

RESEARCH ARTICLE

Md. Monirul Islam
Md. Enamul Hoque
Ripon Kumar Ray
Md. Shamiul Haque
Md. Shamsheer Ali

DNA fingerprinting and diversity analysis in Aus genotypes using microsatellite markers

Authors' address:

Biotechnology Division
Bangladesh Rice Research Institute,
Gazipur-1701, Bangladesh.

Correspondence:

Md. Monirul Islam
Biotechnology Division
Bangladesh Rice Research Institute,
Gazipur-1701, Bangladesh.
e-mail: mislambrrri73@gmail.com

Article info:

Received: 16 October 2014

Accepted: 29 January 2015

ABSTRACT

DNA fingerprinting and genetic diversity of 94 Aus (6 BRRI released Aus variety and 88 local Aus landraces) genotypes were carried out to protect the Aus landraces from biopiracy. A total of 91 microsatellite markers were tested for screening the genotypes. Among 91 amplified products, 56% have polymorphic bands giving 195 alleles. The number of alleles per locus ranged from four (RM25 and RM147) to twenty seven (RM519), where average allele number was 9.76. The Polymorphism Information Contents (PIC) lied between 0.455 (RM5) to 0.934 (RM519). Most robust marker was found RM519 since it provided the highest PIC value (0.934). Pair-wise genetic dissimilarity co-efficient showed the lowest genetic dissimilarity was found BRRI dhan42 and BRRI dhan43 and the highest genetic dissimilarity was found local landraces each other. Here it is shown that most Aus landraces is recognized to have broad genetic base. Thus it is recommended to use these landraces for future breeding program or include new and untouched local landraces to incorporate new genes and broaden genetic base.

Key words: Aus rice, genetic diversity, microsatellite markers, DNA fingerprinting

Introduction

Rice (*Oryza sativa*) is one of the most important food crops in the world and about three billion people, nearly half the world population, depend on rice for survival. Rice occupies 77% of total cropped area in Bangladesh. It provides 75% of the calories and 55% of proteins in the average daily diet of the Bangladeshi peoples (Bhuiyan *et al.*, 2002). Many scientists in the world research have been conducted in the Bangladeshi local landraces, but research on indigenous landraces in our research organization is very limited. Amanda *et al.* (2004) were conducted a study on 234 rice landraces in Plant breeding division, Cornell University and they identified five distinct groups corresponding to *indica*, *aus*, *aromatic*, *temperate japonica* and *tropical japonica* rice's. They also found that Aus group had very high diversity with 98% of loci polymorphic out of all four groups. Although the Aus groups have a historically smaller

geographical distribution and receive less attention than *indica* and *japonica* rices in breeding programs, their drought tolerance and early maturity are adaptive traits that could be usefully targeted in breeding application. Bashar *et al.* (2000) reported that there are four distinct ecotypes of rice – Boro, Aus, transplanted Aman and Deep water aman in Bangladesh. Only few years ago a large number of farmers grow these local cultivars as their main crop. These cultivars have good adaptation but are poor yielder. Actually, cultivation of these landraces gradually replace through high yielding variety last twenty years. Bangladesh has a good source of indigenous rice cultivars. About 4000 T. Aman, 2500 Boro and 1500 Aus landraces present in BRRI rice germplasm gene bank. These landraces are adapted in different parts of the country, some of which have very nice quality, fineness, aroma, taste and high protein contains (Dutta *et al.* 1998).

