2003 and Leenakumari *et al*., 1998) can further be used as parental line in breeding programme. In the present study it has been observedrestoring fertility gene (R*f*) gene is present in the rice varieties *viz*., Remya, Jayathi, Neeraja, Aruna, Swarnaprabha, Manupriya, Annapoorna, Jyothi, PTB10, Bharathy, Kanakom, PTB-32, Aiswarya, Pavizham, PTB9, Varsha, Uma and Mattatriveni. Among the identified varieties for R*f* gene Remya, Jayathi, Swarnaprabha, Uma, Jyothi, Bharathy, Kanakom, Pavizham, Mattatriveni, Annapoorna and Aruna which is of farmers choice can be used as a probable restorer in hybrid seed production programme, but only after the field evaluation of their F_1^s with A lines (CMS),to confirm their restoring ability.

REFERENCES

Ahmadikhah, A. and Alavi, M. 2009. A coldinducible modifier QTL affecting fertility restoration of WA CMS in rice. *International J. Genetics and Molecular Biology.* **1(5):** 089-093.

Ahmadikhah, A. and Karlov, G. I. 2006. Molecular mapping of the fertility-restoration gene Rf4 for WA-cytoplasmic male sterility in rice. *Plant Breeding* **125:** 363-367.

Alahgholipour, M. Rabiei, B. Hosseini, M. Dorosti, H. and Mohammadi, M. 2007. Study of General and Specific Combining Ability of Traits in Parental Lines of Hybrid Rice. *J. Agric.* (in Persian) **9:** 1-12.

Anandakumar, C. R. and Subramaniam, S. 1992. Genetics of fertility restoration in hybrid rice. *Theoretical and Applied Genetics.* **83:** 994-996.

Bazrkar, L., Ali, A. J., Babaeian, N. A., Ebadi, A. A., Allahgholipour, M., Kazemitabar, K. and 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.

Cao, L. Y. and Zhan, X. D. 2014. Chinese experiences in breeding threeline, two-line and super hybrid rice. *In*: Yan W G, Bao J S. Rice: Germplasm, Genetics and Improvement. *Rijeka, Croatia: InTech*: 279-308.

Chauhan, D. A. PATEL, R. R. and Bhimani, G. J. 2015. Heterosis on grain yield and qualitative traits in hybrid rice (*oryza sativa l.).The Ecoscan.* **9(1&2):** 499-502.

Dellaporta, R. P. Wood, J. and Hicks, J. D. 1983. A plant DNA

minipreparation: version II. *Plant Molecular Biology Reports*. **1:** 19-21.

El-Namaky, R., Sedeek, S., Dea Moukoumbi, Y., Ortiz, R. and Manneh, B. 2016. Microsatellite-Aided Screening for Fertility Restorer Genes (*Rf*) Facilitates Hybrid Improvement. *Rice Science*. **23(3):**

Huang, J. Hu, J. Xu, X. Li, S. Yi, P. Yang, D. Ren, F. Liu, X. and Zhu, Y. 2003. Fine mapping of the nuclear fertility restorer gene for HL cytoplasmic male sterility in rice. B*otanical Bulletin- Academia Sinica Taipei*. **44:** 285-289.

Jing, R., Li, X., Yi, P. and Zhu, Y. 2001. Mapping Fertility Restoring Genes of Rice WA Cytoplasmic Male Sterility Using SSLP Markers. *Bot. Bull. Acad. Sin*. **42:** 167-171.

Leenakumari, S., Valarmathi, G., Tessy Joseph., Knakamony, M. T. and Nayar, N. K. 1998. Rice Varieties of Kerala as restorers and maintainers for wild abortive cytoplasmic male sterile lines. *Int. Rice Res. Notes*. **22(2):** 11-12.

Rosamma. S. Elsy, C. R. Balachandran, P. V. And Francies, R. M. 2003. Technical Bulletin, Pattambi Rice Varieties. *RARS, Pattambi, Kerala Agricultural University*.

Mackill, D. J. and Ni, J. 2001. Molecular mapping and marker assisted selection for major-gene traits in rice. In: Khush GS, Brar DS, Hardy B, eds. Rice genetics IV. Los Baños (Philippines), Int. *Rice Res. Inst*. pp. 137-15.

Moukoumbi, Y. D. Ortiz, R. and Manneh, B. 2016. Microsatellite-aided screening for fertility restorer genes (Rf) facilitates hybrid improvement. *Rice Science*. **23(3).**

Nematzadeh, A and Kiani, G. 2010. Genetic analysis of fertility restoration genes for WA type cytoplasmic male sterility in Iranian restorer rice line DN-33-18. *Afr. J. Biotech*. **9:** 6273-6277.

Sambrook, J. Fritsch, E. F. and Maniatis, T. 1989. *Molecular Cloning: a laboratory manual. 2nd ed. N.Y., Cold Spring Harbor Laboratory, Cold Spring Harbor Laboratory Press*. pp.16-59.

Sheeba, N. K., Viraktamath, B. C., Sivaramakrishnan, S., Gangashetti, M. G., Pawan, K. and Sundaram, R. M. 2009. Validation of molecular markers linked to fertility restorer gene(s) for WA-CMS lines of rice. *Euphytica*. **167:** 217-227.

Singh,A. K. Revathi, P. Pavani, M. Sundaram, R. M. Senguttuvel, P. K. B.Kemparaju, Hari Prasad, A. S. Neeraja , C. N. Sravan Raju, N. Kotewara Rao, P. Suryendra, P. J. Jayaramulu, K. and Viraktamath, B. C. 2014. Molecular Screening for Fertility Restorer Genes *Rf3* and *Rf4* of WA-CMS and Evaluation of F1 hybrids in Rice (*O. sativa* L.). *J. Rice Research*. **7:** 1-2.

Virmani, S. S. Sun, Z. X. Mou, T. M. Jauhar, A. A. and Mao, C. X. 2003. Two-line Hybrid rice breeding manual. *Los Baños (Philippines), International Rice Research Institute*.

Yao, F. Y. Xu, C. G. Yu, S. B. Li, J. X. Gao, Y. J. Li, X. H. and Zhang, Q. F. 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.