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Genetic diversity analysis of traditional Sali rice (*Oryza sativa* L.) germplasm of Assam through RAPD markers

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Abstract

Genetic diversity of Assam rice collection was analyzed using 51 Sali rice accessions were characterized based on 72 random amplified polymorphic DNA (RAPD) markers. Among the 11 primers, the percentage of polymorphic bands ranged from 33% (OPK-14) to 100% (OPK-19), showing a high degree of molecular variation. The Jaccard's similarity co-efficient used for RAPD data was found to be 0.515, indicating a high level of diversity. Cluster analysis of RAPD data using the UPGMA revealed 11 pairs of accessions might be considered as suspected duplicates. The potentiality of using molecular marker in rice germplasm management of Assam rice collection is discussed.

Key words: Assam rice collection, RAPD, duplicate accession, Jaccard coefficient.

Introduction

North East India is considered as one of the centres of origin of rice (*Oryza sativa* L.). Variations in ecological conditions, ethnic diversity, diverse cultural practices and different quality preferences contribute to different types of rice grown in this region [1]. Among these, Sali rice of Assam is the largest and most diverse class of rice [2]. The traditional Sali rice varieties are very popular among the farmers. The conservation and characterization of Sali rice germplasm of Assam is important. Sali rice accessions maintained in Assam Agricultural University (AAU), and elsewhere may include duplicate accessions collected in different field trips and from different regions or genetically different samples having similar names. Increasing collection size and decreasing financial resources have stimulated gene banks to identify and remove duplicate germplasm in order to increase efficiency [3]. So, proper characterization and classification of rice germplasm is imperative for their effective management and subsequent utilization. Traditionally, rice germplasm in AAU is characterized based on passport data and morphology. But both the approaches have their limitations. For instance, passport data may show that accessions are conserved under identical or similar names, that samples may have been collected at nearby localities or that pedigree data indicate a common genetic background. Similarly, morphological variation is often found to be restricted and can be strongly influenced by environment. Therefore, studies to identify redundant accessions often combine molecular and morphological characterization [3,4] or are carried out exclusively with molecular markers [5,6]. There is very little information available on the nature and extent of genetic diversity of Sali rice of Assam, particularly using molecular markers. This information is quite valuable for rationalization of Sali germplasm conservation and their utilization in a breeding programme. Hence present study was conducted to understand the pattern of genetic variability in Sali rice accessions based on RAPD markers.

Material and methods

Fifty one Sali rice accessions were obtained from Regional Agricultural Research Station, Assam Agricultural University, Titabar. In this station, the accessions with same name have been maintained because of inability to distinguish them on the basis of morphology. For RAPD analysis, genomic DNA was isolated from seeds following Plaschke *et al.* (1995) with minor modification [7] Two to three seeds of each accession were crushed with a mortar and pestle to a fine powder for DNA extraction using 1000 ul of extraction buffer (1M Tris-Cl, pH 8.0, 5M NaCl; 500mM Na2 EDTA; 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 assayed by running DNA on a 0.8% agarose gel alongside a known quantity of lambda uncut DNA. Amplification reaction was carried out in a 25µl reaction volume containing 10ng of template DNA, 50pM of primer, 200uM of dNTPs, 0.5 unit of Taq polymerase (Bangalore Genei Pvt Ltd, Bangalore, India) and 1XPCR Buffer (50mM Kcl, 10mMTris-Cl, 1.5 mM $Mgcl_2$ 0.01 % gelatin). Initially, fifteen decamer random primers (Operon Technologies, Inc., Alamanda, CA, USA) were used for RAPD amplification as described by Williams *et al.* (1990) with minor modification [8]. Amplification

