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Short Communication

Genetic diversity analysis in indigenous deep-water rice of Assam using RAPD and ISSR markers

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Deep-water rices, locally known as *bao* and *asra,* are an important crop in flood plains of Brahmaputra valley and Barak valley of Assam. Farmers are still growing different deep-water rice landraces, which constitute an important source of genetic variation for utilization in breeding programme of high yielding deep-water rice varieties with tolerance to flooding. So, conservation and characterization of these landraces is an important step towards this direction. The use of molecular markers is considered superior over morphological markers for analysis of genetic diversity and variety identification because some inherent problems of the later. There is very little information available on the nature and extent of genetic diversity in deep-water rice of Assam, particularly that based on molecular markers. This information is quite valuable for rationalization of deep-water rice germplasm conservation and their utilization in a breeding programme. Hence, the present study was conducted to understand the pattern of genetic variability in few deep-water rice accessions based on Randomly Amplified Polymorphic DNA (RAPD) and Inter-Simple Sequence Repeats (ISSR) markers.

The present investigation on the genetic variation was conducted with 63 deep-water rice accessions collected from Brahmaputra valley (Lakhimpur and Dhemaji districts) and Barak valley (Karimganj district) of Assam (Table 1). The identity of accessions was maintained as given by farmers, when same name was observed a number was added after the name to treat them as different genotype for analysis. The total genomic DNA from each of the genotypes included in the present study was extracted following the protocol of Plaschke *et al*. with slight modification [1]. Primers for RAPD-PCR and ISSR-PCR, used in the study, are listed in Table 2. RAPD assay was carried out based on the method given by Barooah and Sarma [2]. The amplification for ISSR marker was carried out in a 25µl reaction volume containing 50ng of template DNA, 0.2mM of ISSR primer, 200mM of each dNTPs, 1.5 mM of $Mgcl_2$ and 0.5 to 1.0 units of Taq DNA polymerase. The amplification profile was programmed to include pre-denaturation at 94°C for 5min, followed by 40 cycles of denaturation at 94°C for Imin, varying annealing temperature for different primers (i.e. from 38°C to 58°C) 2 min and extension at 72°C for 2 min, and finally an additional cycle of 5 min at 72°C. The products were analyzed by electrophoresis through 1.5% agarose gel in 1X TBE buffer and ethidium bromide stained gel was photographed with a digital gel documentation system. The amplified products were scored as present (1) or absent (0) for each genotype-primer combination. The binary data matrix was used to measure the genetic coefficient of similarities among 63 deep-water rice accessions using based on the Jaccard's coefficient [3] and clustered with UPGMA (Un-weighted Pair Group Method using Arithmetic Averages) algorithm [4]. All analyses were performed using NTSYS-PC 2.10 software [5].

The RAPD profiles usually represent available portion of the genome while the ISSR profiles are generated from microsatellite rich regions only. Thus, the two methods involve regions having substantially different evolutionary history and have different genome coverage [6, 7]. Further a collective analysis of data by the two methods together would result in a comprehensive analysis of the accessions relatedness

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Table 1. List of deep-water rice accessions included in the study

S.No.	Name of the accession	Districts of collection	S.No.	Name of the accession	Districts of collection
1	Rangili Bora	Lakhimpur	32	Kholihoi-bao	Lakhimpur
2	Lal badal	Karimganj	33	Maguri bao	Lakhimpur
3	Lal bora	Karimganj	34	Kheseng bao	Dhemaji
4	Kanla	Karimganj	35	Herepi bao	Dhemaji
5	Bogadah kokua	Karimganj	36	Bokajahaingia	Dhemaji
6	Sali badal	Karimganj	37	Kola pakhi	Dhemaji
7	Chakua	Lakhimpur	38	Biria bhanga	Dhemaji
8	Boginadi	Lakhimpur	39	Bogi bao	Lakhimpur
9	Panchanan	Karimganj	40	Rangoli bao	Lakhimpur
10	Ekar chali	Karimganj	41	Soru maguri	Lakhimpur
11	Bagdar	Karimganj	42	Suagmoni	Lakhimpur
12	Kacha badal	Karimganj	43	Dhepa	Karimganj
13	Kali mukri	Karimganj	44	Basudev	Lakhimpur
14	Dud lakhi	Karimganj	46	Sagar Sali	Lakhimpur
15	Harsh badal	Karimganj	47	Pomai bora	Lakhimpur
16	Panindra	High yielding variety	48	Badal	Lakhimpur
17	Pakhari bora	Dhemaji	49	Khuti bao	Lakhimpur
18	Bihari Sali	Dhemaji	50	Kola amona	Lakhimpur
19	Silsili bao	Dhemaji	51	Gajab sali	Lakhimpur
20	Laki	Karimganj	52	Haldharan	Lakhimpur
21	Parar_neghari	Lakhimpur	53	Miyan bao-1	Dhemaji
22	Kekoa bao- 1	Lakhimpur	54	Miyan bao-2	Dhemaji
23	Kekoa bao-2	Lakhimpur	55	Podumoni	Karimganj
24	Dolmora bao	Dhemaji	56	Borphakhori	Dhemaji
25	Kola Sali	Lakhimpur	57	Bihari bao	Lakhimpur
26	Dia bao	Dhemaji	58	Bogijol	Lakhimpur
27	Bega amona	Dhemaji	59	Garo Sali	Dhemaji
28	Dol bao	Dhemaji	60	Sonamukhi	Dhemaji
29	Jeng bao	Dhemaji	61	Sunamukhi	Karimganj
30	Amona bao	Dhemaji	62	Sikan kola	Lakhimpur
31	Nania	Lakhimpur	63	Madab sali	Lakhimpur

