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INHERITANCE OF RAPD MARKERS IN THE F_1 INTERSPECIFIC HYBRIDS OF RICE

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Abstract

Random amplified polymorphic DNA markers were generated in a diploid and two tetraploid salt-tolerant accessions of wild rice species *Oryza punctata, O. sativa* cultivars Basmati-198, IR-6 and F_1 hybrids between Basmati-198 and IR-6 with three accessions of *O. punctata* used as male parent. The PCR mediated DNA amplification profiles produced with different 10 mer primers were generally reproducible and differentiated tetraploid accessions from diploid and the hybrids from their parents. Of the total 263 markers that were generated with 11 different primers, 164 markers (62%) were exhibited by 5 parents and 102 markers (38%) by 3 different F_1 hybrid combinations. In 2 of the 3 hybrids, frequency of the markers inherited from *O. punctata* was above 70%, as most of the primers amplified in the hybrid, DNA fragments inherited from *O. punctata* (male parent). Most of the markers transferred from the male parent to the hybrids were of the same intensity while those transferred from the female parent (IR-6 and Basmati-198) were of low intensity compared to their intensity in the female parent itself. The detection of the male and female parent specific fragments in the hybrids was primer dependent. Our study indicated the reproducibility and reliability of the RAPD markers in identification of wild and cultivated rice varieties and their F_1 hybrids. The significance of RAPD markers in monitoring specific characters transferred from the wild parents to the cultivated rice varieties is discussed.

Introduction

Molecular markers such as RFLP: Restriction Fragment Length Polymorphism (Jeffreys *et al.*, 1985) and RAPD: Random Amplified Polymorphic DNA (Rafalski *et al.*, 1991) are capable of producing unique pattern of bands that are being extensively used in rice breeding programs for different purposes (McCouch & Tanksley, 1991). Since RAPD markers are technically simple, quick to perform with small amount of DNA and do not require radioactive libeling (Michelmore *et al.*, 1991) therefore, they are being preferred over RFLP and are being used for tagging genes for disease resistance (Klein-Lankhorst *et al.*, 1991, Martin *et al.*, 1991, Rafalski *et al.*, 1991), for detection of genetic polymorphism in cere ls (Devos & Gale, 1992, D' Ovidio *et al.*, 1990, Weining & Langridge, 1991), for identification of cultivars (Hu & Quirose, 1991), and for fingerprinting of genomes (Nybom *et al.*, 1989, Welsh & McClleland, 1991).

In our molecular breeding programme, we have identified several salt tolerant wild rice species (Farooq *et al.*, 1992) and have used RAPD markers for differentiating salt tolerant accessions from one another. Two different tetraploid (2n=2x=48, BBCC) accessions and one diploid (2n=2x=24, BB) accession of *O. punctata* for which cultivar specific and genome specific RAPD markers were identified (Farooq *et al.*, 1994a),

were crossed as male parent with salt sensitive *O. sativa* cultivar IR-6 and Basmati-198. The F_1 hybrids appeared salt tolerant upon testing under artificial salinity of EC 12 dS/m (Farooq *et al*., 1994b). To detect the presence of any of the cultivar specific or genome specific marker in the F_1 hybrids, RAPD analyses were performed on 3 F_1 hybrids along with their 3 wild and 2 cultivated parents. Our objectives to use RAPD markers to this programme were to confirm the reproducibility of the RAPD markers specific to *O. punctata* and their pattern of inheritance in the F_1 hybrids and to select, if possible, the RAPD markers that can co-segregate with salt-tolerance in F_2 segregating population. Such markers could be used as probes for salt tolerance screening in rice breeding programs.

