2013 International Conference on Sustainable Environment and Agriculture
IPCBEE vol.57 (2013) © (2013) IACSIT Press, Singapore
DOI: 10.7763/IPCBEE. 2013. V57. 1

Assessment of Purity of F_1 Plants Derived from the Cross of MR264 and Pongsu Seribu 2 Using Microsatellite Markers

H. Nor'Aishah [1], H. Abdul Rahim [2], A. R. Khairuddin [2], H. Sobri [2], M. N. Norain [2] and M. Z Nursamahah [2 +]

[1] Unit Biology, Faculty of Applied Science, Universiti Teknologi MARA, Malaysia
[2] Division of Agrotechnology and Bioprocess, Agency Nuclear Malaysia, Malaysia

Abstract. Microsatellite markers (SSR) are the most promising method to evaluate genetic diversity in germplasm. This study was conducted to identify the rice line of F_1 plants of MR 264 x Pongsu Seribu 2 using SSR markers. F_1 population derived from a cross between a resistant variety, Pongsu Seribu 2 and a susceptible rice cultivar, MR 264. Parental polymorphism analysis was assayed by ninety eight SSR markers and twenty one markers namely RM413, RM168, RM101, RM128, 138, RM144, RM109, RM179, RM18, RM19, RM10, MRG1022, RM187, RM167, RM148, RM120, RM72, SRF5, RM8226 and RM234 showed a reproducible and clearly band and selected to evaluate F_1 plants. Out of twenty one markers, only RM 413 successfully distiguish two parents clearly with specific bands electrophoresed in 3 % agarose gel. In respect, twenty lines were identified as F_1 plants. Thus, these markers could be efficiently used in quantitative trait loci mapping and the selected F_1 could be used for blast rice disease.

Keywords: Microsatellite, MR 264, polymorphism, Pongsu Seribu 2, SSR markers

1. Introduction

Rice or scientifically known as *Oryza sativa* L. is a staple food for the majority for the world population. In Malaysia rice are consumed approximately 1.95 million tonne and this number are estimated to increase every year significantly with the increasing number of population [1]. Nowadays, consumer are demand for a quality rice as rice are the primary source of carbohydrate. Therefore research are crucial to improve rice quality in order to carter the consumer demands. Some recent study [2], verified that the qualities of rice depands on the flavour, frangrance and the texture. With the greater development in majority Asian country, production of quality rice is important and researcher are browsing on genetic analysis to increase the morphology and physiology of rice [3]. Before the use of molecular marker, quality of rice were determine by using conventional method. However, it had many limitation in terms of more time and labour is required if the sample are big and it also not very reliable. On the other hands, molecular marker technologies provides an opportunity in reproduction of rice and information on genome structure. The application of molecular marker will provide information on variation among closely related species that exists in a particular species within a local region as well as among different countries [4]. They serve as a valuable guide for effective collection and use of genetic resources too. Molecular data would provide a basis for better management and conservation of the collection and could be used as reference for its enhanced use in breeding programs. Presently, molecular characterization has high potential to help in the introduction of blast resistant rice cultivars thereby sustaining rice yields.

+ Corresponding author. Tel.: + 60126922863 fax: +6064832449.
E-mail address: aishahng@ns.uitm.edu.my

Among molecular markers available, microsatellite or simple sequence repeat (SSR) are well-known potential to provide more information and useful in molecular technology. SSR is a subset of the satellite DNA containing short DNA sequence that consist many repetitions and varies greatly that making it very valuable as genetic marker [5]. According to previous study [6], there is over 500 microsatellite markers have been developed and used for mapping genetics in rice. Characterization and genetic mapping of SSR markers is not only to identify markers in close proximity to target genes but also to assess germplasm and breeding program material. Many studies have been reported the application of SSRs marker to analyze diversity [7], [8], to locate genes and quantitative trait loci (QTLs) on rice chromosome [9]-[11], to analyze the blast disease in rice cultivar [12] and to identify the salt tolerant rice lines [13]. Expected to rapidly increase the number of SSR markers spread in rice genomics, will provide a useful resource for various applications in the field of genetics and breeding. Therefore this study was conducted to identify the rice line of F_1 progenies of MR264 and Pongsu Seribu 2 using SSR marker. The result of this study may be further strengthening the recommendation for the use of selected SSR marker for blast analysis in F_2 which will contribute to achieve self-sufficiency in rice production in Malaysia.

