*J. Agr. Sci. Tech. (2015) Vol. 17: 1931-1938*

## RESEARCH NOTES

# Validation of SSR Markers Linked to Restoring Fertility (*Rf*) Genes and Genotyping of Rice Lines at *Rf* Loci

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## ABSTRACT

**The present study was carried out with the objective of validating linked SSR markers to *Rf* genes and adopting Marker Assisted Selection (MAS) for restorer/non-restorer line detection in Wild Abortive (WA) type of Cytoplasmic Male Sterility (CMS). Twelve SSR markers reported to be linked to *Rf* genes were analyzed in the mapping population of NedaA/Pajouhesh. Among these, three markers, namely, RM258, RM171, and RM3148 proved to be associated with *Rf* genes. In this study, on a set of rice lines including 2 restorers, 4 maintainers, and 9 conventional varieties (totally 15 genotypes of rice), MAS with RM258 and RM171, a major *Rf* locus on chromosome 10, and RM3148, another *Rf* locus on chromosome 1, both of the *Rf* alleles in Hashemi and Deylamani varieties were amplified the same as restorer lines. However, Pouya, Khazar, and Shastak had one *Rf* locus (partial restorer). Cultivars Shiroudi, Tabesh, Fajr, and Shafaq were identified as non-restorer (maintainer) lines. Results demonstrated that these markers could be used for screening of genotypes to identify restorers and non-restorer lines in hybrid rice breeding programs.**


**Keywords:** Maintainer, Marker assisted selection, Restorer, Rice, WA-CMS.

## INTRODUCTION

Rice is staple food in Iran cultivated on 570,000 hectares of irrigated land and 2.4 million tons of milled rice is produced (Nematzadeh *et al.,* 2006). Iran still largely depends on rice imports amounting to 0.8 million tons each year to meet the domestic consumers' demand. Therefore, hybrid rice with an average 20-25% higher grain yield over conventional varieties seems to be a viable option to enhance the production and productivity levels, since the area under rice cultivation cannot be further increased due to water shortages (Nematzadeh *et al.,* 2006).

Cytoplasmic-genetic Male Sterility (CMS) combined with a fertility restoration system has been found to be the most efficient genetic tool to exploit hybrid vigor on a commercial scale in rice (Lin and Yuan, 1980; Virmani and Wan, 1988). Wild Abortive (WA) is a widely used CMS source that accounted for approximately 90% of the rice hybrids produced in China and 100% of the hybrids developed outside China (Sattari *et al.,* 2008).

The inheritance of fertility restoration in the WA-CMS system has been extensively investigated and genetic analyses have made it clear that two major genes are generally involved (Govinda Raj and Virmani, 1988; Bharaj *et al.,* 1991, 1995; Teng and Shen, 1994). Using RFLP markers, Zhang *et al.* (1997) mapped one of the two *Rf* loci (*Rf3*) on chromosome 1 between RG140 and

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RG532 at a distance of 1.9 cM from each other. Using RAPD and RFLP markers, Yao *et al.* (1997) confirmed the location of *Rf3* on chromosome 1 and mapped the second *Rf* locus (*Rf4*) on chromosome 10 at 3.3 cM from G4003. Jing *et al.* (2001) mapped *Rf4* governing fertility restoration on the long arm of chromosome 10 using SSLP markers. Zhang *et al.* (2002) also mapped the *Rf4* gene on chromosome 10 at 0.9 cM from the marker Y3-8 and anchored to the RFLP marker S10019. Bazrkar *et al.* (2008) tagged four *Rf* genes for WA –CMS system using SSR markers on chromosomes 1 (*Rf3*), 7 (*Rf4*), 10 (*Rf6*) and 12 (*Rf7*) by recessive class analysis.