Table 1. List of Sali accessions used

	Genotypes	Place of collection
1.	Ranga Sali-1	Titabor, Jorhat district
2.	Ranga Sali-2	Golaghat, Golaghat district
3.	Akai Sali-1	North Lakhimpur
4.	Akai Sali-2	Titabor, Jorhat district
5.	Ranga Misi-1	Titabor, Jorhat
6.	Ranga Misi-2	Titabor, Jorhat
7.	Nainadhan-1	Titabor, Jorhat
8.	Nainadhan-2	Titabor, Jorhat
9.	Rohia - Sonahari-1	Karimganj, Karimganj district
10.	Rohia - Sonahari-2	Karimganj, Karlmganj district
11.	Rohia - Sonahari-3	Hailakandt, Cachar district
12.	Bat Kanahi-1	Jorhat, Jorhat district
13.	Bat Kanahi-2	Jorhat, Jorhat district
14.	Sail Borak-2	Karimganj, Karimganj district
15.	Sail Borak-3	Karimganj, Karimganj district
16.	Bateswari-1	Silchar, Cachar district
17.	Bateswari-2	Hailakandi, Karlmganj district
18.	Gauri Sali-1	Sibsagar, Sibsagar district
19.	Gauri Sali-2	Sibasagar, Sibsagar district
20.	Gua Sari-1	North Lakhimpur
21.	Gua Sari-2	Dibrugarh, Dibrugarh district
22.	Siamora-1	Dibrugarh, Dibrugarh district
23.	Siamora-2	Dibrugarh, Dibrugarh district
24.	Siamora-3	Dibrugarh, Dibrugarh district
25.	Latoi-1	Dibrugarh, Dibrugarh district
26.	Latoi-2	Golaghat, Golaghat district
27.	Dholamula Sali-1	North Lakhimpur
28.	Dholamula Sali-2	North Lakhimpur
29.	Kehor Sali-1	Sibsagar, Sibsagar district
30.	Kehor Sali-2	Sibsagar, Sibsagar district
31.	Motanga-1	Sibsagar, Sibsagar district
32.	Motanga-2	Sibsagar, Sibsagar district
33.	Motanga-3	Golaghat, Golaghat district
34.	Gajen Sali-1	Sadiya, Tinsukla district
35.	Gajen Sali-2	Sadiya, Tinsukla district
36.	Gajen Sali-3	Majuli, Jorhat district
37.	Gajen Sali-4	Jorhat, Jorhat district
38.	Runohi Sali-1	Nagaon, Nagaon district
39.	Runohi Sali-2	Nagaon, Nagaon district
40.	Guanakhi-1	Dibrugarh, Dibrugarh district
41.	Guanakhi-2	Dibrugarh, Dibrugarh district
42.	Poal Sali-1	Nagaon, Nagaon district
43.	Poal Sali-2	Golaghat, Golaghat district
44.	Guarai-1	Tinsukia, Tinsukia district
45.	Guarai-2	Tinsukia, Tinsukla district
46.	Guarai-3	Nagaon, Nagaon district
47.	Decemdor Sali-1	Titabor, Jorhat district
48.	Decemdor Sali-2	Golaghat, Golaghat district
49.	Beti Sali-1	Jorhat, Jorhat district
50.	Beti Sali-2	Golaghat, Golaghat district
51.	Ahom Sali-1	Golaghat, Golaghat district

reaction was performed in a thermal cycler (Gene amp PCR2400, Applied Biosystem, USA) for 35 cycles, Each cycle consisted of denaturation for 1 minute at 94°C, 1 minute annealing at 37°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 on 1.5% agarose gel from sigma, in 1 × TBE buffer and ethidium bromide stained gel was photographed with a digital gel documentation system (Ultra-violet products, UK). Reproducibility of RAPD assay was tested by performing duplicate reactions at different times using identical genotypes and primer combinations under strict control of experimental conditions and only the reproducible bands were scored.

The RAPD bands were scored as present (1) or absent (0) for each genotype-primer combination of all the 51 Sali rice accessions, considering each amplified band as unique locus. Band-sharing data were used to calculate genetic similarities based on the Jaccard's coefficient [9] and UPGMA (Un-weighted Pair Group Method using Arithmetic Averages) algorithm was employed to determine the genetic relationships of the 51 Sali rice accessions [10]. All analyses were performed using NTSYS-PC 2.10 software [11].

