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Comparative diversity analysis of indigenous upland rice (*Oryza sativa* L.) of Assam using morphological traits and random amplified polymorphic DNA (RAPD) markers

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ABSTRACT

The genetic relationship of 24 indigenous upland rice comprising 12 *ahu* (summer rice) and 12 *jhum* (hill rice) genotypes of Assam, India was analyzed using 14 diagnostic morphological traits and 15 random amplified polymorphic DNA (RAPD) markers. Considerable morphological variations for different traits were observed among the strains. The 15 random primers showed 92.20% polymorphism with an average polymorphism information content (PIC) value of 0.429. The mean Euclidean distance for morphology and mean Jaccard's coefficient of similarity for RAPD were 5.129±1.423 and 0.493±0.0978, respectively, indicating sufficient genetic diversity among the strains. No ecotype specific clustering was observed based on genetic similarity and distance coefficients using unweighted pair group method using arithmetic average (UPGMA). *Ahu* genotypes were more diverse than the *jhum* genotypes. Mantel test showed no correlation between morphological traits and RAPD, suggesting non-allelic relationship of the two marker systems.

KEYWORDS

Ahu;
Genetic diversity;
Jhum;
Morphological traits;
RAPD;
Upland rice.

INTRODUCTION

Genetic diversity analysis provides useful information on genetic variation of germplasm and facilitates proper conservation, management and utilization of genetic resources. Northeast India including Assam is considered as one of the primary centers of origin of rice representing rich source of genetic diversity and reservoir of valuable gene systems. The cultivation of rice under diverse agro-ecological conditions for continuous period under various biotic and abiotic stresses, specific adaptation through natural selection and farmers' discretion, ethnic migration and immigration over years have resulted in diversification of the rice genetic stock to a great extent[1]. Indigenous upland rice, comprising traditional *ahu* (summer rice) and *jhum* (hill rice) rice, is an important culture in this region after *sali* rice (winter rice). Indigenous *ahu* rice is sown directly to the field in the month of March to 1st week of April under rainfed condition[2]. The hill rice of Assam, an

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another summer rice culture, is grown in an altitude ranging from 95m to1000m above mean sea level in hilly slopes as *jhum*, i.e., shifting cultivation practice through burn and slash method and in terraces as direct seeded rainfed mixed/pure crop[3].

Upland rice of both these situations (upland-plain and upland-hills) share similar morphological and growth characteristics, such as tall plant, broad canopy, drought tolerant capacity, etc. Thus, it is very difficult to distinguish these two groups of cultivars on the basis of morphology. However, adaptation to two growing conditions (monoculture *vs*. mixed cultivation) might have contributed to genetic diversity between *ahu* and *jhum* rice. Substantial differences may be expected between these ecotypes of upland rice. Therefore, knowledge of the nature, extent and distribution of genetic variation in two populations or ecotypes of upland rice of Assam is important for the development of effective management and utilization strategies. Moreover, with the present intellectual property right (IPR) and convention of biological diversity (CBD) regime, it has become imperative to characterize the genetic diversity with regard to important morphological traits and at DNA level for safeguarding the genetic diversity.

Morphological traits have been used to assess variation in *O. sativa* L. and to classify rice genetic resources[4,5]. However, morphological traits are under complex genetic control, subject to environmental effects[4], few in numbers, lack adequate level of polymorphism and hence such markers may not completely represent underlying genetic diversity in rice. Currently, DNA based markers are being used increasingly to estimate the level of genetic diversity in plant populations because of certain advantages of these markers over morphological variables. Among the several DNA based markers, random amplified polymorphic DNA (RAPD) markers[6] are widely used because of its simplicity, speed and efficiency.

Since proper evaluation of genetic diversity is lacking in indigenous upland rice of Assam, an attempt has been made to study the genetic variation of indigenous upland rice and to evaluate the genetic relationship between *ahu* and *jhum* rice using morphological traits and RAPD markers.