Materials and Methods

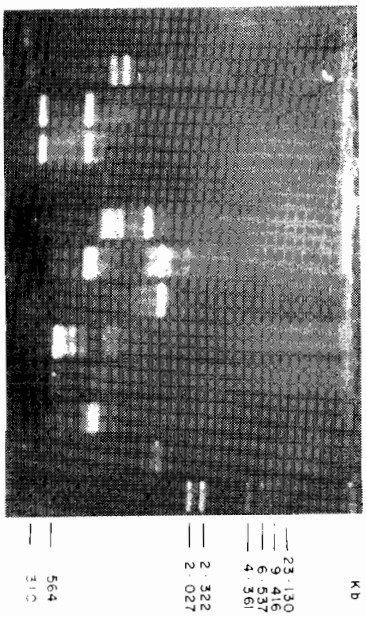
Genomic DNAs were extracted by using CTAB method (Rogers & Bendich, 1988) from 4 week old seedlings (raised in 6" diameter plastic pot filled with sand) of rice cultivars Basmati-198, IR-6, *O. punctata* accessions 101408, 105158, 101389 and different F1 interspecific hybrids made by emasculating the female parents (rice cultivars Basmati-198 and IR-6) and pollinating them with freshly dehiscent pollens collected from three different accessions of *O. punctata* used as male parents. The concentration and quality of the genomic DNAs, composition of reaction mixture, kind and source of primers and amplification conditions were similar to those used earlier (Farooq *et al*., 1994c). All the reactions were repeated twice using fresh DNA samples and data were scored from two good quality photographs.

While scoring the data, lane opposite to the lane showing molecular size markers was considered as lane one while fragment at the top of each lane was considered as fragment-1. All the DNA fragments amplified by a primer in the parents and the hybrids were considered as total fragments amplified by that primer. The amplification profiles were considered as co-dominant when the hybrids exhibited fragments of both the parents and as dominant when exhibited fragments from only one parent. The ambiguous fragments were those for which exact parentage could not be determined while polymorphic fragments were those that were absent in one of the parents. In all the 3 hybrids, fragments inherited from the male (*O. punctata*) and female (IR-6 and Basmati-198) parents were counted separately for every reaction and were compared statistically by using F-test.

Results

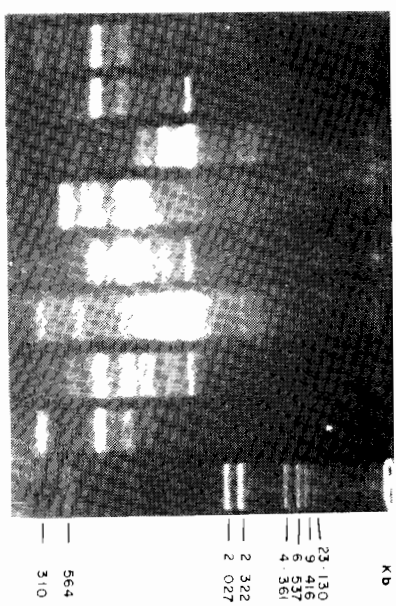
Primer S-20 amplified two high intensity DNA fragments each of approximately 0.947 (Fragment-1) and 0.43 kb (Fragment-2) in two tetraploid accessions (105158 and 101408) of *O. punctata*. In a diploid accession (101389), 5 fragments each of different intensity ranging approximately between 1.21 kb (Fragment-1) and 0.500 kb (Fragment-5) were amplified (Fig.1).

To confirm the reproducibility and the inheritance of these markers, all the 3 accessions were hybridized with cultivated rice varieties and the DNAs extracted from the parents and their F_1 hybrids were amplified again with primer S-20 (Fig.2). The



1. **Pokali**
2. ***O grandiglumis* 105669**
3. ***O. punctata* 105158**
4. ***O. punctata* 101408**
5. **Nonabokra**
6. ***O. malampuzhaensis* 105328**
7. ***Porteresia coarctata***
8. **NR-1**
9. ***O. punctata* 101389**
10. ***O. officinalis* 105322**
11. ***O. latifolia* 100891**
12. **Jhona-349**
13. **Marker**

Fig.1. RAPD profile of different wild and cultivated rice species produced with Primer S-20. Two tetraploid accessions of *Oryza punctata* (lane 3 & 4) each exhibited two major DNA fragments while a diploid accession (lane 9) exhibited 5 fragments.