RESEARCH ARTICLE

Table 1. *List of the Aus genotypes*

| Sl. No. | Name of genotype | Accession No. | Sl. No. | Name of genotype | Accession No. |
|---------|------------------|---------------|---------|------------------|---------------|
| 1 | BR24 (Rahmat) | MV* | 48 | Changdumra | 6199 |
| 2 | BR26 (Sraboni) | MV | 49 | Bateswar | 66 |
| 3 | BRRRI dhan 27 | MV | 50 | Kachilon(2) | 185 |
| 4 | BRRRI dhan42 | MV | 51 | Panki Rat | 189 |
| 5 | BRRRI dhan43 | MV | 52 | Agaua | 191 |
| 6 | BRRRI dhan48 | MV | 53 | Achar Bhog | 566 |
| 7 | Kolar Thor | 2074# | 54 | Lohar Gura | 812 |
| 8 | Ausa Bogi | 2075 | 55 | Nuncha | 942 |
| 9 | Aus Dhan | 2078 | 56 | Aus Baku | 1318 |
| 10 | Lemma | 2080 | 57 | Rangmahal | 1629 |
| 11 | Bogi | 2083 | 58 | Panoik | 1641 |
| 12 | Ausaloi | 2090 | 59 | Irga | 1643 |
| 13 | Aus Gara Binni | 2091 | 60 | Langka Biri | 1645 |
| 14 | Kajli | 2097 | 61 | Lal Golang | 1655 |
| 15 | Gori | 2098 | 62 | Mele | 1671 |
| 16 | Japanese IRRI | 2101 | 63 | Gorisaita | 1675 |
| 17 | Kali Saita | 2102 | 64 | Goyal | 1680 |
| 18 | Narilel Badi | 2106 | 65 | Saita | 1681 |
| 19 | Lemma | 2107 | 66 | Kala Manik | 1682 |
| 20 | Kola Bokri | 2108 | 67 | Kautukomni | 1684 |
| 21 | Kotak Tar | 2117 | 68 | Korchamuri | 1687 |
| 22 | Gyrol | 2119 | 69 | Boilam | 1688 |
| 23 | Prangi | 2124 | 70 | Chaita Boro | 1716 |
| 24 | Putiraj | 2125 | 71 | Bador jota | 1717 |
| 25 | Kala Kitki | 2132 | 72 | Adhakati | 1718 |
| 26 | Khuida Baran | 2133 | 73 | Smriri | 1729 |
| 27 | Sada Aus | 2135 | 74 | Benamuri | 1732 |
| 28 | Maraka Migichak | 2316 | 75 | Chandra Moni | 1733 |
| 29 | Hasha | 2338 | 76 | Madhu Mala | 1737 |
| 30 | Kharai Murali | 3421 | 77 | Huma Gambir | 1738 |
| 31 | Aus Tarabali | 3434 | 78 | Khusni | 1740 |
| 32 | Munshi Murali | 3441 | 79 | Fulkati | 1743 |
| 33 | Saita (sada) | 3547 | 80 | Kele | 1744 |
| 34 | Gorba | 3590 | 81 | Lakhi Kajol | 1746 |
| 35 | Kali Saita | 3738 | 82 | Nara Ganbio | 1750 |
| 36 | Meri Dhan | 3740 | 83 | Badma | 1777 |
| 37 | Atha Gati | 4034 | 84 | Dubraj | 1781 |
| 38 | Pak jota | 4035 | 85 | Gambir | 1783 |
| 39 | Begum Gutu | 4221 | 86 | Kalo Mucha | 1784 |
| 40 | Balujhuri | 4569 | 87 | Ghor Bhai | 1826 |
| 41 | Usha | 4570 | 88 | Goria | 1852 |
| 42 | Begun Bitchi | 4577 | 89 | Kachilon | 1958 |
| 43 | Baldara | 4579 | 90 | Sada Bogi | 1965 |
| 44 | Kalo Chhotna | 4621 | 91 | Bafoi | 2053 |
| 45 | Kalo Jamri | 5627 | 92 | Chaplo | 2058 |
| 46 | Kalo Sate | 4752 | 93 | Boalia | 2063 |
| 47 | Bahoi | 4753 | 94 | Moisha Lama | 2071 |

* = Modern BRRRI released Aus Variety; # = SI No. 7-94 local Aus landraces

RESEARCH ARTICLE

After establishment of BRR I only a small number of local landraces germplasm characterization or DNA fingerprinting has been done. Many countries in the world have characterized their indigenous different crop landraces at both molecular and phenotypic level. This has been done for their crop identity and search a new gene for further crop improvement. But information about the genetic diversity of local landraces as well as Aus rice is very limited. The needs for varieties improvement for such situations are very important. Precise information about the extent of genetic diversity among population is crucial in any crop improvement program, because selection of plants based on genetic diversity has become successful in several crops (Ananda & Rawat, 1984; De *et al.*, 1988). Therefore, research emphasis has been taken on genetic diversity for microsatellite DNA Markers in BRR I released and some local Aus landraces.

Materials and Methods***Plant material and genomic DNA isolation***

The ninety four genotypes, including six BRR I released Aus genotypes were used in this study (Table 1). Genomic DNA was isolated from young leaves from 21 days old plants with minor modification of CTAB method. The concentration of the extracted DNA was estimated by DNA confirmation test by (1.5%) agarose gel electrophoresis with lambda DNA (50 ng/ μ l).

SSR primers analysis

Each PCR was carried out in a 10 μ l reaction volume containing 1 μ l of MgCl₂ free 10X PCR buffer with (NH₄)₂SO₄, 1.2 μ l of 25 mM MgCl₂, 0.2 μ l of 10 mM dNTPs, 0.2 μ l of 5 U/ μ l Taq DNA polymerase, 0.5 μ l of 10 μ M forward and reverse primers (Table 2) and 3 μ l (10 ng) of DNA using a 96 well thermal cycler. The mixture was overlaid with one drop (3 μ l) of mineral oil to prevent evaporation. The temperature profile used for PCR amplification comprised 94°C for 5 minutes (initial denaturation) followed by 35 cycles of 94°C for 1 minute (denaturation), 55°C for 1 minute (annealing), 72°C for 2 minutes (extension) with a final extension for 7 minutes at 72°C at the end of 35 cycles. The annealing temperatures were adjusted based on the specific requirements of each primer combination. The PCR products were mixed with gel loading dye (bromophenol blue, xylene cyanol and sucrose)

and electrophoresed in 8% polyacrylamide gel using vertical polyacrylamide gels for high throughput manual genotyping. 3-4 μ l of amplification products were resolved by running gel in 1X TBE buffer for 1.5 h to 2.5 h depending upon the allele size at around 90 volts and 500 mA electricity. The gels were stained in 1 μ g/ml ethidium bromide and were documented using UVPRO (Uvipro Platinum, EU) gel documentation unit.

