

RAPD-based assessment of diversity in Indica rice genotypes

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Twelve rice genotypes including three basmati indica, eight non-basmati indica, and one japonica possessing one or the other desired character, were subjected to RAPD analysis by using random sequence decamer primers. Thirteen primers generated a total of 146 DNA fragments of which, 91 were polymorphic in at least one pair-wise comparison between 12 rice genotypes. The size of amplified DNA fragments ranged from 0.24 to 3.7 kb. Individual primers showed polymorphic value ranging from 0.034 to 0.421. The average polymorphic value between basmati and non-basmati genotypes was 0.232, whereas among basmati genotypes was 0.144 and among non-basmati was 0.212. The polymorphic value between Azucena (japonica) and indica genotypes was 0.254. The mean polymorphic values were in accordance with their known phylogenetic relationships.

Eco-geographic differentiation of asiatic rice has produced a number of types (sub-species) referred to as indica, japonica and javanica¹. Of these, indica subspecies alone occupies >80% of cultivation area. Basmati rice is famous for its aroma and grain quality and grown in Northern states of India and Pakistan. It has been grouped with indicas. Basmati rice varieties are sensitive to all major biotic and abiotic stresses, have a tall plant phenotype and are poor yielders as compared to non-basmati indica rice cultivars. Basmati rice breeding has been quite cumbersome due to complicated nature of quality traits, its poor combining ability and lower frequency of desirable genotypes in segregating populations from non-basmati x basmati crosses. Hence it has been difficult to select segregants that possess the desired gene(s) from the non-basmati parent while retaining all the important quality traits of basmati rice. The use of molecular markers can greatly accelerate the basmati rice breeding programs. Molecular markers are now available for several important agronomic traits such as disease resistance in non-basmati rice, yet not much information is available on molecular tagging of grain quality and other agronomic traits in basmati rice. Such markers tagged to the desirable genes will

be a useful aid in conventional breeding involving crosses between basmati and non-basmati genotypes.

Randomly amplified polymorphic DNA (RAPD) technique² has been extensively used for studying DNA polymorphism and marker-assisted breeding programs³. The major advantage of RAPD method relies on the fact that it allows the exploration of a much larger genomic portion. In a study, 22 indica rice cultivars could be readily distinguished by using only 10 RAPD primers⁴. For such an application, one need to standardize reaction conditions, as well as to ensure reproducibility and adequacy of RAPDs for studying molecular polymorphism in a particular plant species. Basmati rice has not been much subjected to molecular marker analysis. In this paper, we report the molecular polymorphism as assessed by RAPD analysis between the basmati and non-basmati rice genotypes differing in yield, grain quality and other agronomic traits.

Materials and Methods

The seeds of different rice varieties were obtained either from the CCS HAU, Rice Research Station, Kaul or CSSRI, Karnal (Table 1). The rice seeds were sown in pots and crop was raised in the net-house by following the standard agricultural practices. Leaves of four-week-old plants were used for DNA extraction. Genomic DNA was isolated by the method of Murray and Thomson⁵, using cetyl-trimethyl ammonium bromide-based extraction medium, and its concentration was monitored by electrophoresis on

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0.7% agarose gels in TBE buffer⁶. A total of 20 decanucleotides of random sequence composed of 6 or more G/C bases obtained from Operon Technologies Inc. were used. PCR was carried out in 20 µl containing 50 ng genomic DNA, 100 µM each of dNTPs, 50 mM KCl, 0.1% triton X100, 2 mM MgCl₂, 0.2 µM primer and 0.5 unit of Taq DNA polymerase. The amplification reaction was carried out in PTC-100 MJ Research thermocycler. The amplification conditions were: 94°C 1 min, 40°C 1 min, 72°C 2 min, 40 cycles, 72°C 15 min one cycle, 4°C (for storage). Amplified products were separated on 1% agarose gel electrophoresis. The gels were stained with ethidium bromide and viewed and photographed using 200 ASA colored film and yellow filter under UV light. Amplified DNA bands were analyzed for polymorphism which was calculated as dissimilarity value (1-F) using the formula given by Nei and Li⁷. In the formula, $F = 2A(B+C)^{-1}$, 'A' refers to the number of bands shared by the two genotypes and B and C are the number of bands in each genotype.

Results and Discussion

Rice genotypes used in the present study include basmati, and non-basmati indica and japonica genotypes. The non-basmati genotypes include varieties, which possess important agronomic characters like higher yield potential, salt tolerance, dwarf plant type and disease resistance. For the introgression of these traits from non-basmati into basmati genotypes, application of molecular markers may enhance the efficiency of conventional breeding methods. As a pre-requisite of developing molecular markers, polymorphism between the rice genotypes must be worked out. Of the 20 random primers used for detecting RAPDs in rice genotypes, 4 primers did not amplify DNA of any of the rice genotypes, while 3 primers resulted in amplification in 8 or less number of rice genotypes. The primers, which did not amplify DNA of some or all of the genotypes may not have found the complementary sequences on the genomic DNA. Non-amplifying primers have also been reported for rice⁸ and sorghum⁹. The characteristic fragments generated by random decamer oligonucleotides employed as single, arbitrary primer is illustrated in Fig. 1. 13 primers in 12 rice genotypes amplified a total of 146 fragments (Table 1). The size of amplified DNA fragments ranged from 0.24 to 3.7 kb and number of amplified