2. Materials and Methods

2.1. Plant materials

Seeds (Pongsu Seribu 2, MR 264 and F_1) were obtained from Agrotechnology and Bioprocess Divison, Malaysian Nuclear Agency, Bangi, Malaysia. F_1 seeds were collected from individual plants (derived from cross of Pongsu Seribu 2 x MR264). All the seeds were pre-greminated by soaking in water at 25^{o}C for 48h and then were grown at the Greenhouse.

2.2. DNA extraction

Total genomic DNA was extracted from young and fresh leaves using CTAB method. 1-2g leaves were ground in liquid nitrogen and quickly transferred to 15 ml preheated CTAB (Cetyltrimethylammonium bromide) extraction buffer in Sorvall-centrifuge tube prior to incubate at 60^{o}C for an hour. Then, an equal volume of chloroform-isoamylalcohol 24:1 (v/v) was added to the mixture, prior to centrifuge (Centrifuge 5810, Eppendoft, Germany) at 5000 rpm for 10 minutes at room temperature. After centrifuged, the mixture was separated into two aqueous layers. The top aqueous layer was then transferred to a new 50 ml Falcon tube prior to add 0.6 volumes of cold (-20 ℃) isopropanol. The mixture was gently inverted and incubated on ice for 30 minutes. DNA was spooled out from the tube, transferred to a new micro-centrifuge tube and centrifuge at 12000 rpm for 1 minute. The DNA was washed by adding 100µl of cold 70% ethanol followed by a quick centrifugation and gently removed the supernatant prior to dry the DNA pellet at 37 ℃ for 10 minutes. Dried DNA pellet was re-suspended in 1xTE buffer (approximately 250-500 µl) and leave by overnight at 4^{o}C to dissolve the DNA.

2.3. DNA purification

RNA was removed by adding 2 µl (10 µg/ml) RNAse into dissolved DNA, and incubated for 30 minutes at 37^{o}C. The DNA was re-precipitated by adding 1/10 volume sodium acetate 3M (pH6.8) followed by two volumes of 70% ethanol. The mixture was incubated on ice for 30 minutes prior to centrifuge the mixture at 13000 rpm, room temperature for 1 minute. The DNA pellet was then dried at 37^{o}C for 10 minutes and re-suspended in 250µl 1xTE buffer by overnight incubation at 4^{o}C to dissolve the DNA. In quantifying, the DNA was measured at OD260 by Nano-Drop spectrophotometry (ND1000 Spectrophotometer). Assessment was made in concentration of ng/ul while the DNA quality was checked by DNA gel separation in 1% agarose gel electrophoresis using 1xTAE buffer at 5-8 V/cm for 30 minutes. The separation of the DNA fragments was visualized under UV light by the addition to the gel mixture of 0.1 µg/ml ethidium bromide.

2.4. PCR amplification using microsatellite markers

PCR as described in Archak et al. (2007) with some modification. A total of 98 of SSR markers were tested to determined the discriminatory and stability of F_1 plants. The PCR assay was performed in a 25 µl volume containing 5 µl of 5× PCR buffer, 0.5 µl of 10 mmol l−1 dNTPs (Promega, Madison, USA), 0.1 µl of 1.5 units of Taq DNA polymerase (Promega, Madison, USA), 10.4 µl of sterile ultrapure deionized water, 2