Results and Discussion

The ninety-four Aus genotypes including six BRR I released Aus varieties were assessed for DNA fingerprinting and genetic variability study. Ninety one microsatellite markers were used initially from which fifty one primers gave polymorphism (Table 3). One hundred ninety five alleles were detected at the loci of 51 microsatellite markers across ninety four Aus genotypes. The highest amplicon size was produced by RM171 (333 bp) and the lowest by RM413 (71 bp). These fingerprinting data will identify the genotypes very easily and protect the intellectual property rights (IPR). The highest range of band sizes was found for RM171 (289-333) followed by RM484 (290-319) and RM489 (248-314) (Table 3). Results suggested that these markers may have produced more alleles. This information could be used in further molecular characterization with other local landraces. The number of alleles per locus ranged from 4 (RM25 and RM147) to 27 (RM519) with an average of 9.77 alleles across the 51 loci. The frequency of the most common allele at each locus ranged from 13.83% (RM153) to 70.21% (RM489). On an average, 36.55% of ninety four Aus genotypes shared a common major allele at any given locus. Polymorphism Information Content (PIC) values ranged from 0.4549 to 0.9338 with an average of 0.7327. The highest PIC value (0.9338) was obtained for RM519 (Figure 1) followed by RM286 (0.9001), RM153 (0.8907) and RM 252 (0.8836), respectively (Table 3). PIC values revealed that RM519 and RM286 are the best markers for distinguishing ninety four Aus landraces. Similar results were found from previous fingerprinting and diversity studies. Number of allele per locus is comparable to 1-8 allele with an average number of 4.58 for various classes of microsatellite (Siwach *et al.*, 2004) and 2-7 as reported by Chakrabarthy & Naravaneni (2006) and also comparable to 3 alleles to 9 alleles, with an average of 4.53 alleles per locus for 30 microsatellite markers as per Hossain *et al.* (2007).

RESEARCH ARTICLE

Table 2. Selected primers, their sequence and chromosome number

| Primer code | Forward primer sequence (5 to 3) | Reverse primer sequence (5 to 3) | Chromosome No |
|-------------|----------------------------------|----------------------------------|---------------|
| RM1 | GCGAAAACACAATGCAAAAA | GCGTTGGTTGGACCTGAC | 1 |
| RM495 | AATCCAAGGTGCAGAGATGG | CAACGATGACGAACACAACC | 1 |
| RM312 | GTATGCATATTTGATAAGAG | AAGTCACCGAGTTTACCTTC | 1 |
| RM283 | GTCTACATGTACCCTTGTTGGG | CGGCATGAGAGTCTGTGATG | 1 |
| RM237 | CAAATCCCGACTGCTGTCC | TGGGAAGAGAGCACTACAGC | 1 |
| RM5 | TGCAACTTCTAGCTGCTCGA | GCATCCGATCTTGATGGG | 1 |
| RM259 | TGGAGTTTGAGAGGAGGG | CTTGTTGCATGGTGCCATGT | 1 |
