Assessment of the Genetic Diversity of Joha Rice Germplasms by using Simple Sequence Repeat Markers

P. Saikia, B. Neog, N. Gogoi, D. Baruah

ABSTRACT

Background: Joha Rice are aromatic rice landraces, having small to medium grain size, indigenous to Assam, India. Due to the introduction of high yielding hybrid varieties, many endemic rice landraces including Joha Rice, are in a verge of extinction, as these can only be conserved and maintained by repetitive cultivation. As there is a conflict of local names for these landraces, many landraces with similar morphological characters have been reported from various parts. Simple sequence repeat (SSR) markers with longer perfect repeats have earlier proved successful and essential in studying the genetic diversity among rice cultivars. The present study is aimed to evaluate the genetic relationship among fifteen (15) aromatic Joha rice landraces endemic to Upper Brahmaputra Valley, Assam.

Methods: In the present investigation, different landraces of Joha rice were surveyed during 2016-2019. 15 landraces were selected, based on their morphological characters and local data. The collected germplasm of Joha rice was grown in the experimental plots and DNA from young, healthy leaves were isolated which were further used for determination of genetical diversity using SSR markers. Thirty-eight SSR markers were used to evaluate the genetic relationship among the fifteen aromatic rice landraces.

Result: A total of 110 polymorphic alleles were detected by 34 markers across all the landraces, with an average of 3.25 per locus. The Polymorphic Information Content (PIC) ranged from 0.24 to 0.83, with an average of 0.5 for each marker. The marker RM154, RM454 and RM489 produced maximum six alleles showing PIC value of 0.82, 0.82 and 0.83, indicating a high polymorphism. UPGMA cluster analysis using Jaccard’s similarity index produced a dendrogram clustering the rice landraces in three major groups and five subgroups. Group II, which consisted of five sub-groups and 12 landraces, showed diverse genotypes. These landraces showed significant genetic similarities.

Key words: Endemic, Genetic relationship, Joha, Landraces, SSR markers.

INTRODUCTION

Rice is an essential food crop and staple food for about 2.5 billion people (Ram et al., 2003). Asia alone consumes 90% of the total rice produced worldwide and as such, the demand for rice continues to rise (Maclean et al., 2002). Of the 10,000 landraces of rice cultivated worldwide, India itself grows about 4000 landraces. India is the second-largest producer of rice, 103,500 million tones and ranks top in the area of cultivation (Childs, 2016).

As rice has a long history of its origin and cultivation in Asia and due to its wide geographical distribution, a significant number of varieties have been developed. Different terms were designated for identical morphological traits leading to variation in nomenclature and miscommunication among the researchers.

Rice has significant diversity among the landraces concerning its growing habit, flowering time, seasonal influence, the incidence of sun rays, phenotype such as plant height, leaf length, breadth, colour, presence of culm, leaf flag, ligule, etc. The seed of rice is also diverse in context to shape, size, the colouration of the grain as well as its covering, aroma, cooking properties, etc. These phenotypic and genotypic characters within the Oryza sativa makes a significant impact in studying the diversity among the rice. A sound scientific procedure is required for checking the morphological characters and the Directorate of Rice Improvement, the Directorate of Rice Research, ICAR has identified certain characters which are used for DUS testing for the Protection of Plant Landraces and Farmers’ Right Act in 2001 which can be further used for accessing the morphological structure of rice plant landraces (Shobha Rani et al., 2004).

How to cite this article: Saikia, P., Neog, B., Gogoi, N. and Baruah, D. (2021). Assessment of the Genetic Diversity of Joha Rice Germplasms by using Simple Sequence Repeat Markers. Indian Journal of Agricultural Research. DOI: 10.18805/IJARe.A-5689.

Research, ICAR has identified certain characters which are used for DUS testing for the Protection of Plant Landraces and Farmers’ Right Act in 2001 which can be further used for accessing the morphological structure of rice plant landraces (Shobha Rani et al., 2004).

Microsatellites, Simple Sequence Repeats (SSR), Short Tandem Repeats (STR) and Simple Sequence Length Polymorphisms (SSLP) are widely found in the genome of prokaryotes and eukaryotes. They are distributed in the euchromatin of eukaryotes and coding and non-coding nuclear and organellar DNA (Pérez-Jiménez et al., 2013; Phumichai et al., 2015). The frequency of SSRs in rice is high in transcribed regions, especially in untranslated regions (UTRs) (Morgante et al., 2002). DNA sequence
information on the rice genome available in public domain shows, SSRs with longer perfect repeats (≥ 20 nucleotides) are highly polymorphic (Temnykh et al., 2001). Such markers can be used to study genetic diversity among different cultivars as well as landraces of rice (Singh et al., 2016).

