Microsatellite Markers for Differentiation of Local Bario Rice Varieties

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Introduction

Three rice varieties, Adan Halus, Adan Sederhana and Padi Tuan, are commonly grown in the Kelabit Highlands of Sarawak. The commercial varieties are Adan sederhana and Adan halus.

Bario rice has earned a name for itself because of its special eating quality. It has carved a niche market in Sarawak and Malaysia. The price of Bario rice ranges from RM 1.90 to RM 12.00. There is no price control on specialty rice. Because of the good price it fetches braio rice is often adulterated. The Department of Agriculture initiated the Bario Rice certification Scheme in year 2003. The objectives of Bario Rice Certification scheme are to maintain the quality and authenticity of Bario Rice and to provide confidence and assurance to consumers of Bario rice that the rice is authentic. Physical, chemical and DNA finger printing results of random samples collected from the farms are currently used to certify the Bario rice.

In this study, microsatellite was selected as the marker to differentiate and identify the local Bario rice varieties. Microsatellite markers were used due to its technical simplicity, high reproducibility, high level of polymorphisms, and the requirement of low quantities of template DNA. Furthermore, thousands of microsatellite rice markers are available for study purposes. (Wu & Tanksley 1993, Chen et al. 1997, Cho et al. 2000, Temnykh et al. 2000). Microsatellites, also known as simple sequence repeats (SSR) or simple sequence length polymorphisms (SSLP), are molecular marker loci consisting of tandem repeat units of very short (1-5 base pairs) nucleotide motif. Microsatellites are based on the difference in the number of the DNA repeat units at a given locus and provide a valuable source of genetic markers. Microsatellite markers have been developed in many crop species, e.g. rice, barley, grapevine, *Brassica*, maize, soybean, and tomato. These markers are useful as a tool in genotype identification and variety protection, seed purity evaluation and germplasm conservation, diversity studies, gene and quantitative trait locus (QTL) analysis, pedigree analysis and marker-assisted breeding, and screening of large insert libraries prior to cloning (Chen et al. 1997).

Material and Methods

The total DNA of local Bario rice varieties, Adan Halus, Adan Sederhana, Padi Tuan, were isolated using CTAB extraction method. DNA fragments containing the targeted microsatellites were PCR-amplified using more than 50 selected primer pairs from Rice Genome Databank (http://www.gramene.org/db/markers). The programme for PCR amplification was: template denaturing at 94°C for 4 min, followed by 30 cycles of (94°C 1 min, 55°C 1 min, 72°C 30 sec), and final extention at 72°C for 5 min. The PCR product was analyzed for polymorphisms by 12% non-denaturing polyacrylamide gel electrophoresis. The gel was then stained by ethidium bromide and visualized by ultraviolet light. To ensure reproducibility, each PCR was preformed with at least three different batches of DNA templates.

Results and Discussion

Generally, SSLP loci with more repeats tend to be more polymorphic and have larger amplitude of variation. Based on this concept, more than 50 primer pairs were selected for this study. Out of these selected rice markers, 6 primer pairs were finally identified for this report. Among them, 3 primer pairs containing dinucleotide repeats sequence, i.e. RM013, RM202, and RM264 which contained (GA)6-16, (CT)30, and (GA)27 respectively. Rice markers RM324, and RM333 are trinucleotide repeats which contain (CAT)21 and (TAT)19-(CTT)19. While rice marker RM304 contained (GT)2(AT)10(GT)33 tandem repeats sequence.

Among the 6 identified rice markers, rice marker RM264 is able to show polymorphism for all the three tested Bario rice varieties (Figure 1). Thus, rice marker RM264 is useful as the genetic marker for the identification and differentiation of all the three local Bario rice varieties. Other rice markers are not unique rice marker as it showed similar PCR amplification pattern for more than one Bario rice varieties, however, some of these rice marker were found specific to only either one Bario rice variety. The Rice markers RM013, RM304 and RM333 are uniques for Padi Taun variety, while rice markers RM202 and RM324 are specific for Adan Halus variety.

For the purpose of rice variety identification, the Adan Halus variety could be easily identified by just using RM202 or RM324. The Padi Tuan variety, on the other hand, can be differentiated from other varieties merely by using RM13 or RM333. However, to identify Adan Sederhana variety, only the rice genetic markers RM264 can be used.

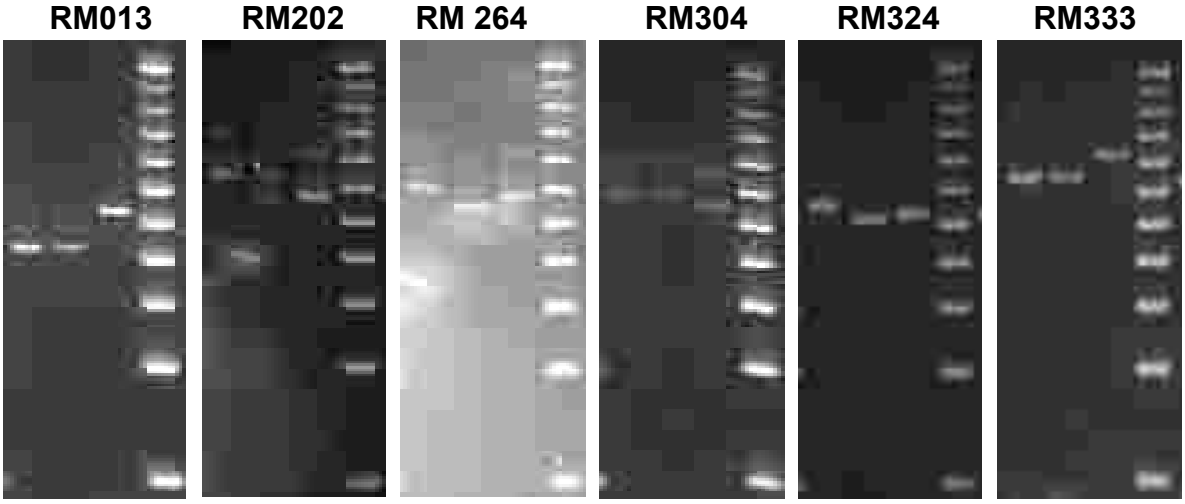

Figure 1. Microsatellite polymorphisms for local Bario rice varieties using rice markers RM013, RM202, RM264, RM304, RM324 and RM333. Lane 1-3 (Start from Left) : Adan Halus, Adan Sederhana, and Padi Tuan. Lane: 3: 25 bp DNA step ladder Markers.

Conclusion

Through microsatellite polymorphisms, local Bario rice varieties, Adan Halus, Adan Sederhana dan Padi Tuan, have been successfully differentiated by using the rice genetic markers RM013, RM202, RM264, RM303, RM324 and RM333.

Reference

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