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Assessment of Nature and Magnitude of Genetic Diversity Based on DNA Polymorphism with RAPD Technique in Traditional Glutinous Rice (*Oryza sativa* L.) of Assam

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Abstract: Extent of genetic diversity based on DNA polymorphism with RAPD technique was studied at Assam Agricultural University, Jorhat during 2004-06 with seeds of 41 indigenous glutinous rice germplasm collected from two diverse agro-climatic zones prevailing in the Brahmaputra Valley and the Barak sub-basin of Northeast India. Ten random RAPD primers generated 214 fragments, out of which 209 were polymorphic (97.77%). With RAPD marker, the index of genetic similarities ranged from 0.078 to 0.623, 0.160 to 0.481 and 0.114 to 0.633 in overall, in Birain and in Bora-Chokuwa groups, respectively. Some of the RAPD fragments could be used to develop group specific markers (for example, OPK-14_{200} in Bora-Chokuwa group and OPK-14_{600} in Birain group).

Key words: PCR, RAPD marker, primers, polymorphism

INTRODUCTION

Indigenous glutinous rice germplasm of Assam is endowed with rich diversity, which necessitates a thorough characterization and classification. DNA markers promote easy identification and documentation of genetic differences and degree of relatedness between different genotypes or cultivars within the germplasm. As a supplement to the classical breeding, present day plant breeders and molecular biologists are exploiting vast array of DNA based markers among which, Random Amplified Polymorphic DNA (RAPD) marker, a PCR based marker, has been of wider acceptance because of its speed, economy and simplicity. This assay, unlike PCR, does not require the knowledge of the target DNA sequence and a simple arbitrary primer will support DNA amplification from a genomic template of binding sites on opposite strands of the template exist within a distance that can be traversed by thermo stable DNA polymerase (Williams *et al*., 1990). RAPD requires minute quantities of DNA for detection of polymorphism based on the presence (dominant) or absence (recessive) of particular bands in electrophoresis. There are some inherent difficulties with RAPDs associated with their reproducibility, dominant nature and uncertain marker homology (Weiseng *et al*., 1995). Despite of these limitations, this marker has been widely used for identification of markers linked to insect and disease resistance, genetic mapping, variety identification and phylogeny analysis. RAPDs have been successfully used for assessing genetic diversity and relatedness in a wide range of grass species including rice (*Oryza sativa* L.) (Virk *et al*., 1995a,b), common wheat [*Triticum turgidum* L. subsp. *dicoccoides*) (Konn ex Asch. and Graebn) Thell.] (Strelchenko *et al*., 1999), *Lolium* species (Huff, 1997). Yamamoto (1994) analysed 35 rice cultivars with 13 RAPD primers and obtained 54 scorable polymorphic bands to calculate genetic distance. Yu and Nguyen (1994) reported that RAPD analysis is useful in determining genetic variation at DNA level among rice cultivars and the technique is sensitive and powerful. Using RAPD marker, Ko *et al.* (1994) confirmed that commercial Australian and USA lines and their relatives are closely related, with similarity indices of 88-97%. The genetic relationship between seven Japonica, two Indica and one tropical Japonica rice varieties was analysed using RAPD method (Nadarajan *et al*., 1999). Ravi *et al.* (2003) assessed the genetic diversity among 40 cultivated varieties and five wild relatives of rice involving RAPD markers. They observed 90% polymorphism from 499 RAPD markers. Barooah and Sarma (2004) demonstrated the potentiality of the use of RAPD markers in characterization of duplicated accessions of Assam rice germplasm. Sarma and Bahar (2005) reported genetic diversity in 23 Bora rice (Glutinous rice) landraces of Assam at DNA level using RAPD makers. Shivapriya and Hittalmani (2006) reported the extent of genetic diversity in a set of 65 local rice accessions and three improved rice varieties at molecular level using RAPD markers.

Therefore, keeping this in view, the present investigation was carried out to assess the nature and magnitude of genetic diversity in traditional glutinous rice of Assam of North East India based on DNA polymorphism with RAPD technique.