DNA fragments ranged from 80 in HKR120 to 104 in Taraori Basmati and MI48 genotypes. Primer OPG-17 generated maximum (17 bands) while OPG-12 generated minimum (4 bands) number of amplified DNA bands (Table 2). In pair-wise comparison of the twelve rice genotypes, among the 146 bands scored, 55 were monomorphic and 91 were polymorphic, thus the proportion of polymorphic bands was nearly 60%. The RAPD polymorphism reported by others in rice is as varied as 30%⁸, 50%¹⁰ and even 80%¹¹. Such variation in amplification and polymorphism is observed due to a competition between primer for priming sites rather than the total number of potential priming sites available¹². For this reason the mean polymorphic value for different primers ranged in the present study from a minimum of 0.034 for primer OPG-16 to a maximum of 0.421 for primer OPG-09. There was no sequence relatedness among the different primers, which generated nearly similar polymorphic values.

Polymorphic or the dissimilarity values (1-F) calculated from the pairwise comparison of RAPD bands among the 12 genotypes, are shown in Table 3. The mean (1-F) value of all of these pairs was 0.22. The highest (1-F) value, 0.316, was observed between Taraori basmati and HKR120. The lowest (1-F) value, 0.064, was obtained between CSR10 and CSR11. These results can be further used for the selection of parents in a breeding program suitable for generating high level of genetic polymorphism. For

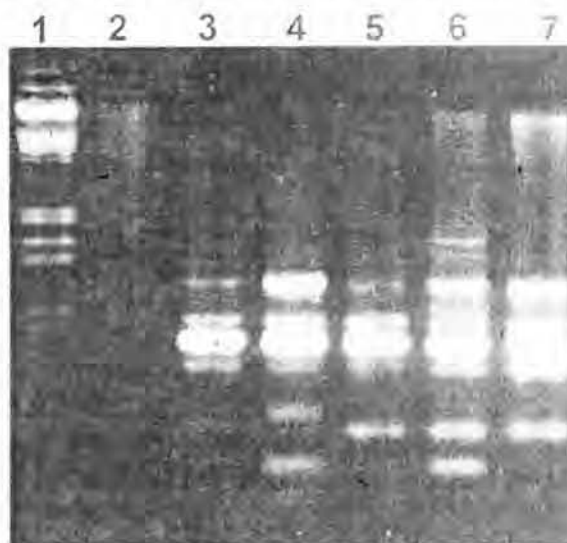


Fig. 1—Electrophoretic pattern of PCR-amplified DNA fragments of rice genotypes generated by primer OPG-04. 1 A DNA double digest marker, 2 un-amplified rice DNA, 3 MI48, 4 Gohind, 5 CSR10, 6 Azucena, 7 Taraori Basmati.

example as primer OPG-09 generated maximum polymorphic value, 0.800, between the pair Taraori basmati (salt-sensitive) and pokkali (non-basmati, highly salt tolerant), these be selected as parents in a cross for tagging salt-tolerant genes using the bulked segregation method.

As RAPD bands which are polymorphic in the parent are inherited as dominant genetic markers, the results of Table 3 can be used as a basis for the

rational calculation of the number of primers required to generate a satisfactory RAPD polymorphism either for genetic mapping or screening F₂ and backcross families, near isogenic lines or bulked segregants for tagging a gene of interest⁹. The genotypes used in the present investigation can be grouped into different clusters. Cluster analysis of the 1-F values showed a high degree of polymorphic value, 0.232, between the basmati genotypes and non-basmati indica genotypes.

Table 1—Agronomic characteristics and RAPD data of rice genotypes

Genotype	Source	Characteristics	RAPD data	
			Total bands	Mean dissimilarity value
<i>Basmati indica</i>				
Basmati 370	a	Tall, low yield, salt-sensitive, susceptible to diseases	97	0.214
Pusa Basmati 1	a	Improved, semi-dwarf, salt sensitive, susceptible to diseases	102	0.209
Taraori Basmati	a	Superior grain quality, tall, salt-sensitive, susceptible to diseases	104	0.228
<i>Non-basmati indica</i>				
HKR120	a	Semi-dwarf, high yield	80	0.236
HKR126		Semi-dwarf, high yield	96	0.236
IR36		Semi-dwarf, high yield	91	0.215
Gobind	a	Dwarf, BLB resistant	95	0.205
Pokkali	b	Highly salt-tolerant	100	0.208
CSR10	b	Salt-tolerant	95	0.200
CSR11	b	Salt-tolerant	97	0.197
MI48	b	Salt-sensitive	104	0.227
<i>Non-bas. Japonica</i>				
Azucena	b	Stress-tolerant, deep root system	97	0.254

BLB, Bacterial leaf blight.