Joha rice (Oryza sativa) is short grain rice landraces intermediate of both indica and japonica variety (Roy et al., 2015). These landraces are indigenous to Assam and have a characteristic aroma like basmati or jasmine rice in terms of aroma but are different from the later because of their small size, high gluten content, unique aroma, superfine kernel, good cooking qualities and excellent palatability (Raina et al., 1987). It is not a preferred variety for cultivation due to its low productivity and yield. And for this reason, very little research has been carried for the conservation of these landraces (Roy et al., 2020). The germplasm of 42 landraces of Joha landraces is maintained by the Assam Agriculture University (Das et al., 2010). There has been a treat of extinction of these indigenous landraces due to the introduction of high yielding landraces (Saikia and Neog, 2020).

MATERIALS AND METHODS

Plant material
In this study, 15 indigenous aromatic rice landraces were collected from various region of Upper Brahmaputra Valley, Assam India, during the period from 2016-2019 (Table 1). The collected germplasm were grown in an experimental designed plot of Dibrugarh University, India. The sprouted seedlings (21 days old) of the 15 accession were transplanted in the field with 15 replications each.

Genomic DNA extraction
The lab experiment was carried out during 2019, in the Plant and Molecular Biology Laboratory, Department of Life sciences, Dibrugarh University. Genomic DNA was isolated from fresh, young leaves of aromatic rice genotypes using CTAB method (Doyle and Doyle, 1987). The quality of DNA was determined by running it on 0.8 % agarose gel with 1x TBA buffer (pH 8.0) at 90 V for 30 minutes and observed in a UV/Vis Gel documentation system (Alphalmager Mini, USA). The concentration of the DNA was measured for the absorbance ratio A260/A280 using UV/Vis microplate Spectrophotometer (Multiskan Sky, Thermo Fisher) and diluted to a final concentration of 100 ng/µl for use in PCR amplification.

SSR genotyping
A total of thirty-eight (38) SSR primers (Table 2) were selected from the rice genome database (http://www.gramene.org/) to carry out PCR on the entire 15 rice accession (Table 1). The SSR primer chosen sets were distributed evenly on the rice chromosome (Park et al., 2019) and had high repeatability, high band reproducibility and a high degree of polymorphism. The PCR reaction was carried out in a 200 µl PCR tube with 20 µl reaction containing 2 µl PCR buffer without MgCl₂ (SRL, BioLit), 1 µl template DNA, 1 µl forward primer (Eurofins), 1 µl reverse primer (Eurofins), 2 µl of 2 M MgCl₂ (SRL, BioLit), 1 µl Taq DNA polymerase (SRL, BioLit), 1 µl dNTPs (SRL, BioLit) and 11 µl of DNase free water. Annealing temperature ranged from 50° to 61°C depending upon the Tₘ of the primer sets. PCR amplification was performed in a thermocycler (Eppendorf, Master cycler nexus) by an initial denaturation at 94°C for 4 min, followed by 36 cycles of 94°C for 30 sec, 53-60°C for 45 sec, 72°C for 1 min and a final extension at 72°C for 10 min. The PCR amplicons (5ml) were stained with the ethidium bromide (10mg/mL) and electrophoresed alongside with 100 bp molecular DNA ladder (Biolt, ProxiO 100bpDNA ladder, SRL) on a 1.5% Agarose gel in 1X TBA buffer (pH 8.0) at 90 V for 30 minutes and observed in a UV/Vis Gel documentation system (Alphalmager Mini, USA). The number of alleles and approximate size of alleles was determined for each marker.