EXPERIMENTAL

Plant material

A set of 24 traditional upland rice strains comprising twelve *ahu* and twelve *jhum* genotypes were collected from the Regional Agricultural Research Stations (RARS) of Assam Agricultural University at Titabar and

TABLE 1 : List of 24 indigenous upland rice strains used in the study

Name of cultivar	Ecotype	Source of collection
Ahu-II	*Ahu*	Regional agricultural research station, Titabor, India
AS-56/2	*Ahu*	Regional agricultural research station, Titabor, India
Bangal ahu	*Ahu*	Regional agricultural research station, Titabor, India
Changa ahu	*Ahu*	Regional agricultural research station, Titabor, India
Charimahia ahu	*Ahu*	Regional agricultural research station, Titabor, India
Cheni ahu	*Ahu*	Regional agricultural research station, Titabor, India
Ikoraguni	*Ahu*	Regional agricultural research station, Titabor, India
Koimurali	*Ahu*	Regional agricultural research station, Titabor, India
Kola ahu	*Ahu*	Regional agricultural research station, Titabor, India
Maibee	*Ahu*	Regional agricultural research station, Titabor, India
Bizor-II	*Ahu*	Regional agricultural research station, Titabor, India
Doga ranga	*Ahu*	Regional agricultural research station, Titabor, India
Bairing	*Jhum*	Regional agricultural research station, Diphu, India
Bairing-I	*Jhum*	Regional agricultural research station, Diphu, India
Bairiring-II	*Jhum*	Regional agricultural research station, Diphu, India
Ranga izong	*Jhum*	Regional agricultural research station, Diphu, India
Dimrou-I	*Jhum*	Regional agricultural research station, Diphu, India
Galengra	*Jhum*	Regional agricultural research station, Diphu, India
Glanchra	*Jhum*	Regional agricultural research station, Diphu, India
Miren abora	*Jhum*	Regional agricultural research station, Diphu, India
Rebon	*Jhum*	Regional agricultural research station, Diphu, India
Sakcharap	*Jhum*	Regional agricultural research station, Diphu, India
Sakcharap-II	*Jhum*	Regional agricultural research station, Diphu, India
Sokbothung-I	*Jhum*	Regional agricultural research station, Diphu, India

Diphu, India (TABLE 1). Considering Northeast India as one of the diversity hot spots for rice, considerable efforts have been made for conserving the traditional rice germplasm of this region. A variety of rice genetic stocks have been maintained at different RARS of Assam Agricultural University, India to be used as a core collection for different groups of rice. The upland rice strains used in the study were collected from the two research stations as mentioned. All the strains were self-pollinated several times for genetic purification.

Morphological studies

For studying genetic variation among the traditional upland rice cultivars, morphological traits and RAPD markers were used in the present investigation. Observations on 14 diagnostic morphological traits were recorded following the descriptor for rice (*Oryza sativa* L.) approved by IBPGR-IRRI Rice Advisory Committee. Among them, eight were quantitative (stem thickness, ligule length, 100-grain weight, panicle length, leaf width, grain width, grain length and culm length) and six were qualitative (culm angle, ligule colour, internode colour, panicle type and awning) in nature. For each morphological trait, the average of three records was used for data analysis. Germinated seeds of each accession were planted in earthen pots of 20 cm diameter and grown in the net house in two summer seasons separately. Single plant per pot and ten pots for each accession were maintained to record the morphological traits.

RAPD analysis

The total genomic DNA was isolated from seed following a protocol[7] with minor modification by avoiding the use of liquid nitrogen. A set of 15 random primers obtained from Operon Technologies Inc. were used for polymerase chain reaction (PCR) amplification. These were OPH-12, OPH-04, OPD-18, OPD-01, OPD-03, OPD-19, OPK-14, OPK-20, OPK19, OPA-01, OPA-03, OPA-10, OPM-01, OPM-19, OPL-07. PCR amplification was performed in a 25µl reaction volume containing 2.5µl 10X PCR buffer, 200µM each dNTPs, 35 pM primer, 2mM $MgCl_2$, 0.5 u Taq DNA polymerase and 20 ng DNA template. After an initial denaturation step at 95°C for 5 min, 40 cycles of 1 min at 94°C, 1 min at 35°C and 1 min at 72°C were performed, followed by a final extension of 5 min at 72°C. The amplification products were separated by electrophoresis on 1.5% agarose gel with a known molecular weight marker as standard.