Attempts were made to use these two major *Rf* genes (*Rf3* and *Rf4*) for Marker Assisted Selection (MAS) to identify restorer lines possessing *Rf* genes for WA-CMS to expedite phenotype-based screening. Ichikawa *et al.* (1997) proposed a simple PCR-mediated system for the selection of rice lines containing the *Rf1* gene. In the study of Prakash (2003), microsatellite marker RM6100 located on chromosome 10 was identified as closely linked to *Rf* gene at a distance of 7 cM. Assessment of this marker for utilization in identification of restorers with a set of 175 lines showed high accuracy of 97.4% in identifying restorer lines.

Microsatellite (RM6100/RM25654) and GC based marker systems (TMPPR3) were also evaluated for their selection efficiency by Sheeba *et al.* (2009) and Ngangkham *et al.* (2010). Suresh *et al.* (2012) validated selection efficiency of SSR markers in a set of restorer lines. They reported DRCG-RF4-14/DRCG-RF4-8 for the *Rf4* locus and DRRM-RF3-5/DRRM-RF3-10 for the *Rf3* locus, with the maximum efficiency of 92 percent for identification of restorers.

The markers which have been reported to be linked to the *Rf* genes have not been validated in alternate populations and have not been characterized for their allelic status with respect to these markers. Therefore, the present study was undertaken to validate linkage of these markers in mapping populations involving fertility restorer lines Pajouhesh for WA-cytoplasm and to analyze the allelic status of the closely linked markers in a set of rice lines used for hybrid rice breeding in Iran.

## MATERIALS AND METHODS

### Plant Materials and Population Development

Plant materials used in this study are presented in Table 1. In this study, CMS-WA line 'Neda A' was crossed with restorer line 'Pajouhesh' and $F_1$ seeds were obtained. The $F_2$ population of this cross, including 328 plants, was used for genetic mapping of the restorer gene and validation of the candidate restorer gene-based markers. 'Pajouhesh' (Nematzadeh *et al.,* 2010) improved through pedigree-backcross method from the cross between 'Sepidroud' and 'Sang Jo'. 'Neda A' is a CMS line with WA type of cytoplasm improved through backcross using 'IR58025A'. Also, two known restorers, namely, Pajouhesh and Sepidroud (Alahgholipour *et al.,* 2007) along with 4 maintainer lines, namely, Neda, Nemat, Dasht, and Champa and 9 conventional rice lines including Pouya,

**Table 1.** Plant materials used in this study with their pedigree and origin.

| Variety name | Pedigree | Origin |
|---|---|---|
| Neda A | Improved cultivar | Mazandaran, Iran |
| Pajouhesh | Improved cultivar | Mazandaran, Iran |
| Sepidroud | Improved cultivar | Guilan, Iran |
| Neda | Improved cultivar | Mazandaran, Iran |
| Nemat | Improved cultivar | Mazandaran, Iran |
| Dasht | Improved cultivar | Mazandaran, Iran |
| Champa | Local | Guilan, Iran |
| Pouya | Improved cultivar | Mazandaran, Iran |
| Hashemi | Local | Guilan, Iran |
| Shiroudi | Improved | Mazandaran, Iran |
| Tabesh | Improved cultivar | Mazandaran, Iran |
| Shastak | Local | Guilan, Iran |
| Fajr | Improved cultivar | Mazandaran, Iran |
| Khazar | Improved cultivar | Guilan, Iran |
| Shafaq | Improved cultivar | Mazandaran, Iran |
| Deylamani | Local | Mazandaran, Iran |