1. ***O. punctata* 101408**
2. **IR-6 x *O. punctata* 101408**
3. **IR-6**
4. ***O. punctata* 101389**
5. **B-198 x *O. punctata* 101389**
6. **Bas-198**
7. **Bas-198 x *O. punctata* 105158**
8. ***O. punctata* 105158**
9. **Marker**

Fig 2. RAPD profile of three accessions of *Oryza punctata* and their F_1 hybrids with rice cultivar IR-6 and Basmati 198. Primer S-20 reproduced major DNA fragments specific for tetraploid accessions (lane 1 & 8) and diploid accessions (lane 4). The DNA fragments specific for *Oryza punctata* and rice cultivars are present in the F_1 hybrids.

two high intensity markers each specific for accessions 101408 and 105158 and 5 for accession 101389 re-appeared at the same place in their respective accessions (Fig.2, lane 1, 4 and 8). Marker-1 from each of accessions 101408 and 105158 and markers 2 and 3 of accession 101389 also re-appeared in their respective hybrids with rice cultivars IR-6 (lane 2) and Basmati-198 (lane 6). Fragment-1 (low intensity) in all the 3 hybrids (lanes 2, 5 and 7) seemed to be inherited from the female parent (lanes 3 and 6 where they were highly intense). The amplification profile of the hybrids thus showed a co-dominant appearance.

To confirm the consistency of the inheritance pattern of the RAPD markers and co-dominant appearance of the amplification profile, freshly extracted DNAs from all the parents and the hybrids were amplified again with primer S-20 as well as with primer S-1 and S-13. Primer S-20 again showed co-dominant pattern of the amplification profile however, primer S-1 and S-13 produced different patterns.

Primer S-1 amplified 3 and 6 DNA fragments respectively in two tetraploid and a diploid accession of *O. punctata* (Fig.3, lane 1 and 4) and all of them reappeared in their respective hybrids (lane 2 and 5) while none of the fragments amplified in the rice cultivar IR-6 (lane 3) and Basmati-198 (lane 6) appeared in the hybrid. Since S-1 mediated DNA amplification in accession 105158 (lane 8) was not detected (due to some mistake) and the hybrid between Basmati-198 and *O. punctata* accession 105158 did not show amplification of any DNA fragment specific to Basmati-198 either, therefore, it was presumed that all the DNA sequences amplified by primer S-1 in the hybrid were perhaps from the accession 105158 (Fig.3).

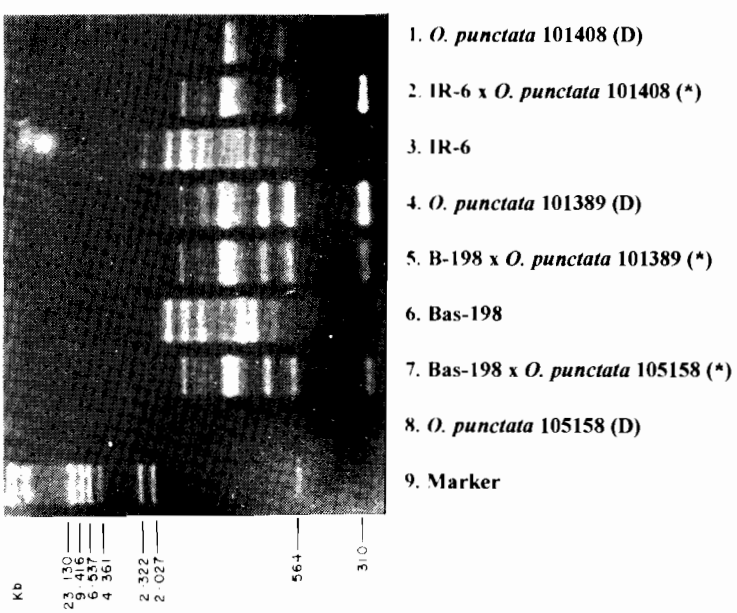


Fig.3. RAPD profile produced with primer S-01. The DNA fragments amplified in all the hybrids are from *Oryza punctata* accessions only.

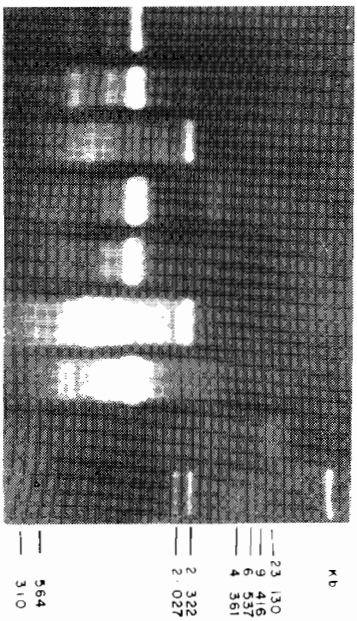


Fig.4. RAPD profile produced with primer S-13. The three F_1 hybrids are showing one major fragment of their respective *Oryza punctata* accessions (male parent). There is no visible fragment either from IR-6 or Basmati-198 (female parents) in any of the F_1 hybrids.