The DNA isolated from single plant was used in all random amplified polymorphic DNA (RAPD) analyses. Amplification of each RAPD primer was repeated three times, and the bands consistently detected with similar intensity in all experiments were selected as reproducible fragments.

Data analysis

Reproducible RAPD bands were scored in a binary format, i.e., presence of band was scored as unity and its absence as zero. The binary data generated by RAPD were used to detect percent polymorphism and polymorphism information content (PIC). The percent polymorphism was calculated by dividing polymorphic amplified fragments to total number of amplified fragments multiplied by hundred. The polymorphic information content (PIC) was calculated as $PIC = 1- \sum(Pi)^2$, where Pi is the proportion of the population carrying i^{th} allele, calculated for each marker locus[8].

The data on morphological traits and RAPD were analyzed using a software package called NTSYS-PC version 2.1[9]. The Euclidean distance for morphological trait is a dissimilarity coefficient; that is, larger is the value greater is the distance between pairs of accessions[10]. For RAPD data, genetic relationship among the accessions was computed using Jaccard's coefficient of similarity[11].

The phenetic representation of genetic relationship among the genotypes as revealed by Euclidean distance and Jaccard's similarity coefficient was performed by cluster analysis using unweighted pair group method using arithmetic average (UPGMMA). The degree of association between the similarity matrix for RAPD and distance matrix for morphological traits was done by Mantel test[12].

RESULTS AND DISCUSSION

Pattern of morphological variation

The present study revealed significant difference for quantitative traits except grain width and grain length. Of the 24 genotypes under study, 14 genotypes re-

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corded the same grain width (0.30cm) and eight genotypes recorded the same grain length (0.70cm), showing least statistical difference (data not shown). Such a high degree of variation in morphological traits e.g. plant height, plant habit, tillering capacity, leaf characteristics, grain size and duration collection was previously reported in Assam rice[2,13].

Comparative analysis of morphological traits between *ahu* and *jhum* genotypes revealed that there was no significant difference between these two ecotypes with reference to quantitative traits except for stem thickness and 100 grain weight (TABLE 2). The mean stem thickness of *jhum* genotypes (3.21cm) was found to be more than that of *ahu* genotypes (2.59cm), whereas the mean 100-grain weight of *ahu* genotypes (2.04g) was found to be more than *jhum* genotypes (1.50g). However, qualitative traits under study failed to distinguish two ecotypes. In case of panicle type, only intermediate type was observed in *jhum* genotypes, however that type was also present in *ahu* accessions along with open type (TABLE 3). It warrants inclusion of few more easily distinguishable traits for quick characterization of indigenous upland rice of Assam. Moreover, the observations were recorded on potted plants which might have influenced the development of morphologi-

TABLE 2 : Comparative variations for quantitative traits in *ahu* and *jhum* genotypes

Traits	Mean ± SD		
	Ahu	*Jhum*	*Ahu + Jhum*
Stem thickness (cm)	2.59 ± 0.529	3.21 ± 0.589	2.8 9 ± 0.634
Ligule length (cm)	2.09 ± 0.517	2.03 ± 0.496	2.06 ± 0.497
100 Grain wt. (g)	2.04 ± 0.261	1.50 ± 0.224	1.77 ± 0.364
Panicle length (cm)	22.38 ± 2.362	22.48 ± 2.467	22.43 ± 2.369
Leaf width (cm)	2.12 ± 0.191	1.93 ± 0.205	2.03 ± 0.221
Grain width (cm)	0.30 ± 0.033	0.29 ± 0.042	0.30 ± 0.038
Grain length (cm)	0.77 ± 0.040	0.70 ± 0.054	0.74 ± 0.058
Culm length (cm)	84.85 ± 16.725	84.82±11.533	84.8 ± 14.050

TABLE 3 : Morphological variations of twenty four indigenous upland rice for qualitative traits