µl of 50 ng DNA template and 4.0 µl of 10 pmol µl of each primers (forward and reverse). A negative-DNA control was performed by adding 1 µl of sterile deionized water. Amplification was performed in personal Eppendorf thermal-cycler (Eppendorf, Germany) with a temperature program consisting of the initial denaturation at 94 ℃ for 5 minutes followed by 30 cycles of denaturation at 94 ℃ for 1 minute, annealing for 1 minute at different temperature (55 ℃, 61 ℃ and 67 ℃) and polymerization at 72 ℃ for 2 minutes. Final elongation was at 72 ℃ for 7 min. The amplification products were analyzed by electrophoresis in a 3.0 % agarose in 1xTBE at 100 V for 1 hour. Gels were stained with ethidium bromide. The amplified fragments were visualized with UV transilluminator (Syngene, USA). The 100 bp DNA ladder (Promega, USA) was used as a DNA size marker.

3. Results and Discussions

Identification of cultivar are crucial to varietal improvement and in the breeding programme. It is mandatory to maintain the genetic purity of seed for the successful crop production. According to recent study [14], uequivocal of characteristic pattern of progenies can be obtained using DNA markers and it has distinct advantages over morphological and biochemical methods. Morphological markers are influenced by the environment conditions, labour intensive time consuming while biochemical markers such as isozyme and protein profiles exhibit limited polymorphsim and often do not allow discrimination between closely related inbred lines. On the other hands, DNA markers overcomes most of these disadvantages and useful to distiguish progenies, its parental lines and off-types. According to previous study [15], usefulness of DNA fingerprinting technique for cultivar identification in rice was reported for the first time. The present study utilized the SSR marker technique for identification of 32 of F_1 population with its parental lines, it has proved that this technique can be successfully applied to distinguish and identify the progenies from their parental lines.

3.1. Genomic DNA

A total genomic DNA was successfully extracted and based on the results, isolated DNA of both parent and F_1 plants demonstrated a clearly and reproducible bands. NanoDrop analysis demonstrated all the DNA extracted consists > 1.8 ng/µL which indicated a good quality of DNA samples.

3.2. Parental polymorphism analysis

Currently, SSR marker is the preferred molecular marker for purity identification in some crops [16] due to its highly desirable properties; high efficiency and simplicity. In this study, polymorphism of 98 SSR primers was examined in two parents and F_1 plants. Out of 98 SSR primers, only 21 SSR primers namely RM413, RM168, RM101, RM128, 138, RM144, RM109, RM179, RM18, RM19, RM10, MRG1022, RM187, RM167, RM148, RM120, RM72, SRF5, RM8226 and RM234 showed a positive result on 3% of agarose gel. Others SSR markers showed a negative results. Although the PCR products were appeared on agarose, they failed to show any polymorphism among parental lines. Probably the differences in the base pair sizes are too small to resolve and identify on the agarose. Some studies [17], reported that the primers which were not able to resolve and show polymorphism in agarose gel were found clearly resolving in polyacrylamide gel of six per cent.

Table 1: List F_1 plants analysis using SSR marker, RM 413.

Results	Total	F_1 plants
Total F_1 plants similar with donors (MR 264 and Pongsu Seribu 2)	20	2, 3, 5, 8, 9, 10, 11, 12, 13, 14, 15, 16, 18, 20, 24, 25, 26, 27, 30, 32
Not Detected	12	1, 4, 6, 7, 17, 19, 21, 22, 23, 28, 29, 31

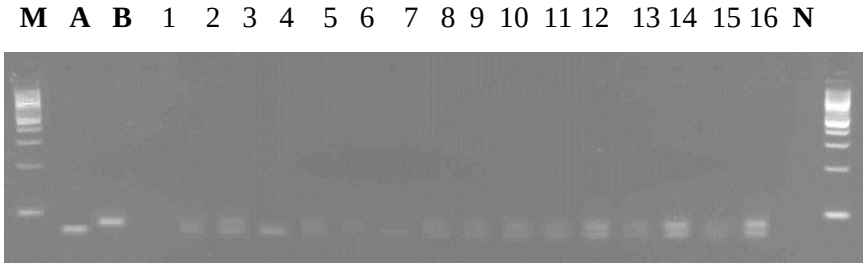

Fig. 1(a): Marker banding patterns in an F_1 population derived from Pongsu Seribu 2 × MR 264 for SSR marker; RM 413 electrophoresed on 3% agarose gel. Lane M: 100 bp DNA ladder; Lane A: MR 264; Lane B: PS2; Lanes 1 -16: progenies; Lane N: Negative control.

