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# DNA fingerprinting of brown planthopper resistant rice cultivars

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## ABSTRACT

*Forty two primers specific for rice microsatellite markers were used for fingerprinting and assessing genetic diversity of 19 rice cultivars differing in resistance to brown planthopper. Thirty six primers revealed polymorphism among cultivars. A total of 128 alleles were amplified, of which 120 were polymorphic. The number of alleles varied from 1 to 13 with an average of 3.05 alleles per locus. The polymorphism information content (PIC) ranged from 0 to 0.952 with an average of 0.633 per polymorphic locus, indicating the suitability of the microsatellite markers for detecting genetic diversity among these rice cultivars. Genetic similarities among cultivars varied from 0.38 to 0.897 with an average of 0.604. All the cultivars could be differentiated and grouped into two major clusters at 54% level of genetic similarity. Seven unique alleles were identified which would be useful for developing diagnostic markers*

***Key words:*** *rice, brown planthopper, genetic diversity, microsatellite, molecular marker*

One of the major constraints for stable higher rice production is the biotic stress. Rice is attacked by several insect pests both in field and storage conditions (Barrion and Litsinger, 1994). Brown planthopper (*Nilaparvata lugens* Stal ) is one of the major pests of rice in South-East Asia and can cause significant yield loss up to 60% in susceptible rice varieties. Conventional methods of controlling BPH depend on spraying of insecticides, which is costly in terms of labour, money and environment. Furthermore, overuse of insecticides has killed natural enemies of BPH and enhanced its resistance to insecticides, which stimulated BPH resurgence (Heinrichs and Mochida, 1984). Host-plant resistance seems to be the best alternative and is likely to be more durable if it employs an array of resistance genes encoding diverse mechanisms of resistance. Durability of resistance depends on the knowledge of genetics of resistance and diversity present in the rice cultivars. Consistent efforts have been made to identify genes for BPH resistance from various sources. Twenty five major genes and many QTLs controlling resistance to BPH has been identified both in cultivated and wild rice (Jena *et al*., 2006; Liu *et al*., 2009; Rahman *et al*., 2009a; Yara *et al*., 2010).

DNA fingerprinting is a simple, reliable and cost effective molecular marker technique for varietal identification, diversity analysis, assessment of phylogenetic relationships, prevention of unauthorized commercial use of varieties and protection of intellectual property rights through Plant Breeders' Rights (PBR). Several molecular marker techniques have been developed during last two decades. Microsatellite or simple sequence repeat (SSR) markers are considered to be the best as they are co-dominant, multi-allelic, highly polymorphic, reliable, abundant, distributed throughout the genome and can be easily analysed using polymerase chain reaction (Mc Couch *et al*., 1997). These molecular markers have been extensively used for identification and assessment of genetic diversity of varieties, gene and genome mapping.

The present study was undertaken to assess the genetic diversity of rice cultivars having different degrees of resistance to BPH, to identify genotype specific markers and to develop DNA fingerprint/ profile data set using molecular markers.

## MATERIALS AND METHODS

A set of 19 rice cultivars having different degree of resistance to BPH were selected for DNA fingerprinting (Table 1). Fifteen seeds per cultivar were

**Table 1.** List of rice cultivars used for DNA fingerprinting

| Sl. No. | Name of cultivars | Reaction to BPH* |
|---|---|---|
| 1 | Lalat | S |
| 2. | Pooja | HS |
| 3 | Samba Mahsuri | S |
| 4 | Sonasali | MS |
| 5 | Triguna | HS |
| 6 | Mansarovar | MS |
| 7 | Uday | MS |
| 8 | Daya | S |
| 9 | Sahiba | MR |
| 10 | Jhup jhup | MR |
| 11 | Banaspati | R |
| 12 | Lahudi | MR |
| 13 | Baidyaraj | R |
| 14 | Swarna | S |
| 15 | TN1 | HS |
| 16 | IR64 | MR |
| 17 | PTB-33 | R |
| 18 | Dhobanambari | R |
| 19 | Salkathi | R |

* R.= resistant S= susceptible, MR= moderately resistant , MS= moderately, susceptible, HS= highly susceptible

germinated in aseptic condition at room temperature. Five day old seedlings were transplanted in individual pots in green house. After 25 days of transplanting, leaves of young plants were harvested from 10 plants and bulked for each cultivar. Genomic DNA was isolated from 3 to 5gram of bulked leaf samples following CTAB method (Murray and Thompson, 1990). The quality and quantity of genomic DNA was estimated by using spectrophotometer and agarose gel electrophoresis. The samples were diluted in $T_{10}E_1$ buffer to get final concentration of 20 ng $\mu l^{-1}$ for PCR amplification.