Name of cultivars Ahu (a), Jhum (j)	Traits					
	Culm angle	Ligule colour	Auricle colour	Internode colour	Panicle type	Awning
Ahu III (a)	Open	White	Pale Green	Light gold	Intermediate	Short and Partly awned
AS 56/2 (a)	Open	White	Pale Green	Purple lines	Open	Short and Partly awned
Bangal ahu (a)	Erect	White	Pale Green	Green	Intermediate	Absent
Changa ahu (a)	Erect	White	Pale Green	Light gold	Intermediate	Short and Partly awned
Charimahia ahu (a)	Open	White	Pale Green	Light gold	Intermediate	Short and Partly awned
Cheni ahu (a)	Open	White	Pale Green	Green	Open	Absent
Ikoraguni (a)	Open	White	Pale Green	Green	Open	Absent
Koimurali (a)	Erect	White	Pale Green	Light gold	Intermediate	Short and Partly awned
Kola ahu (*a*)	Open	White	Pale Green	Purple lines	Intermediate	Absent
Maibee(A) (*a*)	Erect	White	Pale Green	Purple lines	Intermediate	Absent
Bizor II (*a*)	Erect	White	Pale Green	Light gold	Intermediate	Absent
Doga ranga (*a*)	Erect	White	Pale Green	Purple lines	Intermediate	Absent
Bairing (*j*)	Erect	White	Purple	Purple lines	Intermediate	Absent
Bairing I (*j*)	Erect	White	Pale Green	Purple lines	Intermediate	Absent
Bairing II (*j*)	Erect	White	Pale Green	Light gold	Intermediate	Absent
Ronga Izong (*j*)	Open	Purple lines	Purple	Purple	Intermediate	Absent
Dimrou I (*j*)	Open	White	Purple	Purple	Intermediate	Short and Partly awned
Galengra (*j*)	Open	White	Pale Green	Green	Intermediate	Absent
Glanchra (*j*)	Erect	White	Pale Green	Purple lines	Intermediate	Absent
Miren Abora (*j*)	Erect	White	Pale Green	Purple lines	Intermediate	Absent
Rebon (*j*)	Erect	White	Pale Green	Green	Intermediate	Absent
Sakcharap (*j*)	Open	White	Pale Green	Green	Intermediate	Absent
Sakcharap I (*j*)	Erect	White	Pale Green	Purple lines	Intermediate	Absent
Sokbothung II (*j*)	Erect	Purple	Purple	Purple	Intermediate	Absent

cal variation than that usually expressed in natural conditions, resulting in low morphological variation between these two ecotypes (*ahu* and *jhum*). We may expect better results if the genotypes would have evaluated under normal growing conditions with reduced environmental noise.

RAPD assay

The 15 random primers revealed 90.09 percent polymorphism in all 24 upland rice genotypes. The percent polymorphism within *ahu* genotypes (86.79%) was found to be higher than *jhum* genotypes (83.11%), indicting greater level of genetic diversity of *ahu* genotypes than *jhum* genotypes (TABLE 4). The average PIC value revealed by 15 RAPD markers was 0.429 for all genotypes. The average PIC values for both the ecotypes were found to be almost same (0.396 for *ahu* and 0.399 for *jhum* genotypes). The primer OPD-18 and OPA-10 showed highest PIC of 0.499, suggesting greater capacity to distinguish the genotypes under study than the other primers (TABLE 4). Although the RAPD markers showed only two genotype specific bands, however, three ecotype specific bands for *ahu* and two for *jhum* genotypes were also observed.

TABLE 4 : Percent polymorphism and polymorphism information content revealed by RAPD