| RM431 | TCCTGCGAACTGAAGAGTTG | AGAGCAAAACCCTGGTTCAC | 1 |
| RM452 | CTGATCGAGAGCGTTAAGGG | GGGATCAAACCACGTTTCTG | 2 |
| RM154 | ACCCTCTCCGCTCGCCTCCTC | CTCCTCCTCTGCGACCGCTCC | 2 |
| RM327 | CTACTCCTCTGTCCCTCCTCTC | CCAGCTAGACACAATCGAGC | 2 |
| RM514 | AGATTGATCTCCCATTCCCC | CACGAGCATATTAAGTAGTGG | 3 |
| RM489 | ACTTGAGACGATCGGACACC | TCACCCATGGATGTTGTCAG | 3 |
| RM85 | CCAAAGATGAAACCTGGATTG | GCACAAGGTGAGCAGTCC | 3 |
| RM307 | GTACTACCGACTACCGTTCAC | CTGCTATGCATGAACTGCTC | 4 |
| RM252 | TTCGCTGACGTGATAGGTTG | ATGACTTGATCCCGAGAACG | 4 |
| RM119 | CATCCCCTGCTGCTGCTGCTG | CGCCGGATGTGTGGGACTAGCG | 4 |
| RM178 | CAGTGGGCGAGCATAGGAG | ATCCTTTTCTCCCTCTCTCG | 5 |
| RM413 | GGCGATTCTTGGATGAAGAG | TCCCCACCAATCTTGTCTTC | 5 |
| RM169 | TGGCTGGCTCCGTGGGTAGCTG | TCCCGTTGCCGTTTATCCCTCC | 5 |
| RM153 | ACCAACGCCAAAAGCTACTG | TACTCGCCCTGCATGAGC | 5 |
| RM122 | GAGTCGATGTAATTGTCATCAGT | GAAGGAGGTATCGCTTTGTTGGAC | 5 |
| RM161 | AAACTGTTTTACCCTGGCC | ATCCCCTTCTGCGGTAATAAC | 5 |
| RM133 | TGGATTGTTTTGCTGGCTCGC | GGAACACGGGTCGGAAGCGAC | 6 |
| RM541 | TATAACCGACTCAGTGCCC | CCTTACTCCCATGCCATGAG | 6 |
| RM204 | GTGACTGACTTGGTCATAGGG | GCTAGCCATGCTCTCGTACC | 6 |
| RM11 | TCTCCTCTCCCCCGATC | ATAGCGGGCGAGGCTTAG | 7 |
| RM18 | TCCCTCTCATGAGCTCCAT | GAGTGCCTGGCGCTGTAC | 7 |
| RM25 | GGAAAGAATGATCTTTTCATGG | CTACCATCAAACCAATGTTC | 8 |
| RM44 | ACGGGCAATCCGAACAACC | TCGGGAAAACCTACCCTACC | 8 |
| RM284 | ATCTCTGATACTCCATCCATCC | CCTGTACGTTGATCCGAAGC | 8 |
| RM408 | CAACGAGCTAACTTCCGTCC | ACTGCTACTTGGGTAGCTGACC | 8 |
| RM105 | GTCGTCGACCCATCGGAGCCAC | TGGTCGAGGTGGGATCGGGTC | 9 |
| RM215 | CAAAATGGAGCAGCAAGAGC | TGAGCACCTCCTTCTCTGTAG | 9 |
| RM219 | CGTCGGATGATGTAAAGCCT | CATATCGGCATTTCGCTG | 9 |
| RM171 | AACGCGAGGACACGTAATTAC | ACGAGATACGTACGCCTTTG | 10 |
| RM228 | CTGGCCATTAGTCCTTGG | GCTTGCGGCTCTGCTTAC | 10 |
| RM147 | TACGGCTTCGGCGGCTGATTCC | CCCCCGAATCCCATCGAAACCC | 10 |
| RM484 | TCTCCCTCCTACCATTTGTC | TGCTGCCCTCTCTCTCTCTC | 10 |
| RM474 | AAGATGTACGGGTGGCATTCC | TATGAGCTGGTGAGCAATGG | 10 |
| RM216 | GCATGGCCGATGGTAAAG | TGTATAAAACCACACGGCCA | 10 |
| RM536 | TCTCTCCTCTTGTGGGCTC | ACACACCAACACGACCACAC | 11 |
| RM209 | ATATGAGTTGCTGTCGTGCG | CAACTGCACTCCTCCCCTCC | 11 |
| RM167 | GATCCAGCGTGAGGAACACGT | AGTCCGACCACAAGGTGCGTTGTC | 11 |
| RM206 | CCCATGCGTTTAACTATTCT | CGTTCCATCGATCCGTATGG | 11 |
| RM286 | GGCTTCATCTTTGGCGAC | CCGGATTACGAGATAAACTC | 11 |
| RM144 | TGCCCTGGCGCAAATTTGATCC | GCTAGAGGAGATCAGATGGTAGTG | 11 |
| RM287 | TCCCTGTAAAGAGAGAAATC | GTGTATTTGGTGAAAGCAAC | 11 |
| RM20 | ATCTTGTCCCTGCAGGTCAT | GAAACAGAGGCACATTTATTG | 12 |
| RM519 | AGAGAGCCCCTAAATTTCCG | AGGTACGCTCACCTGTGGAC | 12 |
| RM277 | CGGTCAAATCATCACCTGAC | CAAGGCTTGCAAGGGGAAAG | 12 |