A set of 42 primers specific for microsatellite loci distributed over 12 chromosomes of rice were used for PCR amplification (Table 2). The amplification was carried out in a 20 µl reaction mixture volume containing 40 ng of genomic DNA, 1X PCR buffer {75 mM Tris-HCl (pH 9.0), 50 mM KCl, 20 mM $(NH_4)_2SO_4$}, 200 µM dNTP mix (MBI Fermantas, Lithuania, USA), 4 picomole of each of forward and reverse primers, 2mM of $MgCl_2$ and 1U of *Taq (Thermus aquaticus)* DNA polymerase (Biotools, Spain). The PCR was performed in a thermal cycler (Thermal Cycler, Perkin Elmer, Cetus) as per following cycling parameters: initial denaturation at $93^0$C for 3 min followed by 36 cycles of denaturation at $93^0$C for 1 min, annealing at 55-$65^0$C (depending upon primer) for 1 min and extension at $72^0$C for 1.5 min and final extension at $72^0$C for 5 min. The amplified products were separated on 2.5% agarose gel containing ethidium bromide using 1X TBE buffer. The gels were visualized under UV and photographed using a gel documentation system (Fluor Chem™ 5500, Alpha Innotech, USA) to detect polymorphism. The size of amplified fragments was determined by using size standards (50bp DNA ladder, MBI Fermentas, Lithuania).

The amplified bands/alleles were scored as present (1) or absent (0) for each primer genotype combination. The data was entered into a binary matrix (0/1) and subsequently analysed using the computer package NTSYS-pc Version 2.02 (Rolf, 1998). The total number of bands/alleles per locus, percentage of polymorphic alleles, low frequency alleles, high frequency alleles, unique alleles and polymorphism information content (PIC) were calculated to assess diversity of alleles of a marker locus. The polymorphism information content (PIC) was calculated using the formula, $PIC = 1-\sum Pij^2$, where Pij is the frequency of $j^{th}$ allele for the $i^{th}$ locus and summation extends over n alleles (Anderson *et al*., 1993). Dice similarity coefficients were calculated and used to assess the genetic relationship among 19 rice genotypes (Ni and Li, 1979). The genetic distance matrix data were used to construct dendrogram using unweighted pair group method using arithmetic averages (UPGMA) and sequential agglomerative hierarchal nested (SHAN) cluster analysis. The Cophenetic correlation coefficient (Lapointe and Legendre, 1992) was calculated to measure the goodness of fit of clusters. In order to find the efficiency of SSR markers for differentiation of genotypes, the discriminating power (D) of each marker loci was calculated following formula, $Dj = 1-Cj = 1-\sum Pi\ (NPi-1)/(N-1)$, where Dj is discriminating power of $j^{th}$ locus, Pi is frequency of $i^{th}$ allele, Cj confusion probability of $j^{th}$ locus (Tessier *et al*., 1999). Further, in order to know minimum number of marker loci required to identify and differentiate genotypes from each other, total number of non-differentiated pairs (Xj) of genotypes were calculated for the $j^{th}$ locus using formula, $Xj = \{N(N-1)/2\}Cj$. The average similarity index for all pair wise comparisons ($\overline{X}_D$) were calculated and used to estimate the probability of DNA