**Table 2.** List of the used SSR primer pairs with their chromosomal locations (Bazrkar *et al.,* 2008).

| SSR Marker | Chromosome | Forward primer | Reverse primer | Annealing temperature ($^0$C) |
|---|---|---|---|---|
| RM1 | 1 | gcg aaa aca caa tgc aaa aa | gcg ttg gtt gga cct gac | 55 |
| RM443 | 1 | ggg agt tag ggt ttt gga gc | tcc agt ttc aca ctg ctt cg | 55 |
| RM315 | 1 | cgg tca aat cat cac ctg ac | caa ggc ttg caa ggg aag | 55 |
| RM294 | 1 | ttg gcc tag tgc ctc caa tc | gag ggt aca act tag gac gca | 55 |
| RM3148 | 1 | gac tat tgc tcg aac act ttg | ttg tct tgc ttt ggt att tgc | 55 |
| RM6344 | 7 | aca cgc cat gga tga tga c | tgg cat cat cac ttc ctc ac | 55 |
| RM171 | 10 | aac gcg agg aca cgt act tac | acg aga tac gta cgc ctt tg | 67 |
| RM258 | 10 | tgc tgt atg tag ctc gca cc | tgg cct tta aag ctg tcg c | 55 |
| RM244 | 10 | ccg act gtt cgt cct tat ca | ctg ctc tcg ggt gaa cgt | 55 |
| RM591 | 10 | cgg tta atg tca tct gat tgg | ttc gag atc caa gac tga cc | 55 |
| RM3123 | 10 | att tcc cac aca tct cgc tg | gtg tcg ccg gtc aag aac | 55 |
| RM7003 | 12 | ggc aga cat aca gct tat agc | tgc aaa tga acc cct cta gc | 55 |

Hashemi, Shastak, Deylamani, Shiroudi, Tabesh, Fajr, Khazar, Shafaq were totally 16 genotypes used for the study of MAS efficiency using linked SSR markers.

### DNA Extraction and PCR analysis

Total genomic DNA was extracted according to Dellaporta *et al.* (1983). PCR amplification was performed using 12 linked SSR markers (Table 2) in $F_2$ population of 'Neda A'/'Pajouhesh' for validation of their association with *Rf* loci. A 25 µL mixture was prepared for the PCR assay which contained 50 ng template DNA, 2.5 µL of 10X buffer, 0.3 µL of 10 mM dNTPs, 1 µL of 50 mM $MgCl_2$, 1 µL of each primers (2 µM), and 1 unit of *Taq* polymerase. The PCR reaction was performed at 94°C for 5 minutes; then, for 35 cycles of 94°C for 1 minute; 50-67°C for 1 minute; 72°C for 2 minutes followed by 72°C for 5 minutes. PCR products were resolved by electrophoresis in 3.5% agarose gel containing 0.5 µg $mL^{-1}$ ethidium bromide. Restorers were identified by 350 and 150 bp bands using SSR marker RM171 and RM258, respectively.

### Linkage Analysis

Polymorphic primers between parents Neda A and Pajouhesh were used for linkage analysis on completely sterile plants from the $F_2$ population of Neda A / Pajouhesh. Linkage groups were assigned to the corresponding chromosomes based on SSR markers mapped by McCouch *et al.* (2002). For single-marker analysis, the recombination frequency between a positive marker and an *Rf* locus was calculated using maximum likelihood estimator (Allard, 1956), assuming that all the extremely sterile individuals were homozygous at the targeted *Rf* locus.

## RESULTS

### Polymorphism Detection

Twelve 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) were employed for polymorphism survey between the parents of 'Neda A' and 'Pajouhesh'. Microsatellite primers RM171 and RM258, both located on chromosome 10 of rice, and RM3148 on chromosome 1 showed polymorphism for *Rf4* and *Rf3* loci, respectively (Figure 1). In this study, SSR markers RM1, RM443, RM315 and RM294 did not show any polymorphism for *Rf3* located on chromosome 1.