Table 1: List of aromatic rice landraces with their local names and collection place.

| Local Name            | Place of Collection | Grain Character:Grain length, lemma and palea colour |
|-----------------------|--------------------|------------------------------------------------------|
| Kola Kunkuni Joha     | Sonitpur           | Short, black without awn                             |
| Boga Kunkuni Joha     | Sonitpur           | Short, gold furrow without awn                       |
| Padumoni Joha         | Sonitpur           | Long, straw without awn                              |
| Kola Joha (1)         | Sonitpur           | Medium, black without awn                            |
| Kola Joha (2)         | Sibsagar           | Medium, brown furrow without awn                     |
| Dangor Joha           | Dhemaji            | Medium, black without awn                            |
| Ronga Joha            | Jorhat             | Medium, red without awn                              |
| Maniki Madhuri        | Sonitpur           | Short, straw without awn                             |
| Joha Bora             | Sonitpur           | Long, gold furrow without awn                        |
| Boga Kon Joha         | Lakhimur           | Short, yellowish without awn                         |
| Rani Kajol            | Sonitpur           | Short, black without awn                             |
| Boga Bhaboli Joha     | Sibsagar           | Medium, straw with awn                               |
| Ronga Bhaboli Joha    | Jorhat             | Medium, red with awn                                 |
| Tulsi Joha             | Jorhat             | Medium, brown furrow without awn                     |
| Bos Joha              | Jorhat             | Short, gold furrow without awn                       |
| Sl No. | Marker  | Forward Primer | Reverse Primer | T°c  | Chr No | Allele No. | Approximate Allele size in bp | PIC value | MI value |
|-------|---------|----------------|----------------|------|--------|------------|--------------------------------|-----------|---------|
| 1     | RM495   | aatc caaggtgc agatgttg | caac gatgc acacca acc | 55   | 1      | 2          | 150-160                         | 0.391111 | 0.782222 |
| 2     | RM1     | gc gaaacc caacct cagaa | ggct ttgttg gacctg ac | 55   | 1      | 4          | 70-120                          | 0.746667 | 2.986667 |
| 3     | RM283   | gctcaacagcctctgttg g | gcagcctagctctgtagat | 61   | 1      | 4          | 130-180                         | 0.693333 | 2.773333 |
| 4     | RM23    | cattggaggttgagcttg | gtc agccctgc atttc | 55   | 1      | 3          | 110-140                         | 0.48      | 1.44    |
| 5     | RM294   | tgtgccccaggttgctcctcctcac | gagggtagcacaactctgtgac | 55   | 1      | 1          | 180                             | 0         | 0       |
| 6     | RM514   | accctctgcctcctgctcctcc | ctcctctcctgctccacgctc | 61   | 2      | 6          | 150-230                         | 0.817778 | 4.906667 |
| 7     | RM542   | ctgatagcagcagctgcttg | gcgtagcataacagctc | 55   | 2      | 3          | 190-210                         | 0.426667 | 1.28    |
| 8     | RM489   | acttgagaactgcttggacc | tcacccataggtgtctcag | 55   | 3      | 6          | 220-290                         | 0.826667 | 4.96    |
| 9     | RM514   | aggatcttcacacctcccc | caccagcaataacttgctg | 55   | 3      | 5          | 230-280                         | 0.791111 | 3.955556 |
| 10    | RM3134  | gcagggcaacagcaccaggag | aggtggaggtctattgtg | 55   | 3      | 5          | 170-190                         | 0.231111 | 0.462222 |
| 11    | RM124   | atcgctctgggcttctggctc | gcagctgc acgacctccctc | 67   | 4      | 4          | 260-290                         | 0.577778 | 2.311111 |
| 12    | RM307   | gtactac gctacctatccgctcc | ctgctatgc atgaaccttc | 55   | 4      | 4          | 110-190                         | 0.346667 | 1.386667 |
| 13    | RM6250  | aacc tctagttac cctgcacg | gcctcagctgttcagacgg | 50   | 4      | 2          | 170-190                         | 0.124444 | 0.248889 |
