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Studies on genetic relatedness among various genotypes of rice using SSR markers

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ABSTRACT

Microsatellite based analysis was carried out to access the genetic relatedness among eight genotypes of rice viz., IR 58025A, Pusa 6A, KMR 3R, BR 827-35-3-1-1-1R, R 710-437-1-1, PRR 78, Swarna and MTU-1010. Of the 45 microsatellite loci analyzed, only nine were identified as polymorphic among the various genotypes. The degree of similarity ranges from one polymorphic locus to six polymorphism among the genotypes studied.

Key words: *rice, microsatellite markers, polymorphism, genetic relatedness*

The present productivity levels in rice have reached to plateau, which may be attributed to limited use of genetic diversity available in this crop. Despite the richness of genetic resources, only a small proportion of the world rice germplasm collections have been used in breeding programs (Dilday, 1990). As a consequence a high genetic similarity is found within several commercial rice germplasms around the world. The information regarding genetic similarities is needed for the selection and monitoring of germplasm to predict the possible genetic gains (Chakravarthi and Naravaneni, 2006). Conventionally, the studies regarding germplasm similarities and diversity is being carried out by means of morphological and biochemical markers which, in many cases reveals ambiguous and confusing results especially in case of differentiating closely related genotypes (Sonja *et al.,* 2008). In contrast, DNA based molecular markers have proven to be powerful tools for the evaluation of genetic variation. The results derived from analyses of genetic diversity at the DNA level could be used for designing effective breeding programs aiming to broaden the genetic bases of commercially grown varieties (Ghneim *et al*., 2008). Among the available molecular markers, microsatellites or simple sequence repeats (SSRs) have gained considerable importance in plant genetics and breeding owing to many desirable genetic attributes including hypervariability, multiallelic nature, codominant inheritance, reproducibility, relative abundance, extensive genome coverage including organellar genomes, chromosome specific location and amenability to automation and high throughput genotyping (Kalia *et al*., 2011). In rice, microsatellites are abundantly well distributed throughout the genome and can be efficiently used for the assessment of genetic relationship among various genotypes (McCouch *et al*., 1997). In the present study, several microsatellite loci were screened to identify polymorphisms that can be used to establish the genetic relationship among various genotypes of rice.

Eight genotypes (Table I) including, two CMS (IR 58025A and Pusa 6A), four restorer (KMR 3R, BR 827-35-3-1-1-1R, R 710-437-1-1 and PRR 78) and two inbred lines (Swarna and MTU-1010) were analyzed using SSR markers. The experimental seed material was planted in trays and genomic DNA was isolated from young succulent diseases free leaves of 8-10 days old rice seedlings as per the protocol of Xu *et al.* (2005). The DNA samples were quantified, diluted, amplified (using thermocycler) and the electrophoresis of the amplified DNA was carried out for 90 minutes at 140 volts on 6% polyacrylamide gel. The staining of the polyacrylamide gels was done with the help of Ethidium Bromide solution containing 10 µl of 1% EtBr in 300 ml of distilled water. The gels

were visualized under UV trans-illuminator to observe the banding pattern.

Table 1. Genotypes used in the study

CMS line	Developed by
IR 58025A	International Rice Research Institute (IRRI), Manila, Philippines
Pusa 6A	Indian Agriculture Research Institute (IARI), New Delhi
KMR 3R	The Zonal Agricultural Research Station, V.C. Farm, Mandya (UAS, Bengaluru) Karnataka
PRR 78	The Division of Genetics, Indian Agricultural Research Institute, New Delhi
R 710-437-1-1	The Rice Breeder, Indira Gandhi Krishi Vishwa Vidhyalaya, Raipur, C.G.
BR 827-35-3-1-1-1R	The Regional Agricultural Research Station, Karjat (BSKKV), Maharashtra
Swarna or MTU 7029	Andhra Pradesh Rice Research Institute, Maruteru (ANGRAU)
MTU-1010 or Cottondora Sannalu	Andhra Pradesh Rice Research Institute, Maruteru (ANGRAU)