**Table 2.** Details of SSR markers found among 19 rice cultivars

| Sl. No. | Chrom# | Marker loci | TA | PA | LFA | CA | Size(bp) | PIC | D |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 1 | 34 | 2 | 1 | 0 | 2 | 200-900 | 0.145 | 0.149 |
| 2 | 1 | 84 | 3 | 3 | 2 | 1 | 110-130 | 0.796 | 0.805 |
| 3 | 1 | 212 | 2 | 2 | 1 | 1 | 120-130 | 0.578 | 0.585 |
| 4 | 1 | 428 | 2 | 2 | 0 | 2 | 180-300 | 0.240 | 0.246 |
| 5 | 1 | 495 | 2 | 2 | 1 | 1 | 150-175 | 0.694 | 0.705 |
| 6 | 2 | 211 | 3 | 3 | 1 | 2 | 125-250 | 0.869 | 0.881 |
| 7 | 2 | 263 | 3 | 3 | 1 | 2 | 170-200 | 0.866 | 0.877 |
| 8 | 2 | 485 | 5 | 5 | 1 | 3 | 250-400 | 0.801 | 0.850 |
| 9 | 2 | 530 | 3 | 3 | 1 | 1 | 125-235 | 0.759 | 0.764 |
| 10 | 3 | 7 | 2 | 2 | 0 | 2 | 120-125 | 0.716 | 0.728 |
| 11 | 3 | 203 | 13 | 13 | 8 | 4 | 150-800 | 0.905 | 0.915 |
| 12 | 3 | 218 | 3 | 3 | 1 | 2 | 140-170 | 0.851 | 0.862 |
| 13 | 3 | 232 | 3 | 3 | 1 | 2 | 160-175 | 0.851 | 0.862 |
| 14 | 3 | 565 | 3 | 3 | 1 | 2 | 160-550 | 0.831 | 0.825 |
| 15 | 4 | 307 | 5 | 5 | 4 | 1 | 140-360 | 0.779 | 0.786 |
| 16 | 4 | 317 | 2 | 2 | 1 | 1 | 170-175 | 0.666 | 0.675 |
| 17 | 4 | 401 | 4 | 4 | 0 | 4 | 200-380 | 0.640 | 0.652 |
| 18 | 4 | 470 | 2 | 2 | 1 | 1 | 120-130 | 0.679 | 0.690 |
| 19 | 5 | 169 | 2 | 2 | 0 | 2 | 200-240 | 0.733 | 0.746 |
| 20 | 5 | 334 | 4 | 4 | 3 | 1 | 180-225 | 0.913 | 0.923 |
| 21 | 5 | 440 | 1 | 0 | 0 | 0 | 180 | 0 | 0 |
| 22 | 6 | 30 | 3 | 3 | 2 | 1 | 90-115 | 0.780 | 0.788 |
| 23 | 6 | 253 | 2 | 2 | 0 | 2 | 125-150 | 0.679 | 0.690 |
| 24 | 6 | 275 | 1 | 0 | 0 | 0 | 125 | 0 | 0 |
| 25 | 6 | 541 | 3 | 3 | 1 | 1 | 220-250 | 0.692 | 0.696 |
| 26 | 6 | 557 | 1 | 0 | 0 | 0 | 160 | 0 | 0 |
| 27 | 7 | 125 | 1 | 0 | 0 | 0 | 140 | 0 | 0 |
| 28 | 7 | 478 | 5 | 5 | 1 | 4 | 190-420 | 0.662 | 0.673 |
| 29 | 8 | 152 | 3 | 3 | 2 | 1 | 140-180 | 0.844 | 0.854 |
| 30 | 8 | 433 | 2 | 2 | 1 | 1 | 150-170 | 0.494 | 0.497 |
| 31 | 8 | 447 | 2 | 2 | 0 | 2 | 130-140 | 0.744 | 0.757 |
| 32 | 9 | 215 | 1 | 0 | 0 | 0 | 140 | 0 | 0 |
| 33 | 9 | 278 | 1 | 0 | 0 | 0 | 150 | 0 | 0 |
| 34 | 9 | 444 | 5 | 5 | 3 | 2 | 175-360 | 0.906 | 0.915 |
| 35 | 10 | 330A | 3 | 3 | 2 | 1 | 180-300 | 0.633 | 0.645 |
| 36 | 10 | 333 | 7 | 7 | 3 | 2 | 200-750 | 0.952 | 0.96 |
| 37 | 11 | 202 | 2 | 2 | 0 | 2 | 200-210 | 0.654 | 0.667 |
| 38 | 11 | 224 | 2 | 2 | 0 | 2 | 275-500 | 0.733 | 0.746 |
| 39 | 12 | 17 | 4 | 4 | 2 | 2 | 150-190 | 0.922 | 0.931 |
| 40 | 12 | 20 | 2 | 1 | 1 | 1 | 230-280 | 0.488 | 0.491 |
| 41 | 12 | 491 | 7 | 7 | 5 | 1 | 275-500 | 0.864 | 0.871 |
| 42 | 12 | 519 | 2 | 2 | 1 | 1 | 140-150 | 0.633 | 0.64 |