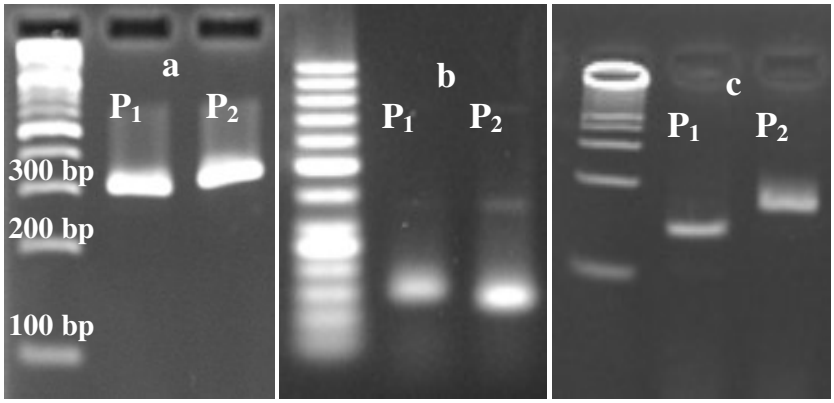


**Figure 1.** Polymorphism detection on parental lines Neda A ($P_1$) and Pajouhesh ($P_2$) using SSR markers RM171 (a), RM258 (b) and RM3148 (c). Lane 1, 100bp ladder.

### Linkage Analysis

We used polymorphic primers for linkage analysis on completely sterile plants from $F_2$ population of 'Neda A' / 'Pajouhesh' and found that RM258 and RM171 were closely linked to restorer gene *Rf4* at the intervals of 3.1 and 6.3 cM from it (Table 3). In addition, SSR marker RM3148, which was previously mapped on chromosome 1, was also proved to be linked with *Rf3* gene at a genetic distance of 19.7 cM.

### Genotyping of Rice Lines for *Rf* Loci

DNA Marker Assisted Selection (MAS) was used for WA fertility restoration in a set of 15 rice genotypes to determine the precise genotype of some rice cultivars in each marker locus. The major gene, *Rf4*, underwent MAS using SSR marker RM171 and RM258. Using these two markers, restorers were identified by 350 and 150 bp bands when resolved in an agarose gel, respectively. Results of this test in comparison with two known restorers (Sepidroud and Pajouhesh) showed that some of these genotypes, such as Pouya, Hashemi, Khazar ,and Deylamani, carried *Rf4* gene on the long arm of chromosome 10 based on presence of restorer bands with either or both of RM258 and RM171 (Table 4). MAS was used for another fertility restoring gene i.e. *Rf4*, using SSR marker RM3148 located on short arm of chromosome 1. Cultivars Hashemi, Shastak and Deylamani possess *Rf4* allele in their genome (Figure 2). This study revealed that cultivars Hashemi and Deylamani carry both of *Rf* genes considered as suspected restorers and suggested to be test crossed with CMS lines to assess the restoration of fertility in their $F_1$ offsprings.

**Table 3.** Recombination frequencies and genetic distances between the positive markers and the *Rf* locus based on the assumption that all the extremely sterile plants are homozygous for the recessive allele at targeted loci.

| Locus | Chromosome | Recombination frequency (%) | Genetic distance (cM) | LOD score |
|---|---|---|---|---|
| RM258 | 10 | 3.12 | 3.13 | 3.85 |
| RM171 | 10 | 6.25 | 6.28 | 3.19 |
| RM591 | 10 | 37.5 | 37.95 | 0.22 |
| RM3148 | 1 | 18.75 | 19.71 | 1.46 |