RESEARCH ARTICLE

Table 3. Data summary for 51 SSR markers across 94 *Aus landraces*

| Marker | Chr. No | Position (bp) | Allele No | Size range | Major Allele | Major Allele Frequency | PIC Value |
|--------|---------|---------------|-----------|------------|--------------|------------------------|-----------|
| RM495 | 1 | 0.21 | 7 | 148-162 | 159 | 0.4149 | 0.6404 |
| RM1 | 1 | 4.63 | 10 | 80-115 | 80 | 0.3723 | 0.7741 |
| RM312 | 1 | 20.69 | 7 | 100-118 | 107 | 0.6064 | 0.5459 |
| RM283 | 1 | 4.88 | 12 | 146-163 | 150 | 0.4255 | 0.7226 |
| RM237 | 1 | 33.29 | 6 | 126-139 | 132 | 0.2660 | 0.7434 |
| RM5 | 1 | 24.13 | 5 | 110-116 | 114 | 0.6596 | 0.4549 |
| RM259 | 1 | 7.44 | 9 | 152-172 | 170 | 0.3511 | 0.7788 |
| RM431 | 1 | 39.22 | 11 | 235-262 | 249 | 0.2766 | 0.8302 |
| RM452 | 2 | 9.50 | 7 | 195-209 | 200 | 0.3511 | 0.6974 |
| RM154 | 2 | 1.08 | 13 | 165-190 | 172 | 0.2766 | 0.8114 |
| RM327 | 2 | 19.49 | 8 | 202-218 | 217 | 0.2660 | 0.8229 |
| RM514 | 3 | 35.22 | 8 | 245-266 | 255 | 0.4894 | 0.6311 |
| RM489 | 3 | 4.31 | 12 | 248-314 | 248 | 0.7021 | 0.4740 |
| RM85 | 3 | 66.76 | 9 | 89-117 | 93 | 0.3511 | 0.7576 |
| RM307 | 4 | 0.00 | 11 | 113-183 | 136 | 0.3830 | 0.7627 |
| RM252 | 4 | 45.21 | 14 | 194-224 | 203 | 0.1702 | 0.8836 |
| RM119 | 4 | 21.22 | 7 | 160-173 | 166 | 0.2660 | 0.8099 |
| RM178 | 5 | 25.08 | 5 | 115-124 | 122 | 0.5213 | 0.6358 |
| RM413 | 5 | 2.19 | 13 | 71-101 | 82 | 0.3723 | 0.7769 |
| RM169 | 5 | 7.47 | 11 | 162-190 | 173 | 0.2553 | 0.8366 |
| RM153 | 5 | 0.67 | 12 | 185-218 | 194 | 0.1383 | 0.8907 |
| RM122 | 5 | 0.29 | 10 | 211-231 | 223 | 0.2979 | 0.7916 |
| RM161 | 5 | 27.89 | 13 | 162-186 | 165 | 0.2340 | 0.8690 |
| RM133 | 6 | 0.00 | 9 | 216-227 | 226 | 0.3404 | 0.7852 |
| RM541 | 6 | 2.72 | 14 | 166-193 | 176 | 0.2447 | 0.8490 |
| RM204 | 6 | 3.17 | 9 | 106-123 | 115 | 0.3298 | 0.7186 |
| RM11 | 7 | 19.25 | 7 | 122-144 | 124 | 0.6170 | 0.5483 |
| RM18 | 7 | 25.65 | 13 | 140-171 | 164 | 0.3085 | 0.8198 |
| RM25 | 8 | 52.2 | 4 | 125-139 | 139 | 0.6489 | 0.4814 |
| RM44 | 8 | 2.88 | 9 | 104-118 | 111 | 0.4255 | 0.6989 |
| RM284 | 8 | 21.08 | 8 | 133-145 | 143 | 0.2660 | 0.8102 |
| RM408 | 8 | 0.12 | 6 | 118-124 | 120 | 0.2660 | 0.7529 |
| RM 105 | 9 | 9.28 | 6 | 135-144 | 140 | 0.4787 | 0.6457 |
| RM215 | 9 | 21.18 | 8 | 142-165 | 154 | 0.3298 | 0.7387 |
| RM219 | 9 | 3.38 | 15 | 191-228 | 204 | 0.2553 | 0.8183 |
| RM171 | 10 | 18.79 | 12 | 289-333 | 301 | 0.3191 | 0.8087 |
| RM228 | 10 | 21.98 | 10 | 104-136 | 114 | 0.6596 | 0.5189 |
| RM147 | 10 | 20.68 | 4 | 93-98 | 94 | 0.4574 | 0.5607 |
| RM484 | 10 | 20.80 | 5 | 290-319 | 299 | 0.5745 | 0.5113 |
| RM474 | 10 | 1.80 | 9 | 185-285 | 195 | 0.2553 | 0.8211 |
| RM216 | 10 | 5.10 | 5 | 127-147 | 128 | 0.5851 | 0.5480 |
| RM536 | 11 | 8.96 | 9 | 222-252 | 238 | 0.2872 | 0.7651 |
| RM209 | 11 | 17.77 | 14 | 124-162 | 154 | 0.2128 | 0.8533 |
| RM167 | 11 | 4.07 | 13 | 121-159 | 127 | 0.2447 | 0.8431 |
| RM206 | 11 | 21.97 | 13 | 126-171 | 134 | 0.4149 | 0.7507 |
| RM286 | 11 | 0.38 | 21 | 98-127 | 108 | 0.1702 | 0.9001 |
| RM144 | 11 | 28.24 | 8 | 216-241 | 228 | 0.3298 | 0.7929 |
| RM287 | 11 | 16.73 | 7 | 97-118 | 100 | 0.2660 | 0.7672 |
| RM20 | 12 | 0.97 | 7 | 155-180 | 155 | 0.3404 | 0.7226 |
| RM519 | 12 | 19.90 | 27 | 116-150 | 121 | 0.1489 | 0.9338 |
| RM277 | 12 | 16.53 | 6 | 116-127 | 124 | 0.4149 | 0.6629 |
| Mean | | | 9.7647 | | | 0.3655 | 0.7327 |