Results and discussion

Out of the 15 primers tested, 11 primers were selected to detect RAPD markers among the 51 Sali rice accessions, based on their reliability of amplification profile (Fig. 1). The amplification profile, generated by the 11 primers, is summarized in Table 2.

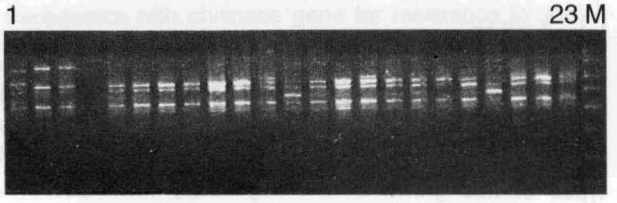

Fig. 1. A representative RAPD banding patterns of 51 Sali rice accessionsof Assam obtained with primer OPK-14. M = molecular weight marker.

Table 2. Primers used in classifying 51 Sali rice accessions

Primer	Sequence	Total band	Polymorphic band	%Polymorphism
OPH-19	5'CTGACCAGCC	9	8	88.89
OPK-14	5'CCCGCTACAC	6	2	33.33
OPK-19	5'CACAGGCGGA	8	8	100.00
OPB-08	5'GTCCACACGG	6	5	83.33
OPH-04	5'GGAAGTCGCC	7	6	85.71
OPH-20	5'GGGAGACATC	7	6	85.71
OPD-20	5'ACCCGGTCAC	5	4	80.00
OPD-03	5'GTCGCCGTCA	8	6	75.00
OPL-17	5'AGCCTGAGCC	4	3	75.00
OPD-01	5'ACCGCGAAGG	7	6	85.71
OPD-18	5'GAGAGCCAAC	5	4	80.00
	Total = 11	72	58	79.33

The average similarity index, based on Jaccard coefficient, was 0.515, indicating a high level of diversity among the Sali accessions. The similarity coefficients ranged from 0.353 (between 'Siamora 3' and 'Gauri Sali 1') to 0.926 (between 'Rohia Sonahari 1' and 'Rohia Sonahari 2'). The phenetic representation of similarity coefficient among the 51 Sali rice accessions is presented in Fig. 2. The dendrogram classified Sali rice genotypes in to two major clusters (A and B) with 23 and 28 Sali rice accessions, respectively. Accessions with similar names ('Ranga Sali 1' and 'Ranga Sali 2'), with 0.82% similarity coefficient, were collected from different districts, which may be considered as possible duplicates. Considering grouping pattern and place of collection, two 'Ranga Misi' accessions ('Ranga Misi 1' and 'Ranga Misi 2' with 0.878% similarity), two 'Nainadhan' accessions ('Nainadhan 1' and 'Nainadhan 2' with 0.818% similarity), two 'Bat Kanahi' accessions ('Bat Kanahi 1' and 'Bat Kanahi 2' with 0.894%), two 'Sail Borak' accessions ('Sail Borak 2' and 'Sail Borak 3' with 0.800%), two 'Guanakhi' accessions ('Guanakhi 1' and 'Guanakhi 2' with 0. 865%), two 'Siamora' accessions ('Siamora 1' and 'Siamora 2' with 0.845,%), two 'Dholamula Sali' accessions ('Dholamula Sali 1' and 'Dholamula Sali 2' with 0.878%) and two 'Kehor Sali' ('Kehor Sali 1' and 'Kehor Sali 2' with 0.923%) may be considered as suspected duplicate. Out of the four 'Gajen Sali' accessions, 'Gajen Sali 1' and 'Gajen Sali 2', collected from same location, were grouped together with 0.855% similarity. So these two accessions can be considered as potential duplicate. However, 'Gajen Sali 3'(collected from Majuli) and 'Gajen Sali 4' (collected from Jorhat) grouped differently, revealing some genetic differences between them. Similarly, two genotypes collected from Cachar district of Assam ('Bateswari 1' and 'Bateswari 2') were also grouped differently with 0.64% similarities, indicating genetic difference between them. Among three 'Guarai' accessions, 'Guarai 1' and 'Guarai 2', collected from same location, were grouped together with a similarity value of 0.828%, suggesting them as potential duplicate. But, 'Guarai 3' can be considered as different genotype based on its grouping pattern from other 'Guarai' accession. The grouping pattern of 'Gauri Sali 1' and 'Gauri Sali 2' (0.867% genetic similarity) with the same place of collection suggests them as potential duplicate. On the other hand, accessions with same name grouped differently like 'Latoi 1' grouped with 'December Sali 2', Latoi 2' grouped with 'December Sali 1', 'Akai Sali 2' grouped with 'Poal Sali 1', and 'Akai Sali 1' grouped with 'Poal Sali 2'. The places of collections were also different for these accessions, indicating them as genetically different. Similarly, two 'Guasari' accessions ('Guasari 1' and Guasari 2') of cluster A collected from different location and two