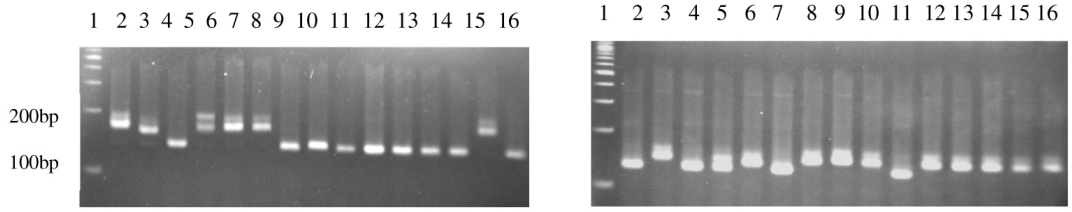


**Figure 2.** Molecular assay using markers RM258 (right) and RM3148 (left) for *Rf* genes in rice lines. Lane 1 is 100 bp ladder, lanes 2 to 16 are Pajouhesh (Restorer), Sepidroud (Restorer), Pouya, Hashemi, Shastak, Deylamani, Shiroudi, Tabesh, Fajr, Khazar, Shafaq, Neda (B-Line), Nemat (B-Line), Dasht (B-Line) and Champa (B-Line), respectively.

**Table 4.** Molecular screening of rice lines for validation of SSR markers for identification of *Rf3* and *Rf4* genes.

| | | Chromosome 10 | | Chromosome 1 |
|---|---|---|---|---|
| Line | Type | RM258 | RM171 | RM3148 |
| Sepidroud | Restorer | NR [a] | R [b] | R |
| Pajouhesh | Restorer | R | R | R |
| Neda | Maintainer | NR | NR | NR |
| Nemat | Maintainer | NR | NR | NR |
| Dasht | Maintainer | NR | NR | R |
| Champa | Maintainer | NR | NR | NR |
| Pouya | Conventional | R | NR | NR |
| Hashemi | Conventional | R | NR | R |
| Shiroudi | Conventional | NR | NR | NR |
| Tabesh | Conventional | NR | NR | NR |
| Shastak | Conventional | NR | NR | R |
| Fajr | Conventional | NR | NR | NR |
| Khazar | Conventional | R | NR | NR |
| Shafaq | Conventional | NR | NR | NR |
| Deylamani | Conventional | R | NR | R |

[a] non-restorer band, [b] restorer band,

## DISCUSSION

Genetic analysis of fertility restoring genes revealed existence of two dominant genes. One *Rf* gene was on rice chromosome 10 and the second on chromosome 1, in accordance with several other researches (Govinda Raj and Virmani, 1988; Teng and Shen, 1994). Conventionally, restorers are identified by test-crossing a large number of genotypes with CMS lines and then evaluating their progeny for pollen and spikelet fertility. This method is laborious, time-consuming, and less accurate. There is, therefore, a need to identify molecular markers that are tightly linked to *Rf* genes so that MAS can be routinely done to identify restorers more quickly and more efficiently. MAS has been successfully used for restorer gene detection by several researchers (Ichikawa *et al*., 1997; Wang *et al*., 2012; Suresh *et al*., 2012).

The result of molecular assay on Pouya cultivar revealed monogenic nature for its fertility restoration ability, which is in consistent with the findings of Bagheri and Babaeian-Jelodar (2011) who have reported a single dominant *Rf* gene for Pouya in $F_2$ and $BC_1$ segregating populations. In

addition, the result of molecular assay on Fajr cultivar revealed maintainer nature for its fertility restoration ability, which is in consistent with the findings of Nematzadeh and Sattari (2003) who have reported maintainer genomic nucleolus for that variety. MAS also showed that cultivars Shiroudi, Tabesh, Fajr, and Shafaq were non-restorer (maintainer) lines. Thus, backcross breeding method can be applied on these lines for transferring sterile cytoplasm to them for development of new CMS lines.

The results this study indicate that the microsatellite markers RM258, RM171 and RM3148 are suited for marker assisted selection of restorer lines from large source nurseries. Using these markers, primary selection can be carried out at seedling stage, hence, works required for test crosses by breeders would be reduced. Further studies are needed to confirm the efficiency of MAS through crossing between suspected restorers with CMS lines and fertility evaluation of $F_1$ plants.

