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# Screening of Lines for Restoring Fertility Genes Rf3 and Rf4 with SSR Markers in Rice (*Oryza sativa* L.)

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**ABSTRACT**

*To study the identification of restorers with different molecular markers which are linked to fertility restorer genes Rf3 and Rf4 of WA-CMS system, seventy two parental lines with no information on fertility restoration were screened with the help of molecular markers linked to major fertility genes Rf3 and Rf4. The SSR primer RM6100 linked to Rf4 gene on chromosome 10 and RM10313 linked to Rf3 gene on chromosome 1 are used efficiently for identifying restorer lines in crop improvement programmes. 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*

## INTRODUCTION

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].

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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 different parental lines. 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.

We made an attempt to confirm the efficiency of previously reported markers linked to *Rf* genes namely, RM6100 and RM10313 in identifying fertility restoration trait by evaluating seventy two parental lines for identification of restoreres.

## MATERIALS AND METHODS

The seventy two genotypes were comprised the materials for present study. Those materials were grown in source nursery at IIRR, Rajendranagar in *kharif,* 2015. Total genomic DNA was isolated from young leaves by c-TAB protocol of Dellaporta *et al*.,[10]. PCR reactions was carried out using 50 ng/l of template DNA, containing 2.5 mM of each dNTP, 0.5 µl of each forward and reverse primer, 0.2 µl of Taq DNA polymerase, 10X PCR reaction buffer in a total volume of 10µl 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 stained with ethidium bromide and documented using Gel documentation system (Alpha Innotech).

## RESULTS AND DISCUSSION

Based on the banding pattern gels were scored for presence and absence of bands as restorers and non- restores. A total 72 breeding lines without prior information about fertility restoration status along with one known restorer (KMR-3) were screened with two SSR primers namely RM6100 linked to fertility restorer gene *Rf4* located on chromosome 10 and RM10313 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 72 lines screened Among the seventy two parental lines screened for the presence of the fertility restorer gene *Rf4* with RM 6100 primer forty six lines were identified to be restorers, 26 lines were non restorers. In same way for *Rf3* gene with RM10313 primer forty seven lines were identified to be restorers and twenty five were non restorer lines. Thirty eight parental lines identified to possess both the *Rf4* and *Rf3* genes (Table 1). Identification of effective restorers and maintainers are the initial steps in three line heterosis breeding. And these lines can be immediately utilized in hybrid rice breeding programme. Therefore these primers are 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% efficiency.

**Table 1: Screening results of *Rf4* (RM6100) and *Rf3* (RM10313).**

| S.No. | Genotype | RM6100 (*Rf4*) | RM10313(*Rf4*) | *Rf4* and *Rf3* |
|---|---|---|---|---|
| 1. | IR-68888B | No | No | No |
| 2. | APMS-6B | No | No | No |
| 3. | IR-80555B | No | No | No |
| 4. | IR-58025B | No | No | No |
| 5. | IR-79156B | No | No | No |
| 6. | IR-80561B | No | No | No |
| 7. | IR-68897B | No | No | No |
| 8. | PUSA-5B | No | No | No |
| 9. | CRMS-32B | No | No | No |
| 10. | TCP-960 | *Rf4* | *Rf3* | *Rf3/Rf4* |
| 11. | AR-9-21 | *Rf4* | No | *Rf4* |
| 12. | TCP-963 | *Rf4* | *Rf3* | *Rf3/Rf4* |
| 13. | AR-19-18 | *Rf4* | *Rf3* | *Rf3/Rf4* |