TA- Total number of alleles, PA-number of polymorphic alleles, LFA-low frequency alleles, CA- common alleles, PIC- polymorphism information content, D-discriminating power

fingerprints of two cultivars being identical by chance as described by Ramakrishna *et al.* (1994) employing formula $(\overline{X}_D)^n$ where, $\overline{X}_D$ = average similarity index and n = the average number of amplified alleles per cultivar.

## RESULTS AND DISCUSSION

A total of 128 reproducible bands/alleles were amplified of which 120 (93.8%) were polymorphic. The number of alleles per locus ranged from 1(RM125, RM215, RM275, RM278, RM440 and RM557) to 13 (RM203) with an average of 3.05 (Table 2). Thirty six out of 42 primers revealed polymorphism between cultivars. The number of alleles per microsatellite locus detected in the present study corresponded well with earlier reports (Nagaraju *et al*., 2002; Ram *et al*., 2007; Joshi *et al*., 2010). The number of alleles detected by a single SSR locus varied from 1 to 31 depending upon the fingerprinting techniques and kind of germplasm used in the studies (Blair *et al*., 2002; Jain *et al*., 2004; Lu *et al*., 2005; Jayamani *et al*., 2007; Kaushik *et al*., 2011).

The overall size of bands/alleles varied from 90bp (RM30) to 900bp (RM34). The size difference between the smallest and largest allele at a given SSR locus varied from 5bp (RM7 and RM17) to 700bp (RM34). The number of alleles per cultivar varied from 48 (Pooja) to 71 (Sambha Mahsuri) with an average on 53.5 alleles per cultivar. Sixty six (49.2%) common alleles and fifty three (40.6%) low frequency alleles were identified among 19 rice cultivars. An allele that was observed in > 30% of 19 rice genotypes was considered to be common allele/ high frequency/ abundant while an allele having frequency between 5% and 30% is called as a low frequency/ intermediate allele. All the 42 primers amplified at least one common allele. On an average, 65.79% of the 19 rice genotypes shared common allele at any of 42 loci. Similar results were also observed by others (Jain *et al*., 2004; Jayamani *et al*., 2007). However, Kaushik et al (2011) observed that 38.5% of 50 rice genotypes shared common alleles at any SSR locus.

The PIC value provides an estimate of discriminating power of a marker locus. The PIC values for 42 microsatellite loci varied from 0 (RM125, RM215, RM275, RM278, RM440 and RM557) to 0.952 (RM333) with an average of 0.684 per polymorphic locus (Table 2). Thirty two loci showed high PIC values (> 0.50). The estimated PIC values are relatively higher and might be due to higher genetic diversity present in selected rice cultivars. Similar to our findings higher PIC values for SSRs were also reported in the literature (Ram *et al*., 2007; Joshi *et al*., 2010; Upadhyaya *et al*., 2011). PIC values showed a positive correlation (r = 0.547, P < 0.01) with number of alleles at a SSR locus. Similar to our observation, Kaushik *et al*.(2011) found a positive correlation between PIC and number of alleles at SSR locus.

Seven diagnostic or unique alleles were identified in six cultivars at six microsatellite loci (Table 3). Two alleles were amplified in resistant cultivar, PTB13 while one allele was amplified in each of resistant cultivar, Salkathi and moderately resistant cultivar, IR64. The primer, RM333 amplified one allele each in high yielding varieties, Lalat and TN1 while RM541 amplified one allele in popular variety, Swarna. These unique alleles/bands would be useful for development of diagnostic markers for identification cultivars.