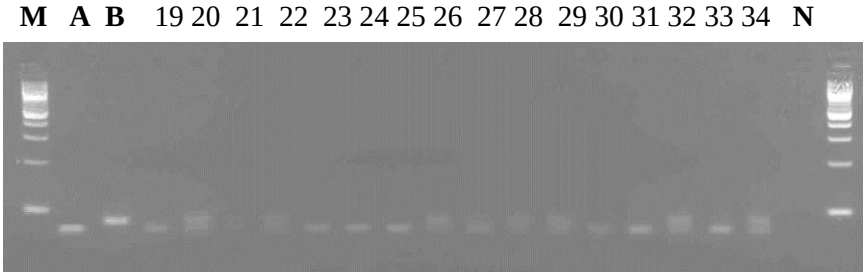

Fig. 1(b): Marker banding patterns in an F_1 population derived from Pongsu Seribu 2 × MR 264 for SSR marker; RM 413 electrophoresed on 3% agarose gel. Lane M: 100 bp DNA ladder; Lane A: MR 264; Lane B: PS2; Lanes 3 -16: progenies; Lane N: Negative control.

3.3. Evaluation on F_1 population

The positive polymorphic markers were screened for purity analysis in F_1 population. In this study, F_1 plants exhibited a wide variation with primer RM 413. A total of 20 F_1 plants (Table 1), demonstrated positive result indicated that there were 2 bands similar to donor; MR 264 and Pongsu Seribu 2. Because SSR markers are codominant, two alleles (one allele per parent) were present in a progenies when polymorphism was detected between the male parent and female parent [18]. Figure 1(a) and Figure 1(b) showed the clearly band in F_1 plants. This result was similar with the study by [12], for identification of rice lines of F_1 progenies; Pongsu Seribu 2 x Mahsuri using RM413 SSR markers. Therefore tested markers could be efficiently used to identify F_1 lines in rice and also can be used in marker assisted selection (MAS) for breeding quantitative trait loci (QTL) mapping and gene pyramiding in rice breeding. Further investigation needed to further backcrossed for the development of F_2 population and used for the beter understanding for plant breeders.

4. Conclusion

RM 413 SSR marker successfully identify 32 F_1 plants derived from cross of MR 264 and Pongsu Seribu 2 and it could also be used in marker assisted breeding for quantitative trait loci (QTL) mapping.

5. Acknowledgement

The authors are grateful to Universiti Teknologi MARA and Malaysian Nuclear Agency for the facilities, support and financial aids during the research work. Research was supported by ScFund MOSTI (06-03-01-SF0110).

6. References

[1] MARDI. Commercial production of high quality rice MRQ 74. (Online). Available at http://www.mardi.my. 2007.

[2] R.A. Henry, and S.A. Knott. A simple regression method for mapping quantitative trait loci in line crosses using flanking markers. *Heredity*, 2006, **69:** 315–324.

[3] S. Chen, J. Wu, Y. Yang, W. Shi and M. Xu. The *fgr* gene responsible for fragrance was restricted within 69 kb.

Plant Science, 2006. **171**: 505-514.

[4] C.J. Bergman, A.M. McClung, S.R. Pinson and R.G. Fjellstrom. Development of PCR-Markers Associated with Cooked Rice Kernel Elongation and Aroma. *Proc. of Rice Technical Working Group Meeting*: 2006, **53**: 536-540.

