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GENETIC DIFFERENTIATION OF RICE MUTANTS BASED ON MORPHOLOGICAL TRAITS AND MOLECULAR MARKER (RAPD)

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Abstract

Rice cultivars IR6 and IR8 were exposed to different doses of gamma radiation and stable mutants along with parents were studied for genetic diversity on the basis of morphological traits and molecular marker (RAPD). Morphological data showed that mutants of IR6 and IR8 performed well as compared to their parents. The genetic variation was determined through RAPD. A total of 74 scorable bands were observed, out of which 47 (63.6%) were polymorphic and 27 (36.5%) were monomorphic. The size of fragments ranged from 201bp – 3.2 kbp. The number of fragments produced by various primers ranged from 1-12 with an average of 4.93 fragments per primer. Maximum 12 bands were amplified with primer A-03 and minimum one band was amplified with primer A-15, A-19 and B-10. Some specific RAPD bands were also identified reflecting the RAPDs application for the identification of rice cultivars/genotypes. Results revealed that variety Sarshar and IR8-178 contain a specific segment of 451bp amplified with primer A-19. The highest similarity was observed between IR8-15A and IR8-178 (96%) and the least similarity was recorded between IR-8 and IR6-15/B (69%). On the basis of RAPD studies, genotypes were grouped on the basis of their similarities and distinctness.

Introduction

Rice as a staple food serves as the main source the calories consumed by human and occupies almost one-fifth of the total land area covered under cereals (Micke *et al*., 1990). Rice was grown on approximately 2.52 million hectares with a total production of 5.02 million tons, out of which 3.69 million tons was exported and earned a foreign exchange worth 69325.1 million rupees (Minfal, 2005-06). Most of the non-aromatic rice varieties have their narrow genetic base of IRRI varieties particularly IR6 and IR8. Hyberdization among IRRI varieties has helped in the manipulation of genes with a limited genetic diversity. Induced mutations has played a very vital role in altering the genetic make-up of genotypes not only at a chromosomal but even at a molcular level (Alan, 2007). Today, mutation induction has become an established tool in plant breeding to supplement conventional crop breeding. Mutations have the potential to alter many morphological and physiological traits (Micke *et al*., 1990; Wang *et al*., 1992). The estimation of genetic diversity on the basis of morphological traits alone does not determine actual level of genetic diversity among germplasm because morphological traits are the product of gene and environmental interactions (Alan, 2007). The degree of gene expression is highly influenced by the conduciveness of the environment and genetic background in which gene is present. Therefore, selection based merely on morphological traits has been often misleading (Kumar *et al*., 1998; Astarini *et al*., 2004; Asif *et al*., 2005). Hence in many instances breeders have been using genetically similar parents in their breeding programmes, leading to a narrow genetic base (Fouilloux &

Bannerot, 1988; Xia *et al*., 2004; Rehman *et al*., 2002). During last ten years, techniques based on DNA markers along with morphological traits have been used to detect variation at DNA level to distinguish closely related genotypes. The development of the random amplified polymorphic DNA (RAPD) markers (Williams *et al*., 1990; Welsh *et al*., 1991; Alan, 2007) allowed genome characterization in several plant groups. The specific objective of this study was to evaluate the genetic relationships among advanced rice germplasm developed by gamma radiation through RAPD marker.

Materials and Methods

Seed of IR6 and IR8 were irradiated with different doses of gamma rays (150, 200 and 250Gy). Four promising mutants, two each of IR6 and IR8 were selected and grown in the field. Observations were recorded for 7 morphological characters viz., days to flowering, plant height (cm), number of tillers per plant, fertile grain per panicle, branches per panicle, 1000 grain weight (g), and paddy yield (kg/ha). Means were compared according to Duncan's Multiple Range (DMR) test (Steel &Torrie, 1980).

DNA extraction: DNA was extracted from fresh leaves of rice genotypes using DNA isolation Kit (Gentra system, Minnesota, USA.)). Fresh leaves (200 mg) were grounded in liquid Nitrogen; 3ml of the cell lysis solution (tris hydoxymethyl aminomethane, ethelenediaminetetra acetic acid and Sodiumdodecyl sulfate) was added with leaf sample to the 15ml centrifuge tube and incubated at 65^{o}C for 60 minutes. Fifteen µl of RNase (Gentra system, Minnesota, USA.) solution was then added to the cell lysate and incubated at 37^{o}C for 30 minutes. Protein precipitation solution (Gentra system, Minnesota, USA.) was added and vortex for 20 seconds and the tubes were placed on ice for 30 minutes. The mixture was centrifuged at 2000 x g for 10 minutes. Supernatant containing DNA was poured in the separate 15ml centrifuge tube and DNA was precipitated by centrifuging at 2000 x g with 3ml of isopropanol absolute.