**Table 3.** SSR markers amplifying diagnostic/unique alleles

| Sl. No. | Markers loci | Allele size (bp) | Name of Cultivars* |
|---|---|---|---|
| 1 | 203 | 620 | PTB33 (R) |
| 2 | 333 | 700 | Lalat (S) |
| 3 | 333 | 750 | TN1(HS) |
| 4 | 485 | 295 | PTB33(R) |
| 5 | 491 | 450 | IR64(MR) |
| 6 | 530 | 125 | Salkathi(R) |
| 7 | 541 | 210 | Swarna (S) |

*Reactions of cultivars to BPH are given in the parenthesis

Ten alleles were amplified only in two or more resistant or moderately resistant cultivars (Table 4). Three primers, RM218, RM232 and RM 433 amplified allelesin Salkathi and Dhobanambari while the primers, RM20 and RM519 amplified alleles in Salkathi and Dhobanambari in addition to Sahiba and Vaidyaraj, respectively. The primer, RM84 amplified an allele in PTB33, Sahiba and Banaspti. However, the relation of these alleles to BPH resistance has to be assessed using mapping populations.

Genetic similarity coefficients varied from 0.38 to 0.897 with an average of 0.604, indicating a wide

**Table 4.** SSR marker amplifying alleles in resistant/ moderately resistant cultivars

| Sl. No. | Marker loci | Allele size (bp) | Name of Cultivars* |
|---|---|---|---|
| 1 | 20 | 280 | Salkathi(R), Dhobanambari ( R), Sahiba (MR) |
| 2 | 84 | 110 | Sahiba (MR), Banspati (R), PTB33 (R) |
| 3 | 203 | 400 | Jhupjhup(MR), Banaspati(R) |
| 4 | 218 | 140 | Salkathi(R), Dhobanabari (R) |
| 5 | 232 | 165 | Salkathi(R), Dhobanambari(R) |
| 6 | 334 | 215 | Salkathi(R), Jhupjhup(MR) |
| 7 | 433 | 150 | Salkathi(R), Dhobanambari(R) |
| 9 | 519 | 140 | Salkathi(R),Dhobanambari(R) and Baidyaraj(R) |
| 10 | 530 | 235 | Jhupjhup(MR), Baidyaraj(R) |

*Reactions of cultivars to BPH are given in the parenthesis

range of genetic variation present in rice cultivars having different degrees of resistance to BPH. Salkathi showed the highest genetic similarity with Dhobanambari (i.e. 0.897) while TN1 showed the least similarity with Salkathi (i.e. 0.238). IR64 showed high level of genetic similarity with Triguna (0.822) and Daya (0.817). Both Salkathi and Dhobanabbari are land races and resistant to BPH while TN1 is highly susceptible to BPH. Similar to our observations, other studies using SSR markers revealed varying degrees of genetic similarity among the accessions of cultivated and wild species of rice (0.22-0.9: Juneja *et al*., 2006; 0.09- 1.0: Jayamani *et al*., 2007).

Cluster analysis based on UPGMA provided a clear resolution of relationships among the 19 rice cultivars. The Cophenetic correlation coefficients (r = 0.783) revealed the reliability and stability of clustering. Two major clusters were observed at 54% of genetic similarity coefficient index (Fig. 2). First major cluster contained five rice genotypes with an average similarity index of 0.697 and further sub-grouped into two sub-clusters, IA and IB. The four varieties, Lalat, Triguna, Daya and IR4 were included in IA while TN1 was included in IB. Interestingly, all the five are released high yielding varieties and are suitable for irrigated and medium land ecosystem (Table 5). Further, these varieties except IR64 are susceptible to BPH. Fifteen genotypes were included second major cluster with an average similarity index of 0.63. Further, it was sub-grouped into six sub-clusters, IIA, IIB, IIC, IID, IIE and IIF having 3, 2, 4, 2, 1 and 2 rice cultivars, respectively (Table 5). The high yielding susceptible cultivars, Pooja and Swarna, and resistant cultivar, Baidyraj were included in sub-cluster IIA. Two moderately resistant cultivars, Sahiba, Lahudi were included in the sub-cluster, IIB while two moderately susceptible cultivars, Sonamani and Mansarovar were included in sub-cluster, IID. The sub-cluster, IIB contained one moderately resistant cultivar, Jhupjhup