| | | | | |
|---|---|---|---|---|
| 14. | ABU-11-37-R | *Rf4* | No | *Rf4* |
| 15. | L2-182 | *Rf4* | *Rf3* | *Rf3/Rf4* |
| 16. | BK-49-53 | *Rf4* | *Rf3* | *Rf3/Rf4* |
| 17. | BK-49-80 | *Rf4* | *Rf3* | *Rf3/Rf4* |
| 18. | TCP-801 | *Rf4* | *Rf3* | *Rf3/Rf4* |
| 19. | TCP-650 | *Rf4* | *Rf3* | *Rf3/Rf4* |
| 20. | Akashyadhan | *Rf4* | *Rf3* | *Rf3/Rf4* |
| 21. | Varadhan | No | No | No |
| 22. | IR-40750R | No | No | No |
| 23. | TCP-657 | *Rf4* | *Rf3* | *Rf3/Rf4* |
| 24. | TCP-661 | *Rf4* | *Rf3* | *Rf3/Rf4* |
| 25. | TCP-585 | *Rf4* | *Rf3* | *Rf3/Rf4* |
| 26. | PSV-41 | *Rf4* | *Rf3* | *Rf3/Rf4* |
| 27. | RPHR-1096 | *Rf4* | No | *Rf4* |
| 28. | RPHR-1004 | *Rf4* | *Rf3* | *Rf3/Rf4* |
| 29. | VG-58 | *Rf4* | No | *Rf4* |
| 30. | TCP-643 | *Rf4* | No | *Rf4* |
| 31. | MTU-9992 | No | *Rf3* | *Rf3* |
| 32. | IB2-57 | *Rf4* | *Rf3* | *Rf3/Rf4* |
| 33. | TCP-783 | *Rf4* | *Rf3* | *Rf3/Rf4* |
| 34. | RPHR-517 | *Rf4* | *Rf3* | *Rf3/Rf4* |
| 35. | RPHR-118 | *Rf4* | No | *Rf4* |
| 36. | SG-27-7-2 | *Rf4* | *Rf3* | *Rf3/Rf4* |
| 37. | SG-26-120 | *Rf4* | No | *Rf4* |
| 38. | TCP-951 | *Rf4* | *Rf3* | *Rf3/Rf4* |
| 39. | SG-27-177 | *Rf4* | *Rf3* | *Rf3/Rf4* |
| 40. | TCP-950 | *Rf4* | *Rf3* | *Rf3/Rf4* |
| 41. | IBL-52-1 | No | *Rf3* | *Rf3* |
| 42. | SG-27-131 | *Rf4* | *Rf3* | *Rf3/Rf4* |
| 43. | NDR-3026 | *Rf4* | *Rf3* | *Rf3/Rf4* |
| 44. | TCP-3005 | *Rf4* | *Rf3* | *Rf3/Rf4* |
| 45. | TCP-432 | *Rf4* | *Rf3* | *Rf3/Rf4* |
| 46. | KMP-128 | *Rf4* | *Rf3* | *Rf3/Rf4* |
| 47. | PSV-15 | *Rf4* | *Rf3* | *Rf3/Rf4* |
| 48. | RNR21252 | *Rf4* | *Rf3* | *Rf3/Rf4* |
| 49. | RNR17422 | No | *Rf3* | *Rf3* |
| 50. | Surekha | No | *Rf3* | *Rf3* |
| 51. | PSV-49 | *Rf4* | *Rf3* | *Rf3/Rf4* |
| 52. | BPT-5204 | No | No | *No* |
| 53. | TCP-964 | *Rf4* | *Rf3* | *Rf3/Rf4* |
| 54. | RNR-21240 | No | *Rf3* | *Rf3* |
| 55. | RNR-11718 | No | *Rf3* | *Rf3* |
| 56. | Rajendra | No | *Rf3* | *Rf3* |
| 57. | TCP-795 | *Rf4* | *Rf3* | *Rf3/Rf4* |
| 58. | Erramallelu | No | No | No |
| 59. | RNR-17497 | No | No | No |
| 60. | JGL-17004 | *Rf4* | *Rf3* | *Rf3/Rf4* |
| 61. | JGL-1798 | No | No | No |
| 62. | AYT-21 | *Rf4* | No | *Rf4* |
| 63. | Anjali | *Rf4* | *Rf3* | *Rf3/Rf4* |
| 64. | TCP-718 | *Rf4* | *Rf3* | *Rf3/Rf4* |
| 65. | Vandana | *Rf4* | *Rf3* | *Rf3/Rf4* |
| 66. | NDR359 | No | *Rf3* | *Rf3* |
| 67. | ABU-10-82R | *Rf4* | *Rf3* | *Rf3/Rf4* |
| 68. | JGL-20171 | No | No | *No* |
| 69. | KMP-153 | No | No | *No* |
| 70. | KMP-175 | *Rf4* | *Rf3* | *Rf3/Rf4* |
| 71. | RNR-21245 | No | *Rf3* | *Rf3* |
| 72. | KMR-3 | *Rf4* | *Rf3* | *Rf3/Rf4* |