Ethanol 70% was used to wash the pellet and the DNA samples were then hydrated with TE buffer. The concentration of isolated DNA was measured with spectrophotometer (BIOMATE 3) at absorbance 260nm and 280 nm. The quality of DNA was checked on 0.8% agrose gel.

PCR with random primers: Forty Primers from Gene Link (New York, U.S.A), each ten bases in length, were used to amplify the DNA. PCR reaction was carried out in 25µl reaction mixture containing 26ng of template (Genomic DNA), 2.5mM MgCl, 0.33mM of each dNTPs, 2.5U of Taq polymerase and 1µM of primer in a 1X PCR reaction buffer (Eppendorph, Hamburg, Germany). The amplification reaction was performed in the Eppendorf Master Cycler with an initial denaturation for 5 minutes at 94^{o}C, then 32 cycles: 1 minute denaturation at 94^{o}C; 1 minute annealing at 52^{o}C; 2 minutes extension at 72^{o}C. Final extension was carried out at 72^{o}C for 10 minutes. Amplified products were analyzed through electrophoresis on 1.5% agarose gel containing 0.5 X TBE (Tris Borate EDTA) and 0.5µg/ml Ethidium bromide to stain the DNA. Gel electrophoresing at 72 volts for 2 hours and photograph was taken under UV light using gel documentation system (Vilber Lourmat, France).

Data was scored as presence of band as (1) and absence of bands as (0) from RAPD of amplification profile. Coefficient of similarity among genotypes was calculated according to Nei & Li (1979). Similarity coefficient was utilized to generate a

dendrogram by means of Unweighted Pair Group Method of Arithmetic means (UPGMA).

Results and Discussion

Morphological analysis: The seven morphological characters showed different degree of variation among genotypes. Mutants of IR6 and IR8 matured earlier than their parents which is desirable for wheat-rice cropping pattern. Plant height also reduced significantly in the mutants as compared to their parents. Reduction in plant height prevents lodging under heavy fertilization and strong winds. The yield contributing traits also improved in the mutants as compared to their parents (Table 3). Number of fertile grains significantly increased in the mutants as compared to their parents. In case of paddy yield mutants of IR6 and IR8 gave 27.37% (IR6-15/A) and 24.62% (IR6-15/B), 25.58% (IR8-15A) and 23.64% (IR8-178) higher paddy yield as compared to their parents respectively. It is evident from the data that the mutant IR8-15A surpassed all other genotypes for its superiority in the yield contributing traits. IR8-15A produced the highest number of tillers per plant (19.0), fertile grains per panicle (152.7), number of branches per panicle (12.33), grains weight (26.67 grams) and highest paddy yield (6656.0 kg/ha). In case of days to flowering, number of tillers per plant, number of branch per panicle, 1000 grain weight and paddy yield, IR6-15/B was followed by IR8-178 which did not differ significantly from one another for these traits. It can be depicted from the agronomic data that the genotype IR8-15A and IR8-178 does not have wide genetic difference. The relative roles of genetic and chromosomal mechanisms on phylogeny and racial differentiation of rice were studied extensively through mutational, molecular and agronomical approaches (Wang *et al*., 1992). Since various physical and chemical mutagens are known to act in different ways to cause DNA lesions, combined effects of mutagens were investigated (Siddiq, 1968; Mahajani &Chopra, 1973; Micke *et al*., 1990). Studies showed that mutant is different with its parent, thus conforming to the expectation that gene duplications provide cushion against harmful effect of mutation but some times both lethal and viable mutants were observed thus conforming that mutations treatment was effective in rice. It was generally believed that polyploids with their duplicated sets of the genes would be less sensitive to mutagenic treatments in terms of realized mutants than their diploid counter parts (Chopra, 2005).

Our knowledge on genetic diversity and relationships of advanced rice genotypes is still limited, hindering the efficient use and conservation of its germplasm. However the data from this study showed that morphological characters alone still cannot be regarded as critical indicators to identify individual rice genotype because most of the traits are influenced by the environment and nutrition available to the plant from the soil where it grows. Compared with the morphological characters, the RAPD data are more powerful technique to study the relationships and variability among rice germplasm and the resolution is much higher to identify individual varieties, even to those with the same label. Therefore, the DNA fingerprinting technology is an effective mean for screening the rice germplasm, due to independent from the environment (Alan, 2007).