Of the 45 microsatellite loci analyzed, only nine (RM201, RM234, RM256, RM260, RM276, RM335, RM410, RM444 and RM1384) were identified as polymorphic (Fig. 1) among various genotypes of rice studied. IR 58025A was found to be genetically highly related with Pusa 6A (only two polymorphic loci) followed by BR 827-35-3-1-1-1R and MTU-1010 (polymorphism at three loci), while as it revealed highest level of variability in relation to PRR 78 and

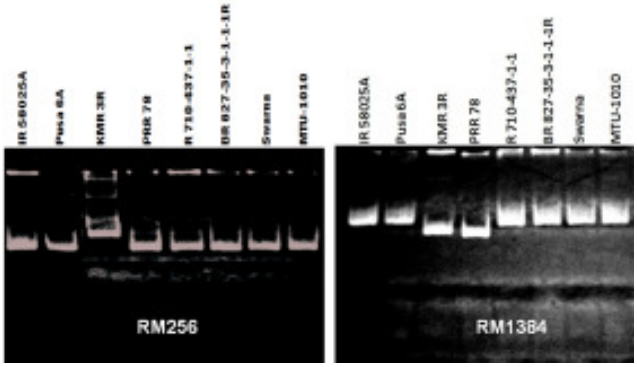

Fig. 1. Amplification pattern obtained using the SSR marker RM256 and RM1384

KMR 3R (six polymorphic loci each). Pusa 6A shows highest degree of similarity with IR 58025A, while as it behaves as genetically most diverse when compared with PRR 78 and Swarna (six polymorphisms each). KMR 3R shows least polymorphism with PRR 78 (only one polymorphic loci), while as it exhibits maximum genetic diversity when compared with IR 58025A, BR 827-35-3-1-1-1R and MTU-1010 (six polymorphic loci each). PRR 78 shows maximum similarity with KMR 3R (only one polymorphic locus), but exhibits highly diverse genotypic relationship with IR 58025A and Pusa 6A (six polymorphic loci each). R 710-437-1-1 revealed maximum (five) polymorphic loci with IR 58025A, whereas it shows close relationship with BR 827-35-3-1-1-1R and MTU-1010 (only two polymorphisms each). BR 827-35-3-1-1-1R shows maximum similarity with R 710-437-1-1 (two polymorphic loci) and exhibits maximum diversity when compared KMR 3R (six polymorphic loci). Swarna resembles genotypically most with BR 827-35-3-1-1-1R, PRR 78 and MTU-1010 (four polymorphic loci each), but shows maximum genetic diversity with Pusa 6A (six polymorphic loci). MTU-1010 shows maximum similarity with R 710-437-1-1 (only two polymorphisms each), while as it shows maximum genotypic differences with KMR 3R (six polymorphic loci).

Despite of the Basmati quality of PRR 78, it was found to be highly related with KMR 3R, suggesting common ancestry of the two genotypes. In general, the results indicated a considerable level of genetic variation among the eight genotypes used. The microsatellite assay generated variety-specific alleles in some of the genotypes screened; these may be used for DNA fingerprinting and purity testing of the variety concerned. Identification of rice varieties and testing their genetic purity plays a significant role in the issues related to plant variety registration and plant breeders' rights. The assessment of genetic diversity is an essential component in germplasm characterization and conservation (Ghneim *et al*., 2008).

Knowledge regarding the extent of genetic variation in germplasm accessions and genetic relationships between genotypes are important considerations for designing effective breeding programs. The classification and quantification of genetic diversity in closely related crop germplasm has

been a major objective for balanced use of genetic resources. Molecular study of germplasm would provide information regarding the extent of similarities within and between species (Shinwari, 1995). This information can then be used for selection of diverse parents for intra-specific crossing and most closely related parents for inter-species crossing, to increase heterosis and to include desirable genes from more diverse backgrounds into the best available germplasm.

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