USE OF MICROSATELLITE MARKERS IN IDENTIFICATION OF VARIETAL ADMIXTURES OF RICE SEEDS

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Introduction

Though the genetic purity is evaluated and maintained prior to release of seed paddy, the morphological similarities of certain rice varieties have led to an under estimation of other distinguishable varieties (ODVs) even in the certified seed paddy samples during seed certification. Seed descriptors of Department of Agriculture reveal that some groups of rice varieties; BG300/BG305, AT303/AT353 and BG359/BG379-2/BG403 are having similar grain morphological characters including grain length, width and colour of glumes (Descriptor of cereals, 2005) and thus may present as varietal admixtures which are not differentiable to the naked eye. Hence, it necessitates the identification of varietal admixtures of seed samples particularly when these seeds are to be used in breeding programmes. There the potential solution would be the use of molecular markers (McCouch et al., 1996). Among various markers available, microsatellites were used due to its associated advantageous features such as high reproducibility, co-dominant inheritance, high precision, high abundance and considerably higher level of polymorphism in rice genome. Therefore, under this investigation, DNA extracted from pure lines of rice varieties BG300, BG305, AT303, AT353, BG359, BG379-2, and BG403 obtained from Rice Research and Development Institute (RRDI) Bathalegoda, were subjected to PCR amplification using 28 microsatellite primers with the objective of developing a fingerprint to identify the varietal admixtures of rice seeds belonging to above three problematic groups of rice varieties at molecular level.

Methodology

DNA of all seven varieties was extracted using CTAB protocol (Mayer et al., 1995) and was quantified using spectrophotometer (UV-1201 SHIMADZU, SHIMADSU cooperation, Japan). PCR amplification of 28 microsatellite loci were performed using primers RM595, RM3614, RM3269, RM11679, RM11715, RM11690, RM8274, RM8096, RM3817, RM1067, RM6569, RM3648 , RM319, RM8232, RM6696, RM6703, RM3362, RM8137, RM325A, RM185, RM334, RM270 , RM184, RM107, RM101, RM160, RM338 and RM108. Amplified PCR products were subjected to agarose gel electrophoresis with ethidium bromide staining followed by polyacrylamide gel electrophoresis with silver staining (Samarasinghe and Perera, 2001).

Results

Amplified loci from microsatellite primers used in this research resulted both polymorphic as well as monomorphic bands. Out of 28 microsatellite primers used, 20 primers resulted polymorphic banding patterns when the seven selected rice varieties were considered as a single population where as, microsatellite loci targeted by eight other microsatellite primers RM319, RM3362, RM3817, RM6569, RM185, RM11715, RM8096 and RM8274 were completely monomorphic for all seven rice varieties (Table 1).

Table 1. Number of microsatellite primers which showed polymorphic and monomorphic bands for three problematic groups of rice varieties.

Group	P*	M*
BG300/BG305	06	22
AT303/AT353	11	17
BG359/BG379-2		
BG359/BG379-	10	18
BG359/BG403	17	11
BG379-	12	16

Discussion

Out of 28 microsatellite primers, RM270, RM3614, RM11690, RM107, RM184 and RM325A could be used to differentiate at least four combinations of problematic groups of rice varieties out of five (BG300/BG305, AT303/AT353, BG359/BG379-2, BG359/BG403 and BG379-2/BG403). Thus, microsatellite regions targeted by these six primers can be considered as the regions with the highest variation in terms of the number of tandem repeats in their respective alleles. Table 2 shows the 20 microsatellite primers which resulted polymorphic bands that enabled the differentiation of the respective morphologically similar groups of rice varieties and Figure 1 shows the DNA fingerprint for the seven rice varieties.

Eight microsatellite primers out of 28 used for this investigation resulted complete monomorphism in all seven varieties and this might be due to highly conserved nature of those alleles found in the particular loci.

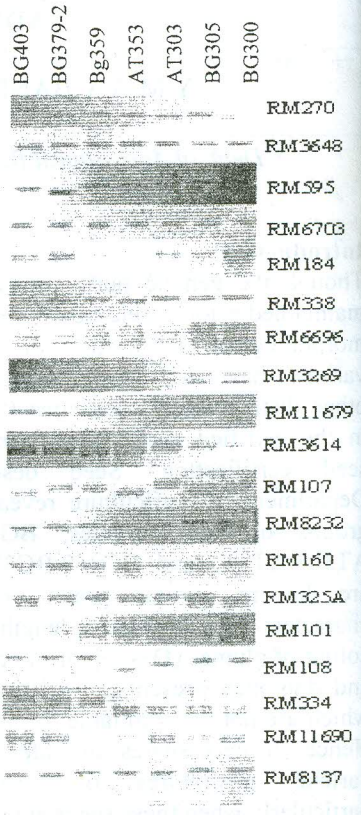

Figure 1. DNA fingerprint of seven rice varie on 10% polyacrylamide gel

Conclusion

Out of 28 microsatellite primers used, 20 primers could be used to develop a DNA fingerprint which enabled the identification of varietal admixtures of tested rice varieties.

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Table 2. Microsatellite primers which enable the differentiation of morphologically similar group of rice varieties

Variety	Name of the Microsatellite Primer
BG300 and BG305	RM160, RM270, RM3614, RM6703, RM11690, RM107
BG359 and BG 379-2	RM184, RM325A, RM3648, RM338, RM3614, RM8137, RM8232, RM595,RM11690, RM101
BG359 and BG403	RM184, RM270, RM325A, RM334, RM3648, RM338, RM3614, RM6696, RM8137, RM8232, RM595, RM3269, RM11679, RM11690, RM101, RM107, RM1067
BG 379-2 and BG403	RM184, RM270, RM325A, RM334, RM3648, RM6696, RM8232, RM595, RM3269, RM11679, RM107, RM1067
AT303 and AT353	RM184, RM270, RM325A, RM338, RM3614, RM6696, RM101, RM107, RM108, RM11690,RM1067

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