[6, 7]. Among 26 RAPD primers and 20 ISSR primers tested, ten each of RAPD and ISSR primers were selected that showed discrete and consistent amplification profile (Table 2). The data presented in the table revealed that the ISSR marker is more informative than RAPD to assess the genetic variability in the deep-water rice landraces of Assam.

The average similarity index was 0.325%, indicating high level of genetic diversity among the accessions. The similarity coefficient ranged from 0.139% ('Haldharan' and 'Dolmora bao') to 0.98% ('Miyan bao-1' and 'Miyan bao-2'). The dendrogram showing the relationships among the genotypes is given in Fig. 1. Three major grouping could be observed among the accessions, where maximum genotypes were grouped together in cluster A. This cluster could be divided again into two sub-clusters (A_1 and A_2) with 38 and 21 genotypes, respectively. The genotypes of cluster C showed maximum genetic dissimilarity from the rest of the accessions. The two genotypes of the cluster B showed similarity in the names as well as they grouped together with a high level of similarity (0.973). So this genotype could be considered as a pair of potential duplicate. Similarly two 'Miyan bao' accessions in cluster A ('Miyan bao-1' and 'Miyan bao-2') could be considered as potential duplicate. But the grouping pattern and similarity coefficient between 'Kekoa bao-1' and Kekoa