| 14    | RM413   | ggccgctatcttgatagagag | tcccacaccactgttctctc | 53   | 5      | 4          | 70-120                          | 0.711111 | 2.844444 |
| 15    | RM161   | tgcagctatgaggagcctgctc | tgtgcacagagctgctccctgc | 61   | 5      | 4          | 150-190                         | 0.72      | 2.88    |
| 16    | RM159   | ggccgctatcttgatagagag | tgtgcacagagctgctccctgc | 55   | 5      | 3          | 240-260                         | 0.631111 | 1.893333 |
| 17    | RM454   | ctctagctatcttgatagagag | tgcagctgacatcatcggag | 55   | 6      | 6          | 250-290                         | 0.817778 | 4.906667 |
| 18    | RM314   | tgcagctatgaggagcctgctc | tgtgcacagagctgctccctgc | 55   | 6      | 2          | 110-120                         | 0.32      | 0.64    |
| 19    | RM469   | agctgtagaggtgcagagctgctc | gcctgacgctgtagctc | 55   | 6      | 2          | 170-190                         | 0.231111 | 0.462222 |
| 20    | RM655   | aaccaccaccctgcgtgctc | aacaggtggagagctgctc | 57   | 7      | 2          | 130-150                         | 0.231111 | 0.462222 |
| 21    | RM712   | tgcagctctacgtgctccacgagctc | caacaggcacagcctgcttg | 55   | 7      | 2          | 160-170                         | 0.32      | 0.64    |
| 22    | RM251   | atgcagctctacgtgctccagcagcc | agaggacatctgctgagaacgcc | 55   | 7      | 3          | 120-140                         | 0.56      | 1.68    |
| 23    | RM343   | tgcagctctacgtgctccacgagctc | agagacacgtgctgctc | 53   | 8      | 4          | 210-250                         | 0.693333 | 2.773333 |
| 24    | RM195   | aaaaagagagcctgctggcccgc | gggtcacc ccccaacctgc | 61   | 8      | 1          | 310                             | 0         | 0       |
| 25    | RM342   | ccactctcctccatcataagaaag | actatcgaggtgtgctc | 55   | 8      | 1          | 140                             | 0         | 0       |
| 26    | RM316   | ctatggtgctgctgctgctg | acgccttatattacgtcaac | 55   | 9      | 4          | 190-220                         | 0.64      | 2.56    |
| 27    | RM321   | ccaacagccctgcgtctgtc | gacggtagcagctctgtc | 55   | 9      | 1          | 200                             | 0         | 0       |
| 28    | RM640   | tgcctagctgcacgctgctc | gcctgccccacaataaag | 55   | 9      | 2          | 150-170                         | 0.32      | 0.64    |
| 29    | RM484   | ttcctctctcttcctctgtgc | tgtgcctctctctctctctc | 55   | 10     | 2          | 280-300                         | 0.48      | 0.96    |
| 30    | RM291   | tgcaggtctacgtgctccacgagctc | ctgctgagacatgctgactgaccc | 55   | 10     | 3          | 80-120                          | 0.64      | 1.92    |
| 31    | RM311   | tgcctatgtagtacatcactaatc | tctctatccatcataacacat | 55   | 10     | 3          | 160-190                         | 0.24      | 0.72    |
| 32    | RM287   | ttcctctctcttcctctgtgc | tgcctgcctccttccttcctc | 55   | 11     | 3          | 80-120                          | 0.24      | 0.72    |
| 33    | RM536   | ttcctctctctctctctgtgc | tgcctgagacatgctgactgaccc | 55   | 11     | 3          | 220-250                         | 0.426667 | 1.28    |
| 34    | RM202   | cagctatgtagtacatcactaatc | tgcctgagacatgctgactgaccc | 55   | 11     | 3          | 150-190                         | 0.497778 | 1.493333 |
| 35    | RM277   | cgaactaactacacctgcagc | caagctgccttgcaaggaag | 55   | 12     | 2          | 100-120                         | 0.48      | 0.96    |
| 36    | RM19    | caaaccagagcgctagtgcagtc | ctaagctgagctggcgtcagtc | 55   | 12     | 4          | 190-250                         | 0.72      | 2.88    |
| 37    | RM270   | ggccgctgttgctctctctctctctctctc | tgcctgagacatgctgactgaccc | 55   | 12     | 2          | 120-140                         | 0.32      | 0.64    |
| 38    | RM7376  | ttcctctctctctctctctctctctctctctctctctctctctc | tgcctgagacatgctgactgaccc | 50   | 12     | 2          | 180-200                         | 0.48      | 0.96    |
Assessment of the Genetic Diversity of Joha Rice Germplasms by using Simple Sequence Repeat Markers