[5] J.M., Brondani, J.M., Bandong, Y.H., Lee, and E.J. Lee, 2001. Race-specific partial resistance to blast in temperate *japonica* rice cultivars. *Plant Dis*, **73**: 496-499.

[6] S. Temnykh, S. Cartinhour, W. Park, N. Ayres, N. Hauck, L.Lipovich, Y. G. Cho, and McCouch, S.R. Mapping and genome organization of microsatellites in rice (*Oryza sativa* L.). 2000, *Theoretical and Applied Genetics*, **100**:563-566.

[7] Y.G. Cho, T. Ishii, S. Temnykh, and X. Chen. Diversity of microsatellites derived from genomic libraries and GenBank sequences in rice (*Oryza sativa* L.). 2000, *Theoritical Application Genetic*, 1**00**: 713-722.

[8] S. Harrington. A survey of genetic diversity of eight AA genome species of *Oryza* using microsatellite markers. 2000, MS thesis Cornell University, Ithaca. Genetic Resources Center. International Rice Research Institute, Manila, 52.

[9] J.H. Zou, X.B. Pan, Z.X. Chen and J.Y. Xu. Mapping quantitative trait loci controlling sheath blight resistance into rice cultivars (*Oryza sativa* L.). 2000, *Theoritical Application Genetic*, **101**: 569-573.

[10] C. Bres-Patry, M. Loreux, G. Clément and M. Bangratz. Heredity and genetic mapping of domestication related traits in a temperate japonica weedy rice. 2001, *Theoritical Application Genetic*, **102**: 118-126.

[11] P. Moncada, C.P. Martinez, J. Borrero and M. Châtel. Quantitative trait loci for yield and yield components in an *Oryza sativa* x *Oryza rufipogon* BC_2F_2 population evaluated in an upland environment. 2001,*Theoritical Application Genetic*, **102:** 41-52.

[12] S. Askani, M.Y. Rafii, M. Sariah, A. Siti Nor Akmar, I. Rusli, H. Abdul Rahim, and A.M. Latif. Analysis of simple sequence repeat markers linked with blast disease resistance genes in a segregating population of rice (*Oryza sativa*). 2012, *Genetic Molecule Residue*, **10**(3): 1345-1355.

[13] M. Moniruzzaman, M.S. Alam, J.A. Rashid, S.N. Begum and M.M. Islam. Marker assisted backcrosing for identification of salt tolerant rice line.2012, *International Agricultural Residues Innovation and Technology*, **2**:1-8.

[14] S. Helia, Z.H. Yu, C.E. Vallejos and S.A. Mackenzie. 2001. Pollen fertility restoration by nuclear gene Fr in CMS common bean: an *Fr* linkage map and the mode of *Fr* action. *Theor Appl Genet*., **90**:1056–1062.

[15] H. Dallas, Y.Yokozeki, A.Inagaki, T.Fujimura. 1998. Highly polymorphic microsatellites of rice consist of AT repeats, a classification of closely related cultivars with these microsatellite loci. *Theor. Appl. Genet*, **94**: 61-67.

[16] J. Yashitola, T. Thirumurugan, R.M. Sundaram, M.K. Naseerullah, M.S. Ramesha, N.P. Sarma, and R.V. Sonti. Assessment of purity of rice hybrids using microsatellite and STS markers, 2002, *Crop Science*, **42**: 1369-1373.

[17] H.M. Pallavi, G. Rame, Y.G. Shadakshari, K. Bhanuprakash, and K. Vishwanath. Identification of SSR markers for hybridity and seed genetic purity testing in sunflower (*Helianthus annuus* L.). 2001, *Helia*, **34**: 59-66.

[18] Y.T. Wu, T.Z. Zhang, W.Z. Guo, and J.M. Yin. Detecting polymorphism among upland cotton (*Gossypium hirsutum* L.) cultivars and their roles in seed purity of hybrids with SSR markers. 2001, Mianhua Xuebao. (*Cotton Science*), **13**: 131-13.