RESEARCH ARTICLE

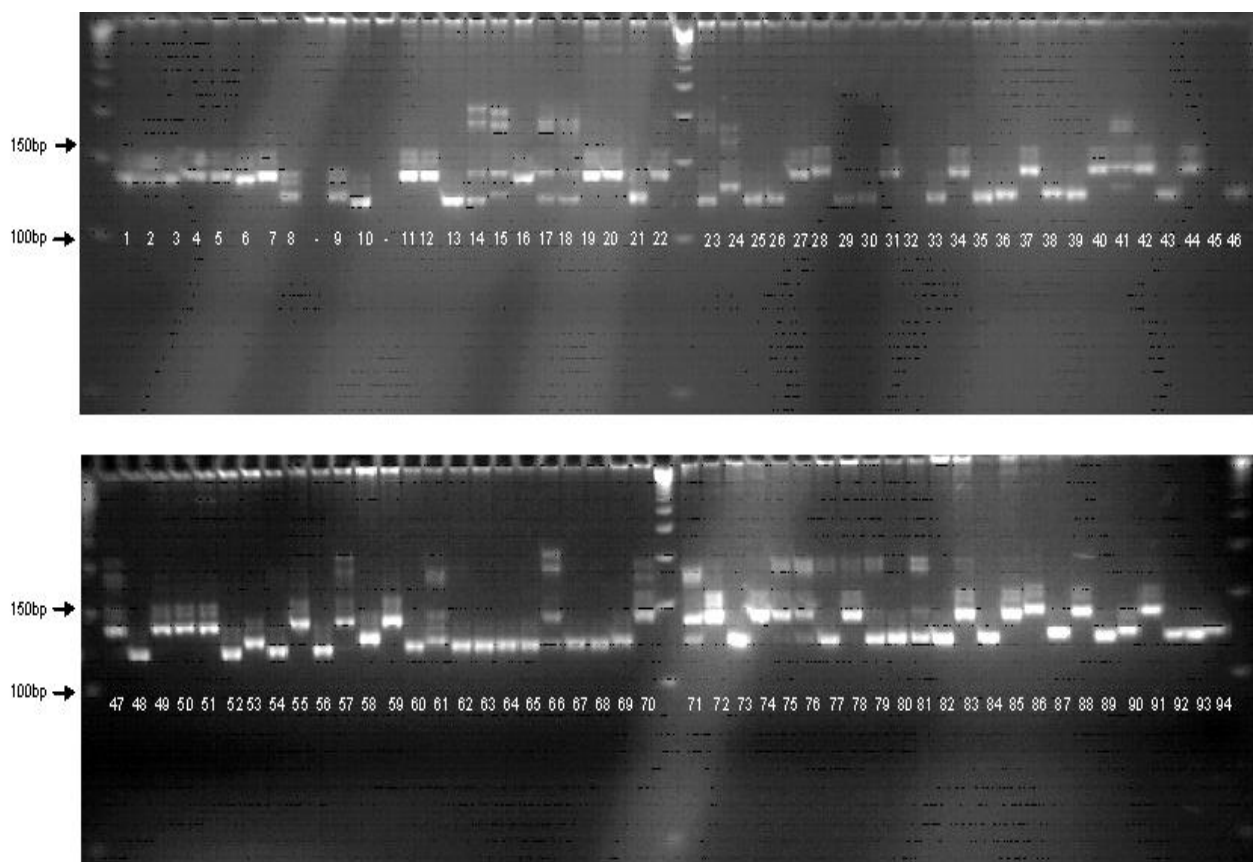


Figure 1. DNA profile of 94 Aus genotypes (6 BRRI released and 88 local Aus) with the SSR marker RM 519.

Legend : Lane1=BR24, 2=BR26, 3=BRRI dhan27, 4=BRRI dhan42, 5=BRRI dhan43, 6=BRRI dhan48, 7=Kolar thor , 8=Aus boga, 9=Aus dhan, 10=Lemma(1) ,11=Bogi, 12=Ausaloi, 13=Aus garra binni, 14=kajli,15=Gori, 16=Japanese IRRI, 17=Kali saita, 18=Narilel badi, 19=Lemma(2), 20=Kola bokri, 21=Kotak tar, 22=Gyrol, 23=Prangi, 24=Putiraj, 25=Kala kitki, 26=Khuida baran, 27=Sada Aus, 28=Maraka Migichak, 29=Hasha, 30=Kharai Murali, 31=Aus tarabali, 32=Munshi murali, 33=Saita(sada), 34=Gorba, 35=Kali Saita, 36=Meri dhan, 37=Atha Gati, 38=Pak jota, 39=Begum Guti, 40=Balujhuri, 41=Usha, 42=Begun Bitchi, 43=Baldara, 44=Kalo chhotna, 45=Kalo Jamri, 46=Kalo sate, 47=Bahoi, 48=Changdumra, 49=Bateswar 50=Kachilon(2), 51=Panki Rat, 52=Agaua, 53=Achar Bhog, 54=Lohar Gura, 55=Nuncha, 56=Aus Baku, 57=Rangmahal, 58=Panoik, 59=Irga, 60=Langka biri ,61=Lal golang, 62=Mele, 63=Gorisatia, 64=Goyal, 65=Saita, 66=Kala manik, 67=Kautukomni, 68=Korchamuri, 69=Boilam, 70=Chaita Boro, 71=Bador jota, 72=Adhakati, 73=Smriri, 74=Benamuri, 75=Chandra Moni, 76=Madhu mala, 77=Huma Gambir, 78=Khusni, 79=Fulkati, 80=Kele, 81=Lakhi kajol, 82=Nara ganbio, 83=Badma, 84=Dudraj, 85=Gambir, 86=Kalo mucha, 87=Ghor Bhai, 88=Goria, 89=Kachilon, 90=Sada Bogi, 91=Bafoi, 92=Chaplo, 93=Boalia, and 94=Moisha Lama.

The Rahman et al. (2009) found an average of 6.33 alleles per locus. They included Bangladeshi high yielding varieties, local cultivars and wild rice for their study. Therefore they found higher of average allele number per locus. We can compare our frequency for most common alleles as Thomson et al. (2007) where they found 21% (RM154) to 73% (RM214). The PIC values observed, are comparable to two previous estimates of microsatellite analysis in rice viz. 0.20-0.90 with an average 0.56 (Jain et al., 2003) and 0.30-0.84 with an average of 0.58 (Hossain et al., 2007).

Pair-wise genetic dissimilarity (Table 4) coefficients were measured among the test entries. Highest genetic dissimilarity (1.000) was found among the Aus genotypes of Ausa Bogi, Aus gram binni, Monshi murali, Kachilon (2), Panki rat, Agaua, Chandra moni and Khusni, whereas lowest genetic dissimilarity was found between BRRI dhan42 and BRRI dhan43 (0.12) followed by Kautukomni and Korchamuri (0.1373). Most local Aus genotypes showed broad genetic base whereas BRRI released modern Aus variety showed narrow genetic base.