Genetic diversity analysis

To study the diversity of microsatellite markers among the fifteen accession, PIC values for the SSR primers were calculated using the following formula:

\[
\text{PIC} = 1 - \frac{1}{n} \sum_{i=1}^{n} P_{ij}^2
\]

Where \( n \) is the number of alleles analyzed per marker and \( P_{ij} \) is the frequency of the \( j \)th common band pattern among the bands of marker \( i \) (Smith et al., 1997 and Park et al., 2019). PIC value was used to calculate Marker Index (MI) using the following formula:

\[
\text{MI} = \text{PIC} \times E
\]

\[ E=\beta \]

Where \( \beta \) is the fraction of polymorphic markers, estimated after considering the polymorphic loci (np) and non-polymorphic loci (nnp) as

\[ \beta = \frac{np}{np+nnp} \]

Microsatellite markers with high reproducibility and high polymorphism were selected for genetic diversity analysis. Past (Version 4.03) program was used to score codominant markers (present=1, absent=0), genetic similarity values were calculated following Jaccard’s method and cluster analysis using UPGMA method. PCR profile of the amplicons generated for each marker was used for PCA analysis using the Past (Version 4.03) program.

RESULTS AND DISCUSSION

Microsatellite analysis

Among the 38 SSR markers used for genetic diversity analysis, only 4 SSR markers were found to be monomorphic viz. RM195, RM294, RM321 and RM342. The remaining 34 SSR markers showed clear and repetitive polymorphic bands and such markers were used for polymorphic analysis among the fifteen aromatic rice landraces. Among the 34 selected markers showing polymorphisms, a total of 110 alleles with an average of 3.25 per locus were recorded with a minimum of 2 alleles in RM172, RM270, RM277, RM314, RM455, RM460, RM469, RM484, RM495, RM 3134, RM 6250 and RM7376 to as high as 6 alleles in RM154, RM454 and RM489. The length of the allele was found to be as long as 310 in case of RM195 and also some of these markers produced shorter alleles of 80 bp in sizes such as RM271 and RM287. The diversity of the length of alleles varied up to 50 bp in a single marker. SSR being short tandem repeats, markers generating 2 to 6 alleles per locus were considered as informative (Vieira et al., 2016). SSR banding profile of RM19, RM311 and RM202 amplicons is shown in Fig 1.

PIC value of a marker is its ability to detect polymorphism within a population, depending on the number

Table 3: Similarity matrix among the 15 aromatic rice landraces using Jaccard coefficient of similarity.

|                  | Kola | Boga | Padumoni | Kola | Kola | Dangor | Ronga | Ronga | Maniki | Madhuri | Bora | Bhaboli |
|------------------|------|------|----------|------|------|--------|-------|-------|--------|---------|------|---------|
| Kola             | 1    | 0.55102 | 0.245902 | 1    | 0.288136 | 0.381818 | 0.265667 | 0.52  | 0.508604 | 0.45283 | 0.47047 | 0.407407 | 0.47047 |
| Boga             | 1    | 1    | 0.52  | 1    | 1    | 0.52  | 0.52  | 0.52  | 0.52  | 0.52  | 0.52  | 0.52  | 0.52  |
| Padumoni         | 0.55102 | 0.52 | 1    | 0.52 | 1    | 0.52 | 0.52 | 0.52 | 0.52 | 0.52 | 0.52 | 0.52 | 0.52 |
| Kola (1)         | 0.288136 | 0.52 | 0.52 | 1    | 0.52 | 0.52 | 0.52 | 0.52 | 0.52 | 0.52 | 0.52 | 0.52 | 0.52 |
| Kola (2)         | 0.288136 | 0.52 | 0.52 | 0.52 | 1    | 0.52 | 0.52 | 0.52 | 0.52 | 0.52 | 0.52 | 0.52 | 0.52 |
| Dangor           | 0.310345 | 0.310345 | 0.310345 | 0.310345 | 1    | 0.310345 | 0.310345 | 0.310345 | 0.310345 | 0.310345 | 0.310345 | 0.310345 | 0.310345 |
| Ronga            | 0.288136 | 0.310345 | 0.310345 | 0.310345 | 0.310345 | 1    | 0.310345 | 0.310345 | 0.310345 | 0.310345 | 0.310345 | 0.310345 | 0.310345 |
| Maniki           | 0.310345 | 0.310345 | 0.310345 | 0.310345 | 0.310345 | 0.310345 | 1    | 0.310345 | 0.310345 | 0.310345 | 0.310345 | 0.310345 | 0.310345 |
| Madhuri          | 0.288136 | 0.310345 | 0.310345 | 0.310345 | 0.310345 | 0.310345 | 0.310345 | 1    | 0.310345 | 0.310345 | 0.310345 | 0.310345 | 0.310345 |
| Bora             | 0.310345 | 0.310345 | 0.310345 | 0.310345 | 0.310345 | 0.310345 | 0.310345 | 0.310345 | 1    | 0.310345 | 0.310345 | 0.310345 | 0.310345 |
| Bhaboli          | 0.288136 | 0.310345 | 0.310345 | 0.310345 | 0.310345 | 0.310345 | 0.310345 | 0.310345 | 0.310345 | 1    | 0.310345 | 0.310345 | 0.310345 |
| Bhaboli (1)      | 0.310345 | 0.310345 | 0.310345 | 0.310345 | 0.310345 | 0.310345 | 0.310345 | 0.310345 | 0.310345 | 0.310345 | 1    | 0.310345 | 0.310345 |
| Bhaboli (2)      | 0.310345 | 0.310345 | 0.310345 | 0.310345 | 0.310345 | 0.310345 | 0.310345 | 0.310345 | 0.310345 | 0.310345 | 0.310345 | 1    | 0.310345 |
| Tulsi             | 0.47047 | 0.47047 | 0.47047 | 0.47047 | 0.47047 | 0.47047 | 0.47047 | 0.47047 | 0.47047 | 0.47047 | 0.47047 | 0.47047 | 1    |
| Ronga Bhaboli    | 0.47047 | 0.47047 | 0.47047 | 0.47047 | 0.47047 | 0.47047 | 0.47047 | 0.47047 | 0.47047 | 0.47047 | 0.47047 | 0.47047 | 0.47047 | 1    |
| Bos             | 0.509612 | 0.666667 | 0.509612 | 0.509612 | 0.509612 | 0.509612 | 0.509612 | 0.509612 | 0.509612 | 0.509612 | 0.509612 | 0.509612 | 0.509612 | 1    |
Assessment of the Genetic Diversity of Joha Rice Germplasms by using Simple Sequence Repeat Markers