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MATERIALS AND METHODS

Seeds of 41 indigenous glutinous rice germplasm were collected from two diverse agro-climatic zones prevailing in the Brahmaputra valley and the Barak sub-basin of Northeast India. The Regional Agricultural Research Station (RARS), Assam Agricultural University (AAU), Titabor provided 26 accessions (viz., Parochakuwa Sali, Kalamdani Chokuwa, Boga Chokuwa, Bor Chokuwa, Maju Chokuwa, Sam Chokuwa, Boga Bora, Bor Bora, Chokuwa Bora-2, Ranga Bora-3, Chokuwa Bora-1, Til Bora, Chandra Bora, Gela Bora, Memon Bora, Joha Bora, Jengoni Bora, Rongali Bora, Kaun Bora, Ronga Bora-1, Bora-2, Ghew Bora, Rupohi Bora, Tangun Bora, Garuchakuwa Bora and Pakhiloga Bora) of the Brahmaputra valley grouped as Bora-Chokuwa and RARS, AAU, Karimganj provided 15 accessions (viz., Chefa Birain, Aki Birain, Kacha Birain, Pusha Birain, Das Birain, Mow Birain, Kala Birain, Tepra Birain, Pani Birain, Jhanki Birain, Agirjal Birain, Akib Birain, Jaisungam Birain, Uba Birain and Garuchakhuki Birain) of Barak sub-basins grouped as Birain , as per local parlance prevailing in Brahmaputra and Barak Valley of Assam, respectively. These were used in the present study during 2004-06 at the Department of Plant Breeding and Genetics of the Assam Agricultural University, Jorhat. The total genomic DNA from each of the genotypes was extracted following the protocol of Plaschke *et al.* (1995) with slight modification. Two to three seeds of each accession were crushed with a mortar and pestle to a fine powder followed by DNA extraction using 1 mL of extraction buffer (1 M Tris-Cl, pH 8.0; 5M NaCl; 500 mM Na_2EDTA;20% SDS). The homogenate was centrifuged to remove cell debris. The supernatant was treated with RNase and DNA was precipitated with chilled 95% alcohol. The quality and quantity was estimated by running DNA on a 0.7% agarose gel alongside a known quantity of lambda uncut DNA. Twenty RAPD primers of arbitrary sequence, obtained from Operon Technologies Inc., USA, were screened initially. The final RAPD analysis of the 41 varieties was carried out using 10 primers only (Table 1). Amplification was carried out in a 25 μL reaction volume containing 10 ng of template DNA, 50 pM of primer, 200 μM of dNTPs, 0.5 unit of Taq DNA polymerase and 1X PCR buffer (50 mM Kcl, 10 mM Tris-Cl, 1.5 mM $MgCl_2$ 0.01% gelatin). The amplification conditions were based on the procedure of Williams *et al.* (1990) and Panaud *et al.* (1996) with denaturation for 1 min at 94°C, 1 min annealing at 32°C, followed by a 5 min rise to 72°C and primer elongation for 1 min at 72°C. The PCR products were separated/resolved by electrophoresis on1.5% agarose gel from sigma, in 1X TBE buffer and ethidium bromide stained gel was photographed with a digital gel documentation system (Ultra-violet products, UK).

Table 1: Arbitrary 10-mer primers (Operon Technologies Inc.,USA) used in RAPD

Primer code	Sequence 5′-3′	GC percentage
OPK-19	-CACAGGCGGA-	70
OPK-20	-GTGTCGCGAG-	70
OPK-14	-CCCGCTACCA-	70
OPK-12	-TGGCCCTCAC-	70
OPK-19	-CCCTACCGAC-	70
OPH-05	-AGTCGTCCCC-	70
OPH-08	-GAAACACCCC-	60
OPH-20	-GGGAGACATC-	60
OPD-08	-GTGTGCCCCA-	70
OPD-07	-TTGGCACGGG-	70

The molecular weight of PCR products obtained for each primer from RAPD analysis was designated based on a ladder of known molecular weight. During band scoring, faint bands and bands with smeared background were avoided and only intense bands were scored. The bands were scored on the basis of presence or absence of an amplified particular DNA fragment. Using polymorphic bands generated by RAPD markers, the graphical representation of DNA fingerprint was developed. RAPD data were analyzed using the software package, NTSYS-pc Version 2.1 (Rohlf, 2000).The binary data generated by RAPD analysis was used to calculate polymorphism percent by dividing amplified polymorphic fragment by total number of bands observed. Polymorphism Information Content (PIC) was evaluated using the formula given by Anderson *et al.* (1993). Genetic relatedness among the genotypes was computed using the Jaccard's coefficient of similarity; SIMQUAL module of NTSYS-pc. The degree of genetic relationship among the studied rice genotypes as revealed by Jaccard's coefficient of similarity was represented through cluster analysis using the algorithm of Unweighted Pair Group Method with Arithmetic Average (UPGMA) by feeding similarity matrix as input data. The degree of fit was interpreted, as if the value was more than the recommended (0.80), it reflected well fit and lower than that showed poor fit (Rohlf, 2000). The degree of association between the similarity estimates based on RAPD was done by Mantel test (Mantel, 1967) using MXCOMP in NTSYS-pc to determine the significance level of correlation coefficient between the above mentioned distance matrices, respectively.