Primer	Sequence	Percent polymorphism			Polymorphism information content		
		All genotypes	*Ahu*	*Jhum*	All genotypes	*Ahu*	*Jhum*
OPH-12	5'ACGCGCATGT3'	100	100	63.63	0.428	0.495	0.339
OPD-18	5'GAGAGCCAAC3'	92.31	90.9	83.33	0.499	0.471	0.473
OPK-14	5'CCCGCTACAC3'	90	87.5	90	0.444	0.345	0.493
OPK-20	5'GTGTCGCGAG3'	90	90	80	0.475	0.466	0.496
OPD-01	5'ACCGCGAAGG3'	100	100	100	0.152	0.218	0.152
OPD-03	5'GTCGCCGTCA3'	85.71	50	80.71	0.476	0.401	0.497
OPL-07	5'AGGCGGGAAC3'	66.66	66.66	50	0.479	0.305	0.481
OPA-10	5'GTGATCGACA3'	100	100	100	0.499	0.517	0.222
OPK-19	5'CACAGGCGGA3'	83.33	83.33	83.33	0.495	0.391	0.446
OPH-04	5'GGAAGTCGCC3'	100	100	100	0.345	0.349	0.393
OPM-01	5'GTTGGTGGCT3'	93.33	88.88	83.33	0.437	0.355	0.397
OPM-19	5'CCTTCAGGCA3'	93.75	66.66	85.71	0.421	0.337	0.35
OPD-19	5'CTGGGGACTT3'	88.88	100	66.66	0.477	0.435	0.426
OPA-01	5'CAGGCCCTTC3'	87.5	85.7	90	0.43	0.396	0.453
OPA03	5'AGTCAGCCAC3'	80	92.3	90	0.381	0.453	0.372
Average		90.098	86.795	83.113	0.429	0.396	0.399

RAPD technique has been used for detecting polymorphism, variety specific band and various genetic diversity analyses in rice[14-16]. The high level of polymorphism in upland rice detected by RAPD in the study is in conformity with various earlier reports in Indian[15,17] and Australian rice[18]. The region with hot spot of genetic diversity also represents vast array of allelic diversity[1], which might be a reason of revealing such a high level of polymorphism.

Analysis of genetic diversity based on morphological traits and RAPD

The genetic distance among the populations based on all 14 morphological traits ranged from 1.643 (between 'Maibee' and 'Doga ranga') to 8.630 (between 'AS-56/2' and 'Bangal ahu') with a mean of 5.129 for 24 upland genotypes under study (data not shown). There was no significant difference in Euclidean distance observed within *ahu* and *jhum* accessions (4.740 and 4.754 respectively).

Cluster analysis of morphological traits using UPGMA (Figure 1) revealed that 'AS-56/2' was outgrouped from the rest of the genotypes as well as from *ahu* genotypes also, indicating considerable dissimilarity of 'AS-56/2' from the other genotypes. It was observed that 'AS-56/2' showed some extreme phenotypes such as highest panicle length, leaf width and grain length, which might be a reason for its separate identity. The rest of the accessions can be grouped into two clusters (A and B). The largest cluster (A) can be sepa-

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rated into many sub clusters, in which a tendency of ecotype specific grouping may be observed with few exceptions (e.g., in case of 'Bizor-II' and 'Glanchra'). The cluster B comprised of only two *jhum* genotypes ('Ronga izong' and 'Sokbothung-I'). Both the genotypes were found to have greater dissimilarity next to 'AS-56/2' and can be separated from rest of the genotypes.

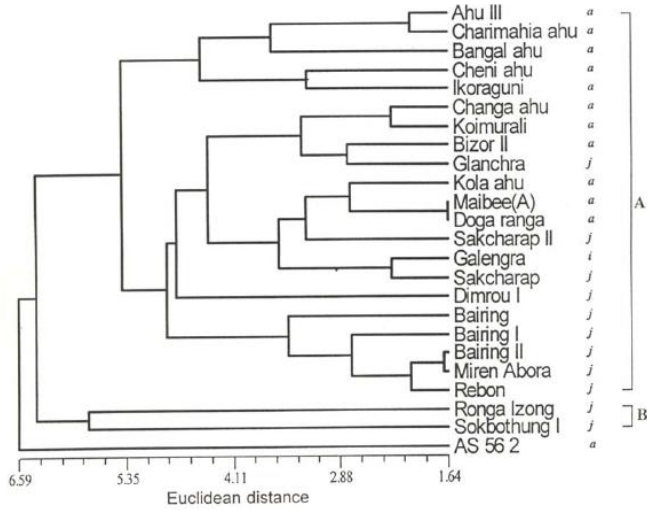

Figure 1 : Dendrogram of *ahu* and *jhum* genotypes based on Euclidean distances of morphological traits. *a* and *j* indicate *ahu* and *jhum* genotypes, respectively