*Rf4* with RM 6100

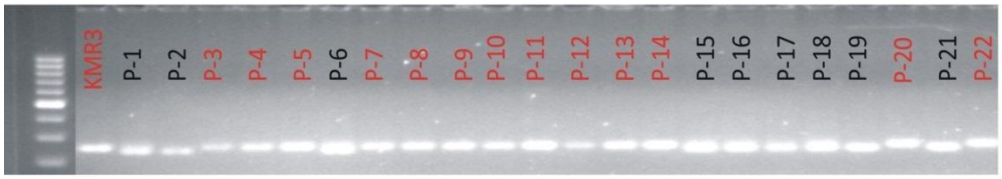


Fig.1 Parental lines confirmed with *Rf4* marker RM6100

Note: Red colour are positive plants

*Rf3* with RM 10313

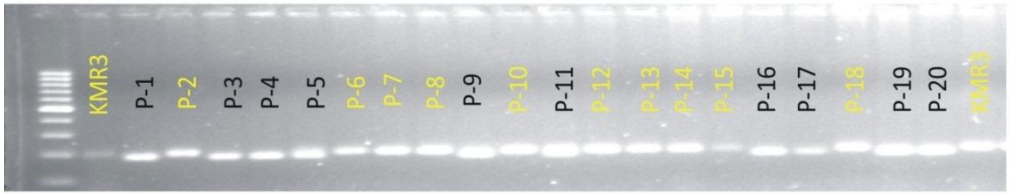


Fig.2 Parental lines confirmed with *Rf3* marker RM10313

Note: Yellow colour are positive plants

## REFERENCES

1. Virmani, S. S. and Kumar, I., Development and use of hybrid rice technology to increase rice productivity in the tropics. Int. Rice Res. Notes, **29:** 10-19 (2004).
2. Lin, S. C. and Yuan, L. P., Hybrid rice breeding in China. In: Innovative Approaches to Rice Breeding, International Rice Research Institute, Manila, pp. 35-51 (1980).
3. Li, Y. C. and Yuan, L. P., Genetic analysis of fertility restoration in male sterile lines of rice. IRRI, Manila, Rice Genetics, pp. 617-632 (1986).
4. Shinjyo , C. and Omura, T., Cytoplasmic-genetic male sterility in cultiated rice (*Oryza sativa* L.) Fertilities of F1, F2 and offspring obtained from their mutual reciprocaal backcrosses; and segregation of completely male sterile plants. *Japan. J. Breed.*, **16:** 179-180 (1966).
5. Govinda Raj, K. and Virmani, S. S., Genetics of fertility restoration of 'WA' type cytoplasmic male sterility in rice. *Crop Sci.*, **28:** 787-792 (1988).
6. Bharaj, T. S., Virmani, S. S. and Khush, G. S., Chromosomal location of fertility restoring genes for 'wild abortive' cytoplasmic male sterility using primary trisomics in rice. *Euphytica*, **83:** 169-173 (1995).
7. Zhang, G., Bharaj, T.S., Lu, Y., Virmani, S.S. and Huang, N., 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 (1997).
8. Zhang, Q .Y., Liu, Y. G. and Mei, M. T., Molecular mapping of the fertility restorer gene Rf4 for WA cytoplasmic male sterility in rice. *Acta Genet. Sin.* **29:** 1001-1004 (2002).
9. Singh, A. K., Mahapatra, T., Prabhu, K. V., Singh, V. P., Zaman, F. U., Mishra, G. P., Nandakumar , N., Joseph, M., Gopalakrishnan, S., Aparajit, G., Tyagi, N. K., Prakash, P., Sharma, R. K., Shab, U. S. and Singh, S.K., Application of molecular markers in rice breeding: progress at IARI. *In*: Advances in marker assisted selection workshop. Trainee's manual, Handouts and references (2005).
10. Dellaporta, S.L., Wood, J., Hicks, J.B., A plant DNA minipreparation version II. *Plant Molecular Biology Reporter* **1:** 19-21 (1983).