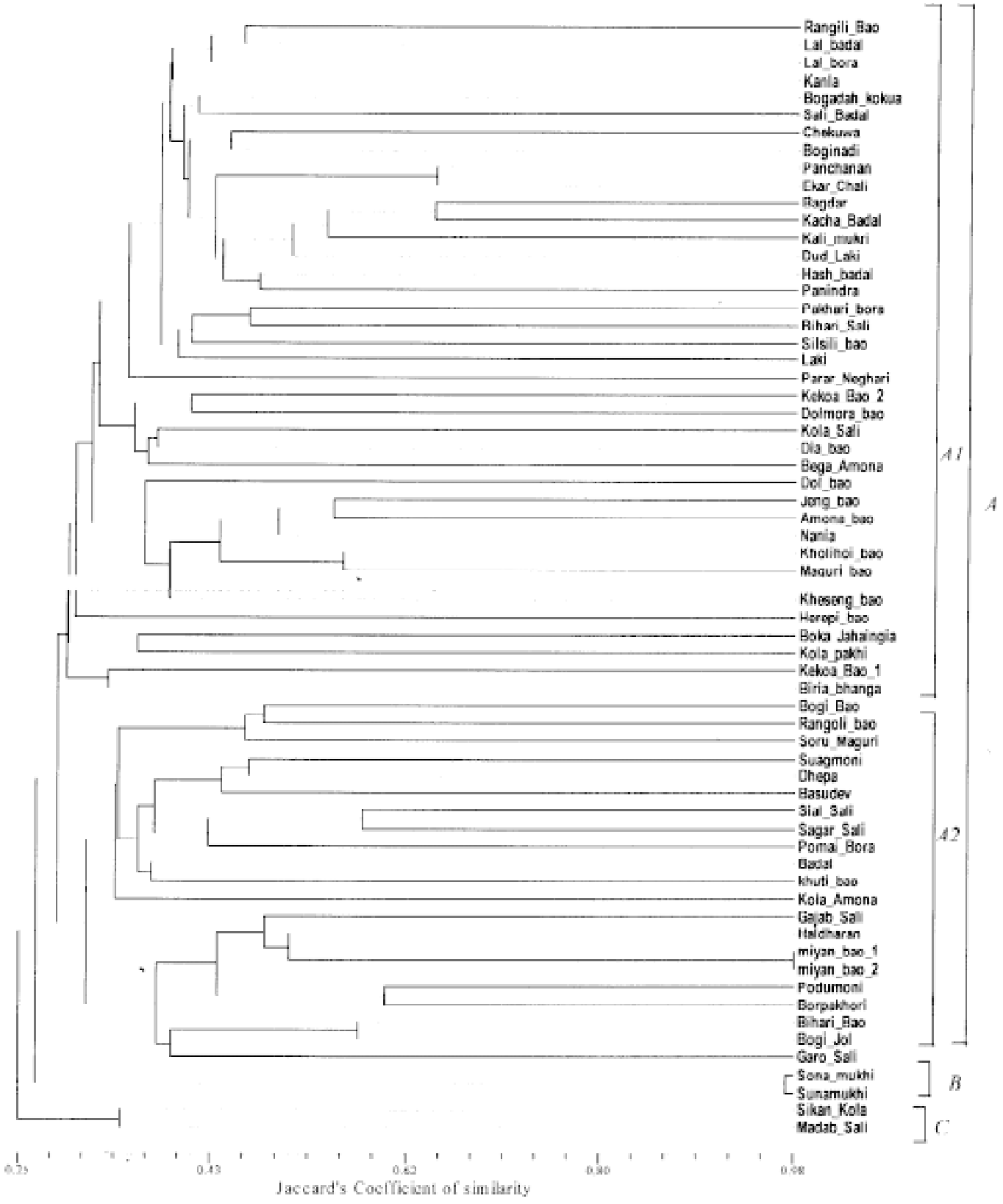

Fig. 1. Dendrogram of 63 deep-water rice genotypes constructed using UPGMA based on RAPD and ISSR markers using Jaccard's coefficient of similarity

Table 2. RAPD and ISSR primers used in classiiying 63 deep-water rice accessions

Primers	Sequence	Total band	Polymorphic band	% polymorphism
OPB-10	5'CTGCTGGGAC	13	13	100.00
OPH-05	5'AGTCGTCCCC	14	13	92.86
OPH-08	5'GAAACACCCC	17	17	100.00
OPH-20	5'GGGAGACATC	5	4	80.00
OPK-14	5'CCCGCTACAC	20	20	100.00
OPK-19	5'CACAGGCGGA	21	21	100.00
OPL-18	5'ACCACCCACC	14	13	92.86
OPP-02	5TCGGCACGCA	15	14	93.33
OPM-16	5'GTAACCAGCC	23	22	95.65
OPD-10	5'GGTCTACACC	10	8	80.00
ISSR-01	5 'GGCGGCGGCGGCGCAT	14	14	100.00
ISSR-02	5 ' AAGAAGAAGAAGAAGGC	22	22	100.00
ISSR-03	5 'AAGAAGAAGAAGAAGTG	20	20	100.00
1SSR-04	5 'AAGAAGAAGAAGA AGCC	14	14	100.00
1SSR-05	5 ' AGCAGCAGCAGCAGCCA	21	21	100.00
JSSR-06	5 ' AGCAGCAGC AGCAGCCG	15	15	100.00
ISSR-07	5 'GGCGGCGGCGGCGGCTA	24	24	100.00
ISSR-08	5 'AGCAGCAGCAGCAGCGA	19	19	100.00
ISSR-09	5 'AAG AAGAAGAAG AAAGCG	14	14	100.00
1SSR-10	5 'CC AGTGGTGGTGGTG	11	10	90.90

bao-2' revealed that they are genetically different despite similarity in their names. The greater role of DNA markers in identification of duplicate accessions in Assam rice collection has already been demonstrated [2, 8]. The present study revealed the existence of sufficient genetic variation in deep-water rice landraces of Assam. The genetic drift and unconscious selection by farmers from original landraces might have resulted such divergence in deep-water rice accessions under study [8]. Since, there is inadequate evaluation data in deep-water and semi deep-water landraces of Brahmaputra and Barak valley, marker data provide only available decision criterion.

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