RESULTS AND DISCUSSION

Out of 20 random primers screened, only 10 primers were selected for further analysis based on reproducibility of amplification profiles. Figure 1 and 2

Fig. 1: Amplification profile of few glutinous rice accessions (§) under study with RAPD primer OPD-08

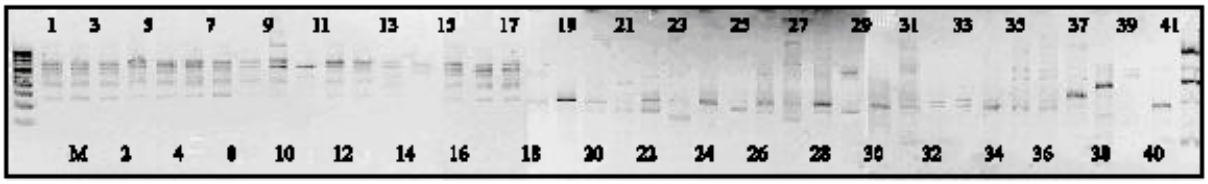

Fig. 2: Amplification profile of few glutinous rice accessions (§) under study with RAPD primer OPD-08

show representative amplification profiles of glutinous rice accessions under study with RAPD marker. The size of amplified fragments ranged from 200 to 2150 bp.

The ten decamer arbitrary primers produced a total of 214 bands out of which 209 were polymorphic (97.77% polymorphism), which is relatively high as compared to several reports of RAPD study in rice. RAPD polymorphism reported by most of the workers varied extensively as 30% (Zhenshan *et al*., 1996), 50% (Parsons *et al*., 1997), 67% (Ko *et al*., 1994), 72.9% (Saker *et al*., 2005), 74.1% (Shivapriya and Hittalmani, 2006), 77.4% (Nadarajan *et al*., 1999), 80% (Yu and Nguyen, 1994), 90% (Ravi *et al*., 2003) and 95.1% (Ragunathachari *et al*., 2000). Such a high level of polymorphism might be due to the reason that intraspecific variation in rice is extensive (Yamamoto, 1994). Moreover, the use of primers with high GC content (60% to 70%), in the present study, might be another reason for higher polymorphism (Yamamoto, 1994). Competition between primers for priming sites rather than the total number of potential priming sites available has been reported to contribute towards variation in polymorphism level (Rafalski *et al*., 1991). However, these reports could not be corroborated from the present study. As North-East India including Assam is considered as rich repository of genetically diverse pool of rice germplasm, detection of such high polymorphism level in the present entries of rice collection is common (Barooah and Sarma, 2004; Sarma and Bahar, 2005). However, none of the primers used were capable of distinguishing the accessions under study alone. Some of the RAPD fragments were amplified, only, in Bora-Chokuwa group (for example, OPK-14_{1000}, OPK-19_{850}, OPH-05_{1000} etc). Likewise, in Birain group one fragment was specifically amplified (OPK-14_{700}). These amplicons can be used as diagnostic markers for Birain or Bora-Chokuwa group. However, no specific amplicon was obtained to differentiate specifically either Bora or Chokuwa genotype. Ko *et al*. (1994) reported few cultivar-specific banding profiles with RAPD markers.

Table 2: Comparative analysis of level of polymorphism as detected by RAPD markers in Bora-Chokuwa and Birain groups of glutinous rice