RESEARCH ARTICLE

Table 4. Pair -wise genetic dissimilarity coefficient (cont.)

| Genotypes | 1 | 3 | 5 | 7 | 9 | 11 | 13 | 15 | 17 | 19 | 21 | 23 | 25 | 27 | 29 | 31 |
|-----------|------|-------------|------|------|------|-------------|-------------|------|------|-------------|------|-------------|------|------|------|------|
| 2 | 0.92 | 0.94 | 0.96 | 0.76 | 0.71 | 0.59 | 0.92 | 0.92 | 0.71 | 0.84 | 0.84 | 0.80 | 0.90 | 0.75 | 0.73 | 0.84 |
| 4 | 0.65 | 0.94 | 0.96 | 0.86 | 0.80 | 0.67 | 0.63 | 0.82 | 0.69 | 0.92 | 0.92 | 0.73 | 0.80 | 0.73 | 0.80 | 0.78 |
| 6 | 0.47 | 0.53 | 0.59 | 0.94 | 0.84 | 0.84 | 0.00 | 0.94 | 0.82 | 0.92 | 0.94 | 0.90 | 0.88 | 0.82 | 0.90 | 0.92 |
| 8 | 0.88 | 0.98 | 0.86 | 0.78 | 0.94 | 0.84 | 0.92 | 0.73 | 0.86 | 0.71 | 0.69 | 0.92 | 0.90 | 0.82 | 0.82 | 0.90 |
| 10 | 0.76 | 0.67 | 0.63 | 0.90 | 0.92 | 0.98 | 0.67 | 0.84 | 0.92 | 0.88 | 0.88 | 0.98 | 0.92 | 0.94 | 0.96 | 0.98 |
| 12 | 0.90 | 1.00 | 0.90 | 0.76 | 0.82 | 0.76 | 0.94 | 0.73 | 0.78 | 0.76 | 0.75 | 0.94 | 0.90 | 0.92 | 0.88 | 0.94 |
| 14 | 0.90 | 1.00 | 0.90 | 0.82 | 0.92 | 0.86 | 0.94 | 0.71 | 0.84 | 0.82 | 0.84 | 0.94 | 0.92 | 0.96 | 0.92 | 0.92 |
| 16 | 0.90 | 1.00 | 0.92 | 0.84 | 0.86 | 0.78 | 0.86 | 0.65 | 0.73 | 0.61 | 0.61 | 0.94 | 0.94 | 0.86 | 0.92 | 0.94 |
| 18 | 0.92 | 0.94 | 0.94 | 0.86 | 0.86 | 0.86 | 1.00 | 0.55 | 0.88 | 0.63 | 0.69 | 0.90 | 0.88 | 0.92 | 0.84 | 0.92 |
| 20 | 0.80 | 1.00 | 0.96 | 0.86 | 0.71 | 0.67 | 0.86 | 0.90 | 0.69 | 0.88 | 0.90 | 0.82 | 0.84 | 0.75 | 0.78 | 0.76 |
| 22 | 0.94 | 0.90 | 0.94 | 0.78 | 0.80 | 0.84 | 1.00 | 0.45 | 0.92 | 0.59 | 0.67 | 0.94 | 0.92 | 0.94 | 0.96 | 0.94 |
| 24 | 0.92 | 0.86 | 0.90 | 0.76 | 0.78 | 0.80 | 0.90 | 0.22 | 0.86 | 0.59 | 0.33 | 0.90 | 0.92 | 0.86 | 0.86 | 0.94 |
| 26 | 0.82 | 0.88 | 0.90 | 0.75 | 0.49 | 0.49 | 0.80 | 0.88 | 0.22 | 0.84 | 0.88 | 0.90 | 0.92 | 0.86 | 0.92 | 0.92 |
| 28 | 0.96 | 0.94 | 0.94 | 0.84 | 0.76 | 0.75 | 0.90 | 0.61 | 0.84 | 0.14 | 0.41 | 0.94 | 0.90 | 0.86 | 0.86 | 0.92 |
| 30 | 0.90 | 0.98 | 0.96 | 0.84 | 0.80 | 0.75 | 0.90 | 0.63 | 0.82 | 0.39 | 0.33 | 0.90 | 0.84 | 0.86 | 0.86 | 0.88 |
| 32 | 0.75 | 0.90 | 0.86 | 0.82 | 0.84 | 0.88 | 0.82 | 0.94 | 0.90 | 0.94 | 0.92 | 0.12 | 0.45 | 0.41 | 0.47 | 0.47 |
| 34 | 0.82 | 0.94 | 0.92 | 0.86 | 0.86 | 0.90 | 0.90 | 0.92 | 0.90 | 0.92 | 0.88 | 0.00 | 0.47 | 0.47 | 0.45 | 0.47 |
| 36 | 0.80 | 0.92 | 0.86 | 0.86 | 0.84 | 0.88 | 0.88 | 0.90 | 0.94 | 0.88 | 0.84 | 0.47 | 0.00 | 0.47 | 0.45 | 0.24 |
| 38 | 0.78 | 0.92 | 0.90 | 0.86 | 0.80 | 0.80 | 0.82 | 0.86 | 0.90 | 0.84 | 0.86 | 0.47 | 0.47 | 0.00 | 0.53 | 0.47 |
| 40 | 0.84 | 0.96 | 0.94 | 0.88 | 0.90 | 0.86 | 0.90 | 0.88 | 0.90 | 0.86 | 0.86 | 0.45 | 0.45 | 0.53 | 0.00 | 0.43 |
| 42 | 0.82 | 0.94 | 0.90 | 0.82 | 0.86 | 0.88 | 0.92 | 0.94 | 0.94 | 0.90 | 0.86 | 0.47 | 0.24 | 0.47 | 0.43 | 0.00 |