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Table 2—Number of amplified DNA bands and mean polymorphic value (1-F) generated by different primers in twelve rice genotypes

Primers		Bands scored			Mean (1-F)
Code	Sequence	Polymorphic	Monomorphic	Total	
G-03	GAGCCCTCCA	8	6	14	0.111
G-04	AGCGTGTCTG	10	1	11	0.312
G-05	CTGAGACGGA	11	4	15	0.345
G-09	CTGACGTCAC	11	1	12	0.421
G-11	TGCCCCTCGT	9	1	10	0.317
G-12	CAGCTCACGA	2	2	4	0.233
G-13	CTCTCCGCCA	6	1	7	0.361
G-14	GGATGAGACC	9	5	14	0.220
G-16	AGCGTCCCTCC	3	10	12	0.034
G-17	ACGACCGACA	7	10	17	0.126
G-18	GGCTCATGTG	5	6	11	0.123
G-19	GTCAGGGCAA	5	5	10	0.171
G-20	TCTCCCTCAG	6	3	9	0.188
Total bands		91	55	146	

Table 3—Mean polymorphic (I-F) values among pairs of rice genotypes based on RAPDs generated by thirteen random sequence decamer primers.

Genotype	B370	PB1	TB	Pokkali	CSR10	CSR11	HKR126	HKR120	IR36	Gobind	MI48	Azucena
B370	—											
PB1	0.142	—										
TB	0.151	0.144	—									
Pokkali	0.248	0.210	0.182	—								
CSR10	0.213	0.201	0.253	0.155	—							
CSR11	0.212	0.195	0.241	0.157	0.064	—						
HKR126	0.213	0.241	0.233	0.226	0.267	0.197	—					
HKR120	0.216	0.274	0.316	0.245	0.244	0.255	0.174	—				
IR36	0.221	0.224	0.299	0.202	0.188	0.202	0.280	0.081	—			
Gobind	0.227	0.242	0.254	0.176	0.177	0.153	0.200	0.197	0.162	—		
MI48	0.285	0.182	0.202	0.223	0.209	0.219	0.289	0.278	0.224	0.234	—	
Azucena	0.234	0.250	0.243	0.258	0.236	0.279	0.291	0.331	0.285	0.233	0.154	—

Within the basmati group the polymorphic value was 0.144, while within non-basmati indica I-F value was 0.212. Japonica rice variety Azucena showed a (I-F) value of 0.258 with rest of the eleven genotypes. Yu and Nguyen¹¹ also detected high degree of polymorphism between japonica and indica subspecies by RAPD analysis. Zhenshan *et al.*⁸ concluded by RAPD analysis, that Chinese common wild rice, indica rice and japonica rice could be clustered into three distinct groups. The RAPD data generated in the present investigation are genuine, as these also reflect the known phylogenetic relationship of rice genotypes¹³. Thus, the primers which have generated higher polymorphism between pairs of genotypes, could be useful directly or after their conversion into STS primers¹⁴ for the analysis of F2 progeny of the cross between desired parents and for tagging of gene(s) of interest.

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References

- 1 Chang TT, *Euphytica*, 25 (1976) 425.
- 2 Williams JGK, Kubelik AR, Livak KJ, Rafalski JA & Tingey SV, *Nucleic Acids Res*, 18 (1990) 6531.
- 3 Mohan M, Nair S, Bhagwat A, Krishna TC, Yano M, Bhatia CR & Sasaki T, *Mole Breed*, 3 (1997) 87.
- 4 MacKill DJ, *Crop Sci*, 35 (1995) 889.
- 5 Murray MG & Thompson WF, *Nucleic Acids Res*, 8 (1980) 4321.
- 6 Sambrook J, Fritsch EF & Maniatis T, (eds) *Molecular cloning—A laboratory manual*, Second edition, (Cold Spring Harbor Laboratory Press, USA) 1989.
- 7 Nei M & Li W, *Proc Natl Acad Sci, USA*, 76 (1979) 5269.
- 8 Zhenshan W, Hong C, Ping Y, Xiangkun W & Lihuang Z, *J Genet Breed*, 50 (1996) 299.
- 9 Tao Y, Manner JM, Ludlow MM & Henzell RG, *Theor Appl Genet*, 86 (1993) 679.
- 10 Parsons BJ, Newbury HJ, Jackson MT & Ford-Lloyd BV, *Mole Breed*, 3 (1997) 115.
- 11 Yu LX & Nguyen HT, *Theor Appl Genet*, 87 (1994) 668.
- 12 Rafalski JA, Tingey SV & Williams JGK, *Agbiotech News & Inf*, 3 (1991) 645.
- 13 Glaszmann JC, *Theor Appl Genet*, 74 (1987) 21.
- 14 Monna L, Miyao A, Inoue T, Fukuoka S, Yamazaki M, Zhong HS, Sasaki T & Minobe Y, *DNA Res*, 1 (1994) 139.