The pair wise genetic relationship among 24 upland rice genotypes based on RAPD data using Jaccard's coefficient of similarity ranged from 0.245 (between 'Cheni ahu' and 'Kola ahu') to 0.804 (between 'Sakcharap' and 'Galengra') with an average of 0.493 (data not shown), indicating high level of genetic diversity in indigenous upland rice of Assam. No two genotypes showed distance of zero or similarity of one, which indicates that the plant material used in the study does not contain any true duplicate. *Jhum* rice showed higher average similarity index (0.562) than *ahu* genotypes (0.458), indicating more diversity in *ahu* genotypes. The population in close neighborhood tended to be uniform because genetic differentiation is often prevented by gene flow[19]. Although RAPD data revealed higher genetic diversity of *ahu* genotypes than the *jhum* genotypes; however, no distinct differentiation between the ecotypes i.e., *ahu* and *jhum* was observed. Rice cultivars of both the ecotypes have been grown for thousands of years and there is no restricted gene flow between *ahu* and *jhum* rice as there is no cross barrier, which might have contributed to indistinct differentiation between *ahu* and *jhum* rice. From adaptive point of view, *ahu* and *jhum* rice cultivars share similar characteristics like drought tolerant capacity, competition for weeds etc., indicating a close relationship in adaptive gene complex. However, microclimatic variation is bound to induce some differences in the genetic make-up of these two ecotypes, which needs further investigation.

The dendrogram of 24 genotypes based on RAPD using UPGMA cluster analysis is presented in Figure 2. Grouping pattern revealed that 'Kola ahu', 'Bairing' and 'Koimurali' were more diverse and could be separated from other genotypes. Rests of the genotypes were separated into four main clusters (A, B, C and D). Ecotype specific clustering pattern was only observed in sub-cluster of A with four *jhum* genotypes ('Bairing-II', 'Ronga izong', 'Glanchra' and 'Sokbotung-I') and in cluster D with four *ahu* genotypes ('AS-56/2, 'Ikoraguni', Bangal ahu' and 'Changa ahu'). For rest of the genotypes no ecotype specific clustering was observed, suggesting close genetic relationship between these two groups of cultivars.

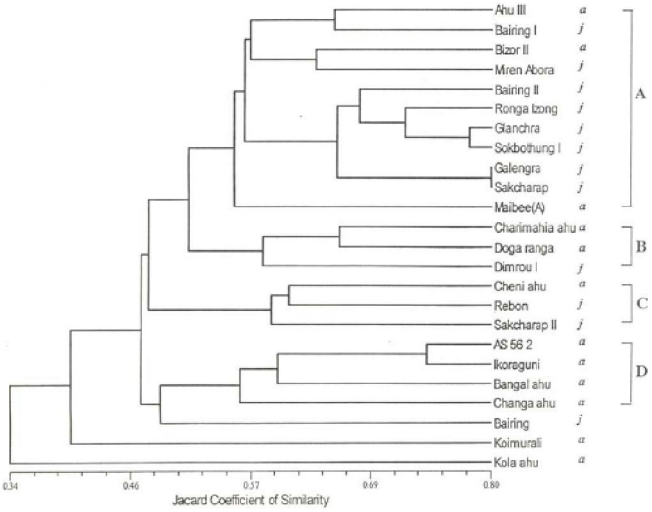

Figure 2 : Dendrogram of *ahu* and *jhum* genotypes based on Jaccard's similarity coefficients of RAPD. *a* and *j* indicate *ahu* and *jhum* genotypes, respectively

Correlation between morphological traits and RAPD

The correlation coefficient between the data matrix and the cophenetic matrix for RAPD data was 0.82 which is considered as high enough to indicate that the clustering dendrogram is a good representation of the

original similarity matrix[10]. However, Mantel test[12] revealed lack of correlation between the genetic diversity indices of morphology and RAPD (r = -0.10923), indicating that the two marker systems represent two areas of genome. Morphological traits are controlled by several genes, which may be highly influenced by environment[4]. Most of these traits also sample a very small region of the genome[17]. Moreover, the diversity of morphological traits reflects the variation in expressed (coding) sequences, while RAPD polymorphism represents variation in both coding and non-coding sequences and distributed throughout the gnome[6,14]. Therefore, to obtain a good correlation between these two marker systems would be very difficult.