Table 1. Sequences of primers and amplified products.

Primer sequence	Range of amplified product	Polymorphic bands	Monomorphic bands	Total no of bands
TGCCGAGCTG	396-1.5	2	2	4
AGTCAGCCAC	201-2.5	8	4	12
AATCGGGCTG	583-3.2	4	2	6
CAATCGCCGT	344-1.5	2	1	3
CAGCACCCAC	203-2.9	6	4	10
TCTGTGCTGG	386-1.2	4	4	8
TTCCGAACCC	714-760	----------	1	1
CAAACGTCGG	451	----------	1	1
GGACTGGAGT	865-1.4		2	2
TGCGCCCTTC	834-2.04	1	2	3
TGCTCTGCCC	331-2.5	2	1	3
GGTGACGCAG	272-1.6	9	1	10
GTCCACACGG	443-1.2	2	1	3
CTGCTGGGAC	309-363	----------	1	1
ACCCCCGAAG	220-2.7	6	1	7
		47 (63.6%)	27 (36.5%)	74

Table 2. Similarity coefficient among the different rice genotypes calculated according to Nei & Li's coefficient.

	IR8-P	IR8-15A	IR8-178	IR6-P	IR6-15/A	IR6-15/B
IR8-P	1					
IR8-15A	0.8	1				
IR8-178	0.793	0.965	1			
IR6-P	0.807	0.85	0.853	1		
IR6-15/A	0.825	0.763	0.758	0.793	1	
IR6-15/B	0.69	0.726	0.753	0.714	0.726	1

Table 3. Performance of important traits of different rice genotypes.

Varieties	Days to flowering	Plant height (cm)	No. of tillers	Fertile grain/ Panicle	Branches / panicles	1000 grain weight (g)	Paddy yield (kg/ha)
IR6	118.7 b	102.0a	16b	128.7c	10.0 b	22.0b	4442 c
IR6-15/A	114.0 c	95.0c	18 a	142.7b	12.67 a	26.0a	5658 b
IR6-15/B	112.0cd	102.0a	17 ab	140.0b	12.0 a	25.67a	5536 b
IR8	122.0 a	103.0a	16 b	106.0d	10.33b	20.67b	5300 b
IR8-15A	114.7 c	99.3b	19 a	152.7a	12.33a	26.67a	6656 a
IR8-178	110.3 d	99.0b	18ab	150.0a	12.0a	26.0a	6533 a

RAPD analysis: Genomic DNAs of rice genotypes produced multiple fragments with 10 arbitrary primers. Of 40 primers, 15 were amplifying the genomic DNA (Table 1). The total number of the amplified DNA products (bands) yielded across the set of 6 rice genotypes was 74, out of which 47 (63.6%) were polymorphic and 27 (36.5%) were monomorphic. Fragments ranged in size from 201bp to 3.2kbp. The number of fragments produced by various primers ranged from 1-12 with an average of 4.93 fragments per primer. Maximum 12 bands were amplified with primer A-03 and minimum one band was amplified with primer A-15, A-19 and B-10.

Some specific RAPD bands were also identified thus, reflecting the RAPDs application for the identification of rice. Results revealed that variety IR8-15A and IR8-178 contain a specific segment of 451bp amplified with primer A-19.