Primers	Range of freguencies amplified fragments	Average frequency	PIC	Size of most frequent fragment (bp)
Bora-Chkuwa group				
OPK-14	0.154-0.731	0.350	0.877	200
OPK-20	0.115-0.731	0.395	0.843	550
OPK-19	0.231-0.538	0.363	0.868	450
OPK-20	0.514-0.538	0.313	0.902	200
OPK-12	0.192-0.500	0.313	0.902	800,750
OPK-9	0.308-0.615	0.481	0.768	1150
OPK-07	0.231-0.500	0.400	0.840	1400
OPK-08	0.192-0.500	0.374	0.860	400,350
OPK-08	0.577-0.846	0.673	0.574	650
OPK-05	0.15-0.731	0.317	0.899	600
Average		0.397	0.830	
Birain group				
OPK-14	0.067-0.467	0.304	0.907	600
OPK-20	0.067-0.800	0.539	0.709	200
OPK-19	0.133-0.600	0.333	0.889	600
OPK-20	0.267-0.600	0.458	0.790	250
OPK-12	0.067-0.533	0.295	0.912	400
OPK-09	0.200-0.800	0.500	0.750	1050
OPK-07	0.067-0.667	0.196	0.961	1050
OPK-08	0.133.0.667	0.311	0.903	1050
OPK-08	0.067-0.667	0.207	0.957	700
OPK-05	0.67-0.733	0.185	0.965	500
Average		0.326	0.874	

PIC: Polymorphic Information Content

The ten primers revealed an average PIC of 0.860 in all the genotypes under study. The primer OPD-08 showed a maximum PIC (0.933), while the primers OPK-09 and OPH-08 showed a minimum PIC (0.761) across all the genotypes. In Bora-Chokuwa group, OPK-20 and OPK-12 generated a maximum PIC (0.902) and OPH-08 revealed a minimum PIC (0.547). Within Birain accessions, OPH-05 revealed the highest PIC of 0.965 and OPH-20 showed a minimum PIC of 0.709 (Table 2). The frequency of

occurrence of each polymorphic bands generated by the decamer primers ranged from 0.115 (OPK-20) to 0.731 (OPK-20 and OPK-14) with an average of 0.364, for all the genotypes under study. If a primer can generate bands occurring less frequently in the genotypes, the discriminatory power of the primer increases with greater possibility of obtaining genotype-specific characteristic banding pattern. The primer OPD-08 exhibited higher PIC across the genotype, thereby revealing more informativeness of the primers in discriminating glutinous rice of Assam. But the primers OPK-12 and OPK-20 had higher discriminatory power to distinguish genotypes of *Bora-Chokuwa* group due to their high PIC values. The primer OPH-05 showed its better utility to distinguish Birain accessions due to its high PIC value.

The indices of genetic relationship based on pair wise Jaccard's coefficient of similarity among the glutinous rice germplasm showed that, in general the genetic similarity ranged from 0.078 to 0.623, with an average of 0.229. The maximum similarity (0.623) was observed between Chokuwa Bora-1 and Chokuwa Bora-2. The minimum similarity (0.078) was observed between Mow Birain and Tangun Bora. Within Birain groups, the similarity value ranged from 0.160 (Aki Birain and Jaisungam Birain) to 0.481 (Agirjal Birain and Akib Birain) with an average of 0.258. In bora and chokuwa groups, the Jaccard's coefficient of similarity ranged from 0.114 (Ranga Bora-3 and Chandra Bora) to 0.623 (Chokuwa Bora-1 and Chokuwa Bora-2) with an average of 0.224. The minimum similarity between Mow Birain and Tangun Bora, suggested maximum genetic difference between the two accessions. The average similarity values based on RAPD markers revealed that Bora accessions are more diverse than Birain group. Wider cultivation of bora accessions in Brahmaputra valley than Birain accessions in Barak valley might be a contributing factor to the more diversity in Bora group.

The dendrogram (Fig. 3) classified glutinous rice genotypes into two clusters (A and B) with 12 Birain accessions in one cluster and rest 29 accessions in