Unlike primer S-1, S-13 amplified one high intensity fragment in all the parents and the hybrids except Basmati-198 where 4 different DNA fragments were amplified (Fig.4). However, like primer S-1, S-13 also did not amplify in the hybrid any of the DNA fragments specific for the female parents. In both these cases, all the DNA fragments present in the hybrid were from the male parents. Since the 3 primers produced different results in the parents and the hybrids therefore, it was decided to use more primers to get clear picture of the results.

Considerable differences were observed in the number of fragments amplified with 11 different primers (Table 1) in the rice cultivars used as female parent (IR-6 and Basmati-198) and the wild rice species used as male parents (*O. punctata* accessions 101408, 101389 and 105158). In hybrid 2H (IR-6 with *O. punctata* accession 101408), 7 of the 11 primers (64%) amplified DNA fragments that were specific for the male parent, one primer (S-9) amplified fragments specific for the female parent while 3 primers (S-16, 19 and 20) amplified DNA fragments specific for both the parents. Similarly, in the hybrid 5H (Basmati-198 with *O. punctata* accession 101389), 8 primers (73%) amplified DNA fragments specific only for the male parent, one primer (S-19) amplified fragments from the female parent while 2 primers (S-16 and S-18) amplified fragments from both the parents. In hybrid 7H (Basmati-198 with *O. punctata* accession 105158) on the other hand, 5 of the 11 primers (45%) amplified fragments from the male parent, 3 primers (S-12, 16, and 19) amplified fragments from the female parent while another 3 primers (S-1, 18 and 20) amplified fragments from both the parents (Table 1).

Table 1. DNA fragments amplified by different primers in 2 rice cultivars, 3 accessions of *Oryza punctata* and their F1 hybrids.

Primer designation and sequence	Distribution of scorable fragments in parents and hybrids							
	1A	2H	3B	4A	5H	6B	7H	8A
S-1 CTACTGCGCT	4	6(4A)	7	6	5(5A)	5	5(4A + 1B)	0
S-2 CCTCGACTG	3	3(3A)	1	3	3(3A)	1	1(1A)	1
S-6 GATACCTCGG	4	4(4A)	2	5	5(5A)	3	2(1A)	1
S-7 TCCGATGCTG	1	1(1A)	7	1	1(1A)	3	1(1A)	1
S-9 TCCTGGTCCC	1	3(2B)	2	3	3(3A)	2	3(3A)	3
S-12 CTGGGTGAGT	3	3(3A)	0	4	4(4A)	4	1(1B)	1
S-13 GTCGTTCCTG	1	1(1A)	1	1	1(1A)	4	2(2A[a])	0
S-16 AGGGGGTTCC	4	3(2A + 1B)	1	4	4(3A + 1B)	3	4(3B)	1
S-18 CTGGCGAACT	6	6(6A)	5	4	5(5A)	7	3(2A + B)	3
S-19 GAGTCAGCAG	4	3(2A + B)	6	4	6(5B)	6	6(5B)	4
S-20 TCTGGACGGA	3	4(3A + 1B)	3	5	3(2A + 1B)	4	5(2A + 3B)	3

A 1,4 and 8 respectively are *O. punctata* accessions 101408, 101389 and 105158.

H refer as Hybrid.

B 3 and 6 respectively are rice cultivars IR-6 and Basmati-198.

[a] Contribution from parent A is not evidenced.

Figures in paranthesis are numbers of fragments transferred from respective parents.