Assessment of the Genetic Diversity of Joha Rice Germplasms by using Simple Sequence Repeat Markers

and size of the alleles and their frequency of distribution; thus, it provides the discriminatory ability of the marker (Nagy et al., 2012). The PIC value gives information about the diversity of the allele and its frequency among landraces. PIC value among the 34 SSR markers ranged from 0.24 in RM287 and RM311 to 0.83 in RM489, with an average of 0.5 for each marker. The marker index (MI) for each marker were ranged from 4.96 in RM489 to 0.64 in RM172, RM270 and RM314, with an average of 1.84 for each marker (Table 2). Among all the markers used in the present study, RM1, RM154, RM489, RM524 and RM454 have PIC values higher than 0.75 which shows a promising result in the study of genetic diversity using these markers. Aljumali et al., 2017 in their work have used 32 SSR markers to study genetic diversity among 50 aromatic rice accession from Malaysia and has shown a promising result using RM195, RM294, RM321 and RM342, but these markers showed a monomorphic band in the present investigation.

Hierarchical cluster analysis

A dendrogram was created (Fig 2) by analysis of similarity matrix among the fifteen landraces using Jaccard coefficient of similarity (Table 3) and unweighted pair group method with arithmetical average (UPGMA) cluster analysis of the 34 SSR markers using Jaccard similarity index. The dendrogram grouped the landraces into three major groups and five subgroups (Table 4). The seven subgroups formed were I-A including Kola Joha (1) and Kola Joha (2), II-B consisting of Dangor Joha, followed by II-A which includes two minor sub-group II-A1 and II-A2. II-A1 comprises of Rani Kajol Joha and Kola Kunkuni Joha whereas II-A2 consist of Boga Kunkuni Joha and Bos Joha. Manikimadhuri Joha and Boga Kon Joha are included in the subgroup II-B as Joha Bora and Podumoni Joha consists of the subgroup III-A. Tulsi Joha solely is present in the sub-group III-B whereas sub-group III-C is further classified into III-C1 comprising of Ronga Bhaboli Joha and Boga Bhaboli Joha and subgroup III-C2 consisting of Ronga Joha only. A similar result was also found by Singh et al., 2015, where 76 rice accession were evaluated for genetic polymorphism using SSR markers and were grouped into two major groups and 14 subgroups. The dendrogram shows a close similarity between Podumoni Joha and Joha Bora of 0.72 while it shows the least similarity (about 0.2) between landraces included in group I and the rest of the landraces. Generation
Table 4: Group-wise distribution of the 15 aromatic rice landraces based on dendrogram generated by UPGMA cluster analysis of the 38 SSR markers amplicons using Jaccard similarity index.