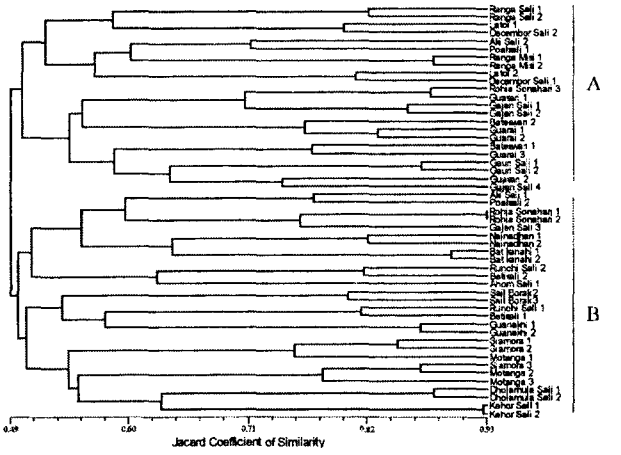

Fig. 2. Dendrogram of 51 Sali rice genotypes constructed using UPGMA based on 72 RAPD bands using Jaccard coefficient of similarity

'Runohi Sali' accessions ('Runohi Sali 1' and 'Runohi Sali 2') of cluster B collected from same location. But they were grouped differently, so they can be considered as genetically different.

The present study revealed existence of sufficient genetic variation at DNA level in Sali rice collection maintained in RARS, Titabar. This information will help breeder to select diverse varieties based on the variation at DNA level to be used in a crossing programme for realization of heterosis, identification of donor parents for useful agronomic attributes and markers linked to different polygenic traits. By identifying 11 pairs of accessions as duplicates, the present study also highlighted the presence of duplicates in Sali rice germplasm maintained in Assam Agricultural University. However, it is impossible to prove that two accessions are genetically identical without sequencing their genomes. But, from gene bank management point of view, samples do not necessarily have to be completely identical in order to be considered as duplicate/ redundant. For instance, quantification of genetic diversity between samples based on screening of germplasm for large number of markers combined with passport data can be used to identify duplicate [6]. This study demonstrated its potentiality of RAPD marker for effective management of rice collection of Assam Agricultural University in terms of estimation of diversity, duplicate identification, thereby reduces the chances of losing useful genotypes in the name of duplicates, and enhancing the use of those collection in crop improvement. But, accessions that are found identical or similar based on molecular genetic data may differ in just one important character. So it will be difficult for germplasm managers for rationalization only on the basis of molecular data as long as additional information

is unavailable. Since, there is inadequate evaluation data in rice collection maintained in AAU, marker data provide only available decision criterion. Core collection could be established by removing the duplicates in the Sali rice collection of Assam with more markers, which will help in their management in a cost effective way and will also for a safe duplication of accessions representing broad diversity of rice collection in other locations or gene banks as a backup conservation material.

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