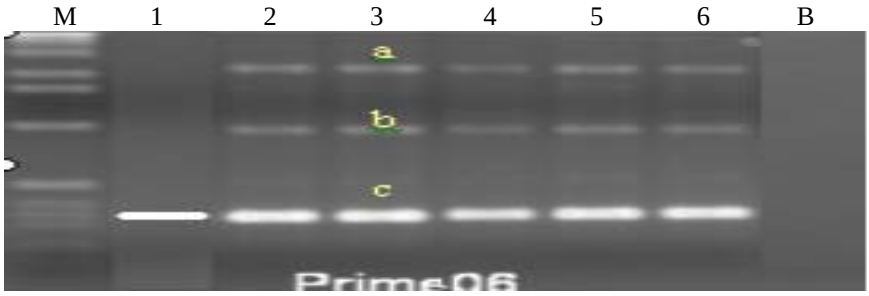

Fig. 1

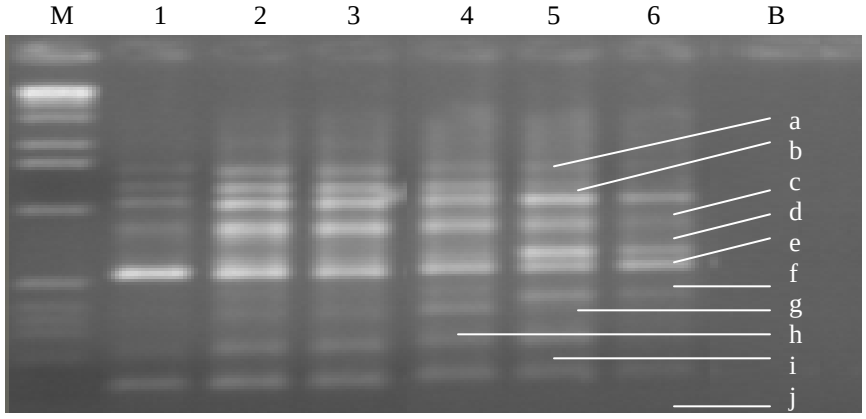

Fig. 2

Figs. 1&2. Results of RAPD-PCR with primer B-06, B-07, M=ladder, 1=IR8-P, 2=IR8-15A, 3=IR8-178, 4=IR6-P, 5= IR6-15/A, 6=IR6-15/B

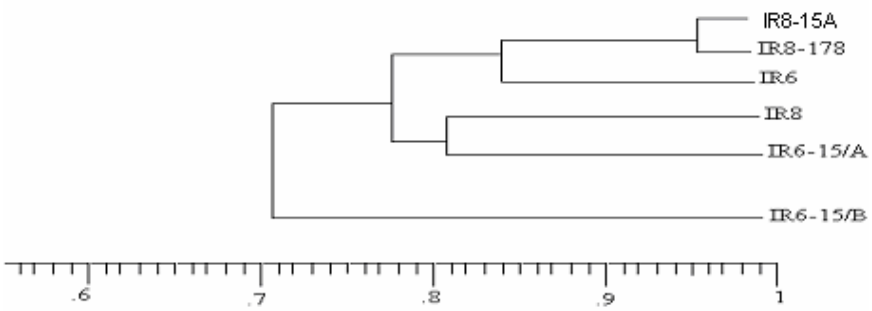

Fig. 3. Dendrogram of six advanced rice genotypes developed from RAPD data using un-weighted pair group method of arithmetic means (UPGMA).

Level of the individual genotypes of the 6 rice genotypes produced polymorphism as shown in Fig. 1 and 2. Three major segments were amplified using primer B-05 (Fig. 1, bands a, b and c). Band c was shared by all 6 genotypes; band a and b disappeared in IR8-P. On the other hand, there were 10 segments amplified using primer B-07 in 6 genotypes (bands a, b, c, d, e, f, g, h, i and j in Fig. 2 and Table 1). Some segments (a, b, d and h) amplified only in one or a few genotypes, but other segments (c, f and j) amplified in all genotypes. Band g appeared only in IR6-15/A, indicating the specificity to sub-species. Different combinations of the 10 segments formed DNA fingerprints in 06 mutants. Polymorphism of amplified fragments was caused by: (i) base substitutions or

deletions in the priming sites, (ii) insertions that render priming sites too distant to support amplification, or (iii) insertions or deletions that change the size of the amplified fragment (Chopra, 2005). RAPD markers provide an efficient way to further saturate the rice genetic map and to select agronomical useful traits in segregating populations.

Genetically most similar genotypes were IR8-15A and IR8-178 (96%) while most dissimilar genotypes were IR8 parent and IR6-15/B (69%) (Table 2). The close genetic relationships are quite alarming and may impede further plant improvement. It has been very well documented that plant improvement is based on the information about the genetic relationships among accession within and between species (Thormann *et al*., 1994). The information about genetic similarity will be helpful to avoid any possibility of elite germplasm becoming genetically uniform.

The dendrogram (Fig. 3) delineated most of the genotypes into groups, which corresponds well with the breeding programmes adopted at different breeding centers, and institutes of release. The varieties could be divided into two groups, IR8-15A, IR8-178 and IR6-P are in A group, IR8-P and IR6-15/A are clustered in B group. Distinct genotype IR6-15/B was falling in both groups i.e., A and B showing 71% similarity with both the groups.

RAPD analysis may be very useful in breeding for rapid and early identification of most diverse genotypes in large population. Keeping in view the useful information about the close genetic relationship, it is suggested that goal oriented breeding programes with the help of RAPD technology will be helpful to produce distinct genotypes with diverse genetic back ground and improve crop productivity (Rout & Lucas, 1996; Asif *et al*., 2005; Alan, 2007). Plants gave phenotypic variability, mainly due to true genetic changes. Thorough knowledge of the genetic diversity of the crop is necessary for parental selection that maximizes genetic improvement.

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