| Landraces          | Group | Sub-group | Minor sub-group |
|--------------------|-------|-----------|-----------------|
| Kola Joha (1)      | I     | I-A       |                 |
| Kola Joha (2)      |       |           |                 |
| Dangor Joha        |       | I-B       |                 |
| Rani Kajol Joha    | II    | II-A      | II-A1           |
| Kola Kunkuni Joha  |       |           | II-A2           |
| Boga Kunkuni Joha  |       |           |                 |
| Bos Joha           |       | II-B      |                 |
| Manikimadhuri Joha |       | II-B      |                 |
| Boga Kon Joha      | III   | III-A     |                 |
| Podumoni Joha      |       |           |                 |
| Joha Bora          |       |           |                 |
| Tulsi Joha         | III-B |           |                 |
| Ronga Bhaboli Joha | III-C | III-C1    |                 |
| Boga Bhaboli Joha  |       |           |                 |
| Ronga Joha         |       | III-C2    |                 |

of minor subgroup indicates that there is an evolutionary significance among all these landraces. This suggests that there is considerable diversity among the studied landraces.

**Principal component analysis (PCA)**

PCA analysis of the amplicons of the 34 SSR markers used for genetic diversity analysis among the fifteen aromatic rice landraces produced a two-dimensional data which corresponds to the result of the dendrogram created by UPGMA clustering using Jaccard similarity matrix (Fig 3). Comparison of the groups created by UPGMA with the PCA analysis shows first quadrant containing group III-C near the centre, I-A and III-B evenly distributed around group III-C. Group I-A which includes Kola Joha (1), Kola Joha(2) and Dangor Joha is plotted with much distance showing distances which resemble the result of the UPGMA cluster and Jaccard similarity matrix of 0.5 among the three landraces. The second quadrant contains group III-A comprising of Podumoni Joha and Joha Bora towards the upper right. Though they are within the same group III in the dendrogram, Jaccard similarity matrix shows similarity less than 0.4 with the rest of landraces belonging to group III and hence placed in a separate quadrant. Group II-B, which consist of Manikimadhuri Joha and Bos Joha, are placed in the third quadrant towards left near the central axis. The fourth quadrant has the group II-A toward left just below the central axis. Though the landraces belonging to the group II are placed on the left side of the two quadrants, they are plotted near each other and thus agree with the finding of the UPGMA and Jaccard similarity index. Similar findings were earlier reported by Park et al., (2019). Therefore, when comparing the result of PCA analysis with that of the dendrogram, it showed similar tendencies and the composition of the group was similar.

**CONCLUSION**

All the collected aromatic rice landraces are endemic to the Upper Brahmaputra Basin, Assam and hence they show a close similarity among themselves and make it difficult to identify with mere physical looks. Few landraces have a conflict of names with the origin of the collected landraces as these landraces were traditionally preserved by locally cultivating and maintaining the germplasm.

The study showed that SSR markers could be used concerning the analysis of the genetic relationship among close landraces belonging to a particular geographical location. As SSR are short tandem repeats and their distribution is highly non-random and they vary a lot among landraces in rice because of their high degree of mutation, they serve as a better tool in genetic diversity study. Moreover, these SSR markers could be used as a selecting tool for genetically diverse germplasm accessions, to carry out an efficient selection method among highly segregating generations.
Assessment of the Genetic Diversity of Joha Rice Germplasms by using Simple Sequence Repeat Markers

REFERENCES

Childs, N. (2016). USDA ERS-Rice Outlook: April 2016. Ers. Usda. Gov.

Das, A., Kesari, V. and Rangan, L. (2010). Aromatic joha rice of Assam-A review. Agricultural Reviews. 31(1): 1-10.

Doyle, J.J. and Doyle, J.L. (1987). A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochemical Bulletin. 19: 11-15.

Jasim Aljumaili, S., Rafii, M.Y., Latif, M.A., Sakimin, S.Z., Arolu, I.W. and Mahi, G. (2018). Genetic diversity of aromatic rice germplasm revealed by SSR markers. Biomed Research International. 2018.