Taking all the primers into consideration a total of 34 DNA fragments were amplified in hybrid 2H of which 26 fragments (76 %) were from the male parent which are significantly higher ($p > 0.01$) than the 5 fragments (15 %) specific for female parents. In addition to this, 3 polymorphic fragments were also amplified specifically in the hybrid that were absent in both the parents (Table 2). Similarly, in the hybrid 5H (Basmati-198 with *O. punctata* accession 101389), of the 36 fragments that were ampli-

fied, 28 fragments (78 %) and 7 fragments (19 %) respectively were from the male and female parents with one polymorphic fragment that was absent in both the parents (Table 2). In hybrid 711 (Basmati-198 with *O. punctata* accession 105158), 17 of the 32 fragments (53 %) were inherited from the male parent, 13 (41 %) from female parent while there were 2 polymorphic fragments in addition to 6 (19%) ambiguous fragments for which correct parentage could not be determined (Table 2).

Discussion

Since RAPD markers are technically simple and do not require radioactive labeling therefore, they are being preferred over RFLP markers. However, there are also some concerns associated with RAPD markers (Devos & Gale, 1992, Heun & Helentjaris, 1993) which included non reproducibility, non reliability and "epistatic" influences on the RAPD markers. From the present study, it is evident that RAPD markers are reproducible and heritable while their detection in the F_1 hybrids is primer dependent.

In wide hybridization, co-dominant phenotype of the hybrid plant indicates expression of the alien gene in the genetic background of the cultivated varieties (Farooq, 1990). The hybrids used in the present study also possessed co-dominant phenotype and the expression of salt tolerance genes transferred from *O. punctata* to cultivated rice varieties were also tested by screening the hybrids for salt-tolerance. Since the cultivar specific RAPD markers were identified for both the parents before hybridization therefore, we anticipated and did succeed to amplify in the hybrid, cultivar specific markers for both parents but, only with 18-27 % of the primers. In most of the hybrids, 70-78 % of the primers used in the study, amplified RAPD markers that were specific for the male parents. Even in the hybrids where amplification profile exhibited markers specif-

Table 2. Details of parent specific DNA fragments amplified in the different F_1 hybrids by different primers.

Hybrid Combinations	Total visible fragments amplified in the hybrid	No. of fragments transferred from male parents (%)	No. of fragments transferred from female parent (%)	Other fragments amplified in hybrids (%)
IR-6 x *Oryza punctata* (101408)	34	26(76.0)	5(15.0)	3(9.0)
Bas-198 x *O. punctata* (101389)	36	28(78.0)	7(19.0)	1(3.0)
Bas-198 x *O. punctata* (105158)	32	17(53.0)	13(41.0)	2(6.0) 6(19.0)

Ambiguous fragments

ic for both the parents (co-dominant pattern of inheritance), the intensity of the markers inherited from the female parent was low as compared with the intensity of the same marker that appeared in the female parent itself (Fig.2). The intensity of the markers specific for the male parent generally remained unchanged. Also, when more than one markers were amplified in the female parent, all of them were not amplified in the hybrid even with the primers that amplified in the hybrids, markers from both the parents. These observations indicated that RAPD markers are though heritable, their amplification in the hybrid is definitely subjected to "epistatic" effects which confirms the reports of Heun & Helentjaris (1993) and Reiter *et al*., (1992) who suggested to use one or two high intensity RAPD markers as most reliable and predictable fragments. In the present study also, high intensity RAPD markers proved reproducible and despite many markers amplified in the parents, the hybrid exhibited only one or 2 of them with greatest intensity.

In the present study, two types or primers were identified: those which amplified DNA fragments from the male parent only and those which amplified in the hybrids DNA fragments from both the parents. Since the inheritance of the markers was studied in the F_1 hybrids where both the parents were in the haploid state, therefore, identification of the primers which amplify fragments specific to only one parent is very important as it is possible that the same primer might again amplify in the BC1 progenies, fragments specific to the male parent only. This situation would be of significance since we are interested only in the markers of the male parents because of their salt-tolerance and identification of such markers in the BC1 population would provide an opportunity to correlate the level of salt tolerance of individual BC1 plant with the marker specific to *O. punctata* (male parent). However, since in the BC1 population, the female parent would be in the diploid state therefore, the markers specific for the female parent might dominate the markers specific for the male parent. Although this possibility still remains to be seen, nevertheless, under these conditions it would be better to label the greatest intensity markers specific to male parent and use them as probes to screen BC_1 population for salt tolerance, which would help tagging genes for salt tolerance.

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