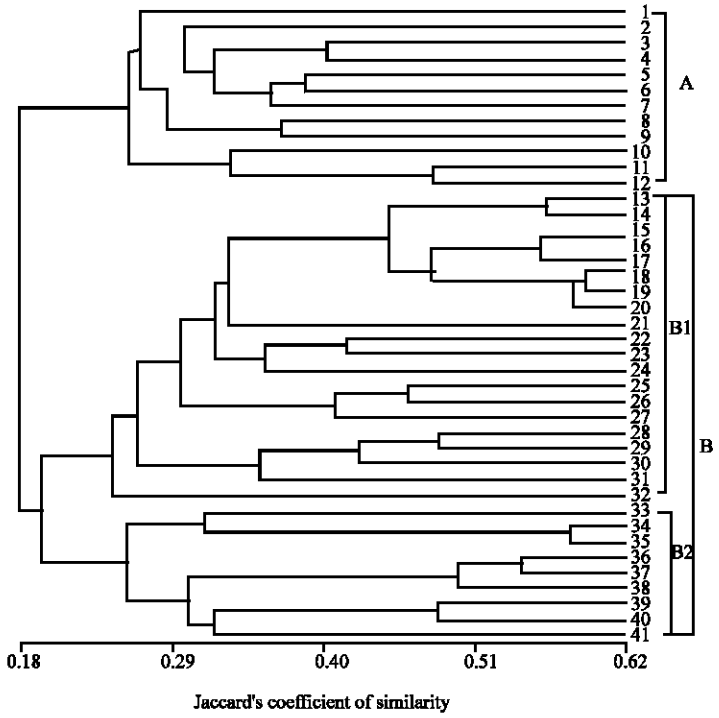

Fig. 3: UPGMA based dendrogram of 41 glutinous rice accessions (§) using Jaccard's coefficient of similarity based on 214 RAPD markers. (§)Glutinous rice accessions: 1: Chefa Birain, 2: Aki Birain, 3: Kacha Birain, 4: Pusha Birain, 5: Das Birain, 6: Tepra Birain, 7: Mow Birain, 8: Kala Birain, 9: Jhanki Birain: 10: Ani Birain, 11: Agirjal Birain, 12: Akib Birain, 13: Jaisungam Birain, 14: Boga Bora, 15: Chokuwa Bora- 2, 16: Chokuwa Bora -7, 17: Kaun Bora, 18: Bor Chokuwa, 19: Maju Chokuwa, 20: Tangun Bora, 21: Pakhiloga Bora, 22: Chandra Bora, 23: Rupohi Bora, 24: Rangali Bora, 25: Ranga Bora-1, 26: Kalamdani Chokuwa, 27: Boga Chokuwa, 28: Parochokuwa Sal, 29: Til Bora, 30: Sam Chokuwa, 31: Ghew Bora, 32: Garuchokuwa Bora, 33: Uba Birain, 34: Garuchakhuki Birain, 35: Bor Bora, 36: Gela Bora, 37: Joha Bora, 38: Bora-2, 39: Memon Bora, 40: Jengoni Bora, 41: Ranga Bora-3

another group. Clustering pattern revealed that genotypes with the same name were included in different sub-clusters like *Ranga Bora*-1 in sub-cluster B_1 and Ranga Bora-3 in sub-cluster B_2. The probability of duplicate accessions in Assam rice with similar names was highlighted by Barooah and Sarma (2004) and Sarma and Bahar (2005). They also reported the potentiality of RAPD marker in identifying duplicates. None of accessions within clusters was found to have 100% similarity. The obtained results indicated that, the plant materials used in the study do not comprise of any true duplicates. The present investigation also suggested two Chokuwa Bora accessions as suspected duplicate based on similarity in names and clustering pattern in RAPD analysis.

There were 209 polymorphic bands in RAPD which were able to distinguish the 41 accessions. The percent genome coverage was found to be 0.44%, assuming all the amplified fragments were non-overlapping. Cophenetic correlation studies revealed RAPD data had a good fit to Mantel test. This suggested that RAPD marker had great influence in the clustering pattern. For variety registration and protection of Plant Breeder's Rights, identification of crop cultivars is pre-requisite. For conclusive definition and for declaring a variety as different, data of DNA fingerprinting in conjunction with agronomic data is widely adapted (Morell *et al*., 1995). Also, Mackill (1995) stated the feasibility of RAPD markers in DNA fingerprinting of rice cultivars. The utility of such fingerprints can be enhanced for the germplasm curator and breeder,when some bands could be linked with some economically important traits.

CONCLUSION

From the aforesaid, it is apparent that with RAPD marker, the index of genetic similarities ranged from 0.078 to 0.623, 0.160 to 0.481 and 0.114 to 0.633 in overall, in Birain and in Bora-Chokuwa groups, respectively. Some of the RAPD fragments could be used to develop group specific markers (for example, OPK-14_{200} in Bora-Chokuwa group and OPK-14_{600} in Birain group). Ten random RAPD primers generated 214 fragments, out of which 209 were polymorphic (97.77%). Variability for the locus/bands as measured by Polymorphism Information Content (PIC) was 0.859. With the marker system, Bora-Chokuwa groups were found to be more diverse than Birain groups. Mantel's test for correlation analysis systems revealed independence of clustering pattern.

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