Maclean, J.L., Dawe, D.C., Hardy, B., Hettel, G.P. (2002). Rice Almanac. Los Ban̄ os, Philippines: International Rice Research Institute, 1–10.

Morgante, M., Hanafey, M. and Powell, W. (2002). Microsatellites are preferentially associated with nonrepetitive DNA in plant genomes. Nature Genetics. 30(2): 194-200.

Nagy, S., Poczai, P., Cernák, I., Gorji, A. M., Hegedqs, G. and Taller, J. (2012). PICcalc: an online program to calculate polymorphic information content for molecular genetic studies. Biochemical Genetics. 50(9-10): 670-672.

Park, J.R., Yang, W.T., Kwon, Y.S., Kim, H.N., Kim, K.M. and Kim, D.H. (2019). Assessment of the genetic diversity of rice germplasms characterized by black-purple and red pericarp color using simple sequence repeat markers. Plants. 8(11): 471.

Pérez-Jiménez, M., Besnard, G., Dorado, G. and Hernandez, P. (2013). Varietal tracing of virgin olive oils based on plastid DNA variation profiling. PLoS One. 8(8): e70507.

Phumichai, C., Phumichai, T. and Wongkaew, A. (2015). Novel chloroplast microsatellite (cpSSR) markers for genetic diversity assessment of cultivated and wild Hevea rubber. Plant Molecular Biology Reporter. 33(5): 1486-1498.

Raina S.K., Sathish, P. and Sarma, K.S. (1987). Plant regeneration from in vitro cultures of anthers and mature seeds of rice (Oryza sativa L.) cv. Basmati-370. Plant Cell Rep. 6(6): 43-45.

Ram, P.C. (2003). Rice Almanac. [Maclean, J.L., Dawe, D.C., Hardy, B. and Hettel, G.P. (eds.)], 3rd edn.

Roy, S., Banerjee, A., Basak, N., Kumar, J. and Mandal, N.P. (2020). Aromatic Rice. In: The Future of Rice Demand: Quality Beyond Productivity Springer, Cham. (pp. 251-282).

Roy, S., Banerjee, A., Mawkhlieng, B., Misra, A.K., Pattanayak, A., Harish, G.D. et al. (2015). Genetic diversity and population structure in aromatic and quality rice (Oryza sativa L.) landraces from North-Eastern India. PloS one. 10(6): e0129607.

Saikia, P. and Neeg, B. (2020). Present status of Joha rice and its diversity in the Upper Brahmaputra valley, Assam. Glimpses of Biological Research in Upper Brahmaputra Basin 25-32. ISBN:9788194692201.

Shobha Rani, N., Shobha Rao, L.V., Virakta, B.C. and Mishra, B. (2004). National Guidelines for the Conduct of Tests for Distinctiveness, Uniformity and Stability. Directorate of Rice Research, 6-13.

Singh, A., Saini, R., Singh, J., Arya, M., Ram, M. and Singh, P.K. (2015). Genetic diversity studies in rice (Oryza sativa L.) using microsatellite markers. International Journal of Agriculture, Environment and Biotechnology. 8: 143-152. doi: 10.5998/2230-732X.2015.00019.

Singh, N., Choudhury, D.R., Tiwari, G., Singh, A.K., Kumar, S., Srinivasan, K. et al. (2016). Genetic diversity trend in Indian rice landraces: an analysis using SSR markers. BMC Genetics. 17(1): 127.

Smith, J.S.C., Chin, E.C.L., Shu, H., Smith, O.S., Wall, S.J., Senior, M.L. et al. (1997). An evaluation of the utility of SSR loci as molecular markers in maize (Zea mays L.): comparisons with data from RFLPs and pedigree. Theoretical and Applied Genetics. 95(1-2): 163-173.

Temnykh, S., DeClerck, G., Lukashova, A., Lipovich, L., Cartinhour, S. and McCouch, S. (2001). Computational and experimental analysis of microsatellites in rice (Oryza sativa L.): frequency, length variation, transposon associations and genetic marker potential. Genome Research. 11(8): 1441-1452.

Vieira, M.L.C., Santini, L., Diniz, A.L. and Munhoz, C.D.F. (2016). Microsatellite markers: what they mean and why they are so useful. Genetics and Molecular Biology. 39(3): 312-328.