## REFERENCES


1. Allard, R. W. 1956. Formulas and Tables to Facilitate the Calculation of Recombination Values in Heredity. *Hilgardia,* **24:** 235–278.
2. 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.,* **9:** 1-12. (in Persian)
3. Bagheri, N. and Babaeian-Jelodar, N. 2011. Genetics and Combining Ability of Fertility Restoration of 'Wild Abortive' Cytoplasmic Male Sterility in Rice. *Afric. J. Biotechnol.,* **10:** 9314-9321.
4. 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.
5. Bharaj, T. S., Bains, S. S., Sidhu, G. S. and Gagneja, M. R. 1991. Genetics of Fertility Restoration of 'Wild Abortive' Cytoplasmic Male Sterility in Rice, *Oryza sativa* L. *Euphytica,* **56:** 199–203
6. Bharaj, T.S., Virmani, S. S. and Khush, G. S. 1995. Chromosomal Location of Fertility Restoring Genes for 'Wild Abortive' Cytoplasmic Male Sterility Using Primary Trisomics in Rice. *Euphytica,* **83:**169–173.
7. Dellaporta, R. P., Wood, J. and Hicks, J.B. 1983. A Plant DNA Mini-preparation: Version II. *Plant Mol. Biol. Report.,* **1:** 19-21.
8. Govinda Raj, K. and Virmani, S. S. 1988. Genetics of Fertility Restoration of WA Type Cytoplasmic Male Sterility in Rice. *Crop Sci.,* **28:** 787–792.
9. Ichikawa, N., Kishimoto, N., Inagaki, A., Nakamura, A., Koshino, Y., Yokozeki, Y., Oka, M., Samoto, S., Akagi, H., Higo, K., Shinjyo, C., Fujimura, T. and Shimada, H. 1997. A Rapid PCR-aided Selection of a Rice Line Containing the *Rf-1* Gene which Is Involved in Restoration of the Cytoplasmic Male Sterility. *Mol. Breed.,* **3:** 195–202.
10. 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.
11. Lin, S. C. and Yuan, L. P. 1980. Hybrid Rice Breeding in China. In: "*Innovative Approaches to Rice Breeding*". International Rice Research Institute (IRRI), Manila, Philippines, PP. 35–51.
12. McCouch, S. R., Teytelman, L., Xu, Y., Lobos, K. B., 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. and Stein, L. 2002. Development and Mapping of 2240 New SSR Markers for Rice (*Oryza sativa* L.). *DNA Res.,* **9:** 199-207.
13. Nematzadeh, Gh. A. and Sattari, M. 2003. A Study of Nucleus Genome of Some High Yielding Rice (*Oryza sativa* L.) Varieties for Application in Hybrid Rice Technology. *Iranian J. Agric. Sci.,* **34:** 213-219. (in Persian)
14. Nematzadeh, G. A., Ali, A. J., Sattari, M., Valizadeh, A., Alinejad, E. and Nouri, M. Z. 2006. Relationship between Different Allogamic Associated Trait Characteristics of the Five Newly Developed Cytoplasmic