One of the important goals in any diversity analysis is to have a core collection for future reference. Usually, core collections are regarded as being a limited set of germplasm accessions from a larger germplasm collection, selected on the basis that they are representative of the diversity within the whole collection, and aim at improving the efficiency of management and use of large germplasm collections[20]. There are a number of ways that a core set of germplasm can be selected, and in our work we tested, on a small scale, the efficiency of two possible methods. This study will guide systematic collection and maintenance of upland rice germplasm of Assam along with their proper exploitation in rice breeding.

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REFERENCES

[1] G.R.Das, T.Ahmed; Conservation of Rice Genetic Resources of N.E.India, In A.C.Thakur Ed. 'Problems and Prospects of Agricultural Research and Development in North East India', Proceedings of Agricultural Science Society, India, **(1978)**.
[2] G.R.Das, T.Ahmed, H.C.Bhttacharya, B.C.Borthakur; J.Res., Assam Agricultural University, **2**, 56-154 **(1981)**.
[3] A.Roy; Collection and Evaluation of Hill Rice Genotypes of Karbi Anglong and North Cachar Hills Districts of Assam, Final Project Report, Director of Research, Assam Agricultural University, India, **(2001)**.
[4] H.I.Oka; Jpn.J.Breed., **3**, 33-34 **(1953)**.
[5] T.Morinaga; Jpn.J.Breed., **4**, 1-19 **(1954)**.
[6] J.H.K.Williams, A.R.Kubelik, K.J.Livak, J.A.Rafalsky, S.V.Tingey; Nucl.Acids Res., **18**, 6531-6535 **(1990)**.
[7] J.Plaschke, M.W.Ganal, M.S.Roder; Theor.Appl. Genet., **254**, 1001-1007 **(1995)**.
[8] D.Botstein, R.L.White, N.Skolnick, R.W.Davis; Am.J.Hum.Genet., **32**, 314-331 **(1980)**.
[9] F.J.Rohlf; NTSYS-pc: Numerical Taxonomy and Multivariate Analysis System, Version 2.0. Exeter Software, New York, **(1998)**.
[10] P.H.A.Sneath, R.R.Sokal; Numerical Taxonomy. W.H.Freeman & Co., San Francisco, **(1973)**.
[11] P.Jaccard; Bulletin De La Société Vaudoise De Sciences Naturales, **44**, 223-270 **(1908)**.
[12] N.A.Mantel; Cancer Res., **27**, 209-220 **(1967)**.
[13] D.V.Seshu, U.P.Rao, T.E.Erinivasan, S.V.S.Shastri; Ind.J.Genet., **34**, 143-149 **(1974)**.
[14] P.S.Virk, H.J.Newbury, M.T.Jackson, B.V.Ford-Lloyed; Heredity, **74**,170-179 **(1995)**.
[15] N.Nadarajan, P.Vivekananda, R.J.Henry; Ind.J. Genet., **59**, 247-259 **(1999)**.
[16] S.Baishya, A.Sachdev, R.P.Johari, S.L.Mehta; J.Plant Biochem.Biotech., **9**, 23-26 **(2000)**.
[17] G.Thomas, L.Joseph, G.Varghese, K.Sreejayan, P.Kuriachan; Canad.J.Bot., **79**, 1090-1098 **(2001)**.
[18] H.I.Ko, D.C.Cowan, R.J.Henry, G.C.Graham, A.B.Blackeney, L.G.Lewin; Euphytica, **80**, 179-189 **(1994)**.
[19] M.D.Loveless, J.L.Hamrick; Annl.Rev.Ecol.Syst., **15**, 65-95 **(1984)**.
[20] A.H.D.Brown; Genome, **31**, 818-824 **(1989)**.