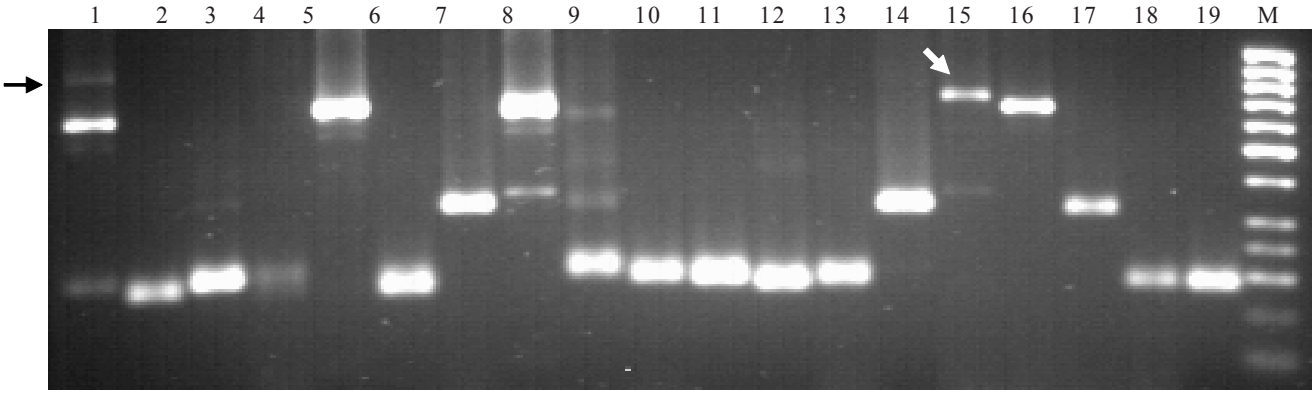


**Fig. 1. DNA profiles of rice cultivars obtained with microsatellite primer, RM 333**

The lane number on the top of gel corresponds to a rice cultivar as given in the Table 1, M-50bp DNA ladder, arrows indicate the diagnostic/ unique alleles.

1-Lalat, 2- Pooja, 3-Samba Mahsuri, 4-Sonasali, 5- Triguna, 6- Mansarovar, 7- Uday, 8-Daya, 9-Sahiba, 10-Jhup jhup, 11-Banaspati, 12-Lahudi, 13-Baidyaraj, 14-Swarna, 15-TN1, 16-IR64, 17-PTB-33, 18-Dhobanambari, 19-Salkathi

**Table 5.** Clustering pattern of rice cultivars differing in resistance to BPH

| Major Clusters | Sub Clusters | No. of cultivars | Name of cultivars* |
|---|---|---|---|
| I | - | 4 | Lalat(S), Triguna(HS), IR64 (MR), TN1(HS), Daya(MS) |
| II | IIA | 3 | Pooja(HS), Baidyraj(R), Swarna (S) |
| | IIB | 2 | Sahiba(MR), Lahudi(MR) |
| | IIC | 4 | Jhuapjhup(MR), Banaspati(R), Salakathi(R), Dhaobanambari (R) |
| | IID | 2 | Sonamani(MS), Mansarovar(MS) |
| | IIE | 1 | Samba Mashuri(S) |
| | IIF | 2 | Uday (MS), PTB33(R) |

*Reactions of cultivars to BPH are given in the parenthesis

and three resistant cultivars, Banaspati, Salakathi, Dhaobanambari. The sub-cluster, IIF contained moderately susceptible cultivar, Uday and resistant cultivar, PTB33. Only one susceptible high yielding cultivar, Samba Mashuri was included in IIE. The present study has resulted in grouping of cultivars based on their reaction to BPH except few exceptions. Based on the above clustering pattern, breeding experiments may be initiated between diverse cultivars with resistance to BPH for getting more genetic variation, transgressive segregants and with genes of diverse mechanisms of resistance to BPH.

All the rice cultivars used in the present study could be distinguished precisely from each other. None of the microsatellite locus could differentiate all the cultivars. The discriminating power of microsatellite loci varied from 0 (RM125, RM215, RM275, RM278, RM440 and RM557) to 0.960(RM333) (Table 2). On the basis of discriminating power, the minimum number of microsatellite loci required to differentiate between genotypes in the present study was found to be four (i.e. RM17, RM203, RM333, and RM334). These four SSR loci amplified a total of 28 alleles, all being polymorphic. The frequency of these alleles ranged from 1/19 to 11/19. Similar results were obtained by others (Olufowote *et al*., 1997; Rahman *et al*., 2009b). Rahman *et al*. (2009b) were able to discriminate twenty eight rice varieties which included thirteen high yielding, fourteen local and a wild rice cultivars using only three