Male Sterile (CMS) Lines in Rice. *J. Cet. Eur. Agric.,* **7:** 49-56.
15. Nematzadeh, G. A., Oladi, M., Kiani, G., Hajipour, A, and Hashemi, S. H. R. 2010. Breeding of Fertility Restoring Variety (Pajouhesh) with Good Morpho-physiological Characteristics in Rice. *11th Iranian Crop Science Congress*, Shahid Beheshti University, Tehran, PP. 533-536.
16. Ngangkham, U., De Sandip, Singh, S. K., Parida, S. K., Raj Kumar, K. A., Singh, A. K. and Mohapatra, T. 2010. Genic Markers for Wild Qbortive (WA) Cytoplasm Based Male Sterility and Its Fertility Restoration in Rice. *Mol. Breed.,* **26:** 275–292.
17. Prakash, P. 2003. Molecular Mapping of *Fertility Restorer* Gene(s) and Validation of *Rf* Gene Linked Markers in Rice. MSc. Dissertation, Indian Agricultural Research Institute.
18. Sattari, M., Kathiresan, A., Gregorio, G. B. and Virmani, S. S. 2008. Comparative Genetic Analysis and Molecular Mapping of Fertility Restoration Genes for WA, Dissi, and Gambiaca Cytoplasmic Male Sterility Systems in Rice. *Euphytica,* **160:** 305–315.
19. 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.
20. Suresh, P. B., Srikanth, B., Hemanth Kishore, V., Subhakara Rao, I., Vemireddy, L. R., Dharika, N., Sundaram, R. M., Ramesha, M. S., Sambasiva Rao, K. R. S., Viraktamath, B. C. and Neeraja, C. N. 2012. Fine Mapping of *Rf3* and *Rf4* 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:** 421-435.
21. Teng, L. S. and Shen, Z. T. 1994. Inheritance of Fertility Restoration for Cytoplasmic Male Sterility in Rice. *Rice Genet. Newslett.,* **11:** 95–97.
22. Virmani, S. S. and Wan, B. H. 1988. Development of CMS Lines in Hybrid Rice Breeding. In: "*Hybrid Rice*". International Rice Research Institute (IRRI), Manila, Philippines, PP. 103–114.
23. Wang, X., Tan, X., Shao, G., Ma, H., Chen, Y., Zhang, C. and Shang, W. 2012. Marker-assisted Selection of Japonica Restorers Used for Yinshui CMS System. *Rice Genom. Genet.,* **3:** 19-24.
24. 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.
25. Zhang, G., Bharaj, T. S., Lu, Y., Virmani, S. S. and 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.
26. Zhang, Q. Y., Liu, Y. G. and Mei, M.T. 2002. Molecular Mapping of the *Fertility Restorer* Gene *Rf4* for WA Cytoplasmic Male Sterility. *Acta Genet. Sin.,* **29:** 1001–1004.

## اعتبار سنجی نشانگرهای SSR همبسته با ژن‌های اعاده کننده باروری (*Rf*) و تعیین ژنوتیپ لاین‌های برنج در مکان‌های ژنی *Rf*

**غ. کیانی**

### چکیده

مطالعه حاضر با هدف اعتبار سنجی نشانگرهای SSR همبسته با ژن‌های *Rf* و استفاده از انتخاب به کمک نشانگر برای تشخیص لاین‌های اعاده کننده و غیر اعاده کننده باروری در سیستم نرعقیمی

سیتوپلاسمی نوع WA انجام گرفته است. دوازده نشانگر SSR که همبستگی آنها با ژن‌های *Rf* گزارش شده است در جامعه نقشه‌کشی ندا/پژوهش مورد ارزیابی قرار گرفتند. از بین آنها سه نشانگر RM258، RM171 و RM3148 با ژن‌های *Rf* همبسته بودند. MAS با استفاده از RM258 و RM171 (مهمترین مکان ژنی *Rf* روی کروموزم ۱۰) و RM3148 (دیگر مکان ژنی *Rf* روی کروموزم ۱) روی مجموعه‌ای از لاین‌ها شامل ۲ اعاده کننده، ۴ نگهدارنده و ۹ رقم معمولی (جمعاً ۱۵ ژنوتیپ)، هر دو آلل *Rf* در ارقام هاشمی و دیلمانی مشابه لاین های اعاده کننده باروری تکثیر شدند. درحالیکه ارقام پویا، خزر و شصتک دارای یک مکان ژنی *Rf* (اعاده کننده ناقص) بودند. ارقام شیرودی، تابش، فجر و شفق به عنوان لاین‌های غیر اعاده کننده (نگهدارنده) شناسائی شدند. نتایج نشان داد که از این نشانگرها برای غربالگری ژنوتیپ‌ها به منظور شناسائی لاین‌های اعاده کننده از غیر اعاده کننده در برنامه‌های اصلاح برنج هیبرید می‌توان استفاده نمود.