Legend 1= Kolar thor, 2=Japanese IRRI, 3=Munshi Murali, 4=Aus dhan 5=Kalo Jamri, 6=Aus boga, 7=Begum Gutti, 8=Bahoi, 9=Kotak tar, 10=Changdumra,11=Kali Saita, 12=Kachilon(2), 13=Ausa Bogi, 14=Panki Rat, 15=Kalo Mucha, 16=Agaua, 17=Kola bokri, 18=Chandra Moni, 19=Kautukomni, 20=Aus gara binni 21=Chaita Boro, 22=Khusni, 23=BRRRI dhan43, 24=Gambir, 25=BR24, 26=Lemma(2), 27=BR27, 28=Korchamuri, 29=BRRRI dhan48, 30=Boilam, 31=BR26, 32=BRRRI dhan42, 34=BRRRI dhan43, 36=BR 24, 38=BR 27, 40=BRRRI dhan48, 42=BR 26

In crop improvement program more genetic diversified Aus landraces could be chosen as parents in the crossing program to create genetic variability on various breeding purpose. In conclusion, the genetic information gathered here provides unique DNA profiles for Bangladeshi Aus landraces, which will serve as a strong weapon to protect our breeders IPR. Moreover, these data will help the breeders to select parents for future breeding programmes as the identification and utilization of diverse genetic resources is a prerequisite for plant improvement. Here it is shown that most Aus landraces is recognized to have broad genetic base. Thus it is recommended to use these landraces for future breeding program or include new and untouched land races to incorporate new genes and broaden genetic base.

Acknowledgement

This research work was funded by “Strengthening and Capacity Building of Biotechnology Laboratory in BRRRI” Project, Ministry of Agriculture, Government of the People’s Republic of Bangladesh.

References

- Amanda JG, Tai TH, Coburn J, Kresovich S, McCouch S. 2004. Genetic structure and diversity in *Oryza sativa* L. Genetics, 169: 1631-1638.
- Ananda IJ, Rawat DS. 1984. Genetic diversity, combining ability and heterosis in brown mustard. Indian J. Genet., 44: 226-234.

RESEARCH ARTICLE

- Bashar MK., Islam O, Nasiruddin M. 2000. Scope and basis of rice improvement through the use of indigenous conservation knowledge in Bangladesh .Scientific basis of participatory plant breeding and conservation of genetic resources, Mexico, October 8–14, 2000. Abstracts. Report No.25. University of California Division of Agriculture and Natural Resources, Genetic Resources Conservation Program, Davis CA USA.
- Bhuiyan NI, Paul DNR, Jabber MA. 2002. Feeding the extra millions by 2025- challenges for rice research and extension in Bangladesh. A keynote paper presented on national workshop on rice research and extension 2002. Held on 29-31 January, 2002, at BRRI, Gazipur.
- Chakrabarthy BK, Naravaneni R. 2006. SSR marker based DNA fingerprinting and diversity study in rice (*Oryza sativa* L). African J. Biotech. 5(9): 684-688.
- De RN, Setharam R, Sinha MK, Banarjee SP. 1988. Genetic divergence in rice. Indian J. Genet., 48: 189-194.
- Dutta RK, Lahiri BP, Baset Mian MA. 1998. Characterization of some aromatic and fine rice cultivars in relation to their phsico-chemical quality of grains. Indian J. Plant Physil., 3(1): 61-64.
- Hossain MZ, Rasul MG, Ali MS, Iftekharuddaula KM, Mian MAK. 2007. Molecualar characterization and genetic diversity in fine grain and aromatic landraces of rice using microsatellite markers. Bangladesh J. Genet. Pl. Breed., 20(2): 1-10.
- Jain S, Mictchell SE, Jain RK, Kresovich S, McCouch SR. 2003. DNA fingerprinting and phylogenetic analysis of Indian aromatic high quality rice germplasm using panels of fluorescent-labeled microsatellite markers. In: Advance in Rice Genetics ed. by Khush *et al.* IRRI, Philippines, 162-166.
- Rahman MS, Molla MR, Alam MS and Rahman L. 2009. DNA fingerprinting of rice (*Oryza sativa* L.) cultivars using microsatellite markers. Australian Journal of Crop Science, 3(8): 122-128.
- Siwach P, Jain S, Saini N, Chowdhury VK, Jain RK. 2004. Allelic diversity among basmati and non-basmati longgGrain indica rice varieties using microsatellite markers. J. Pl.. Biochem. Biotech., 13: 25-32.
- Thomson MJ, Septiningsih EM, Suwardjo F, Santose TJ, Sililonga TS, McCouch SR. 2007. Genetic diversity analysis of traditional and improved Indonesia rice (*O. sativa* L.) germplasm using microsatellite markers. Theor. Appl. Genet., 114: 559-568.