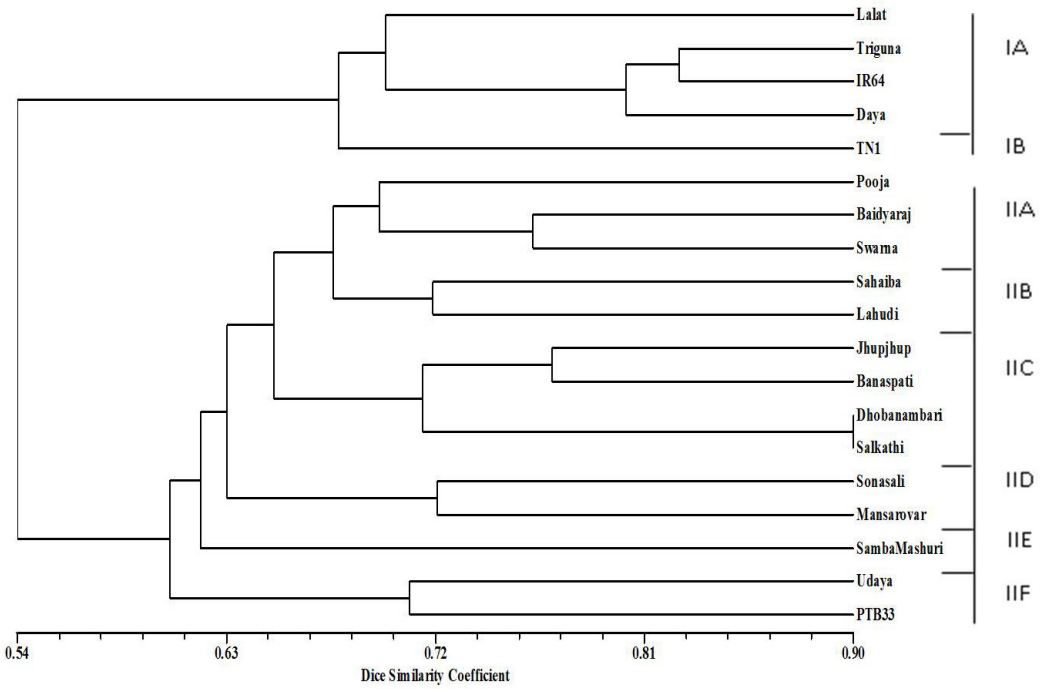


**Fig. 2. Dendrogram showing genetic relationships between rice cultivars differing in resistance to BPH**

Scale on the bottom indicates the Dice Similarity Coefficient. The major clusters and sub-clusters are indicated on right margin.

**Table 6.** Analysis of DNA fingerprints using different sets of SSR loci

| Attributes | 4 SSR loci | 40 SSR loci |
|---|---|---|
| Average bands/alleles for each cultivar | 6.947 | 53.53 |
| Average similarity index ($\overline{X}_D$) | 0.28 | 0.604 |
| Probability of identical match by chance $(\overline{X}_D)^n$ | $1.44X10^{-4}$ | $1.9X10^{-12}$ |

microsatellite markers. The combination of all the polymorphic and non-polymorphic alleles obtained with all the 42 SSR loci enabled development of DNA fingerprint/ profile data set for 19 rice cultivars differing in resistance to BPH (data not shown), which would be very useful for future reference. The probability of identical match was found to be $1.44 \times 10^{-4}$ based on the DNA fingerprints/ profiles generated by the four most discriminating microsatellite loci ( i.e. RM17, RM203, RM333 and RM334), suggesting that $10^4$ rice cultivars can be distinguished by using these four loci. Inclusion of all the 42 microsatellite loci provided a very high resolution power enabling nearly $10^{12}$ cultivars to be precisely identified (Table 6). Several workers have been demonstrated the utility and power of microsatellite markers in establishing distinction of rice varieties (Santhy *et al*., 2000; Singh *et al*., 2004).

The present study indicated that microsatellite markers are efficient in identification and assessment of genetic diversity present in rice cultivars having different degrees of resistance to BPH. A basic molecular data set was created for these rice cultivars which can be used for variety registration and protection of the plant breeders as well as farmers' rights. We suggest a wider survey and collection of BPH resistant rice cultivars from different geographical regions of India in order to conserve maximum diversity and molecular cataloguing.

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