Investigation of population structure and molecular genetic diversity under coastal agro-ecosystem in rice (*Oryza sativa* L.)

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Abstract

Molecular markers are useful tools for evaluating genetic diversity and determining cultivar identity. Thirty nine simple sequence repeat (SSR) markers were selected in order to evaluate the genetic diversity in 88 genotypes. A total of 115 alleles were detected in 39 SSR polymorphic markers. The number of alleles per marker ranged from 1 to 5, with an average of 3 alleles per locus. An average polymorphism information content value of 0.5615 was recorded. It ranged from zero (RM431, RM379 and RM262) to 0.6476 (RM324), indicating significant genetic diversity among and within the rice accessions of the present study. The average observed heterozygosity was 0.1136, while the average expected genetic diversity was 0.6105. A dendrogram constructed using the Unweighted Pair Group Method with Arithmetic means (UPGMA), grouped the 88 rice genotypes into three well differentiated major clusters. The structured program without prior information provided support for the existence of three genetically distinct clusters (K=3).

Key words: Cluster analysis, SSR marker, *Oryza sativa*, Rice breeding, Variability.

INTRODUCTION

Rice (*Oryza sativa* L.) is an annual grass (Gramineae). It is originated in Asia. It is a staple food for peoples of South and South East Asia (Chang, 1976). Rice is cultivated from sea level to around 3000 m altitude and from the 30°S to 49°N. Temperature and day lengths are therefore quite diverse in the growing areas. The wide geographical distribution of rice has resulted in the development of a great diversity of varietal types.

Globally rice is grown in and around 162.06 million ha, occupying one tenth of arable land, with an annual production of 497.7 million tonnes of rough rice (http://www.statista.com, 2019). The average productivity of 3.07 t/ha of rice provides a per capita consumption of 100 to 240 kg/year across the globe. Over 90 per cent of the world’s rice is produced and consumed in Asia and more than half of it comes from India and China, exploiting maximum arable land. More than two billion people obtain their 80 per cent calorie from rice and its derived products from 90 per cent of the world’s rice area (FAO, 1999). Rice based production systems and their associated post harvest operations employ nearly a billion people in rural areas in developing countries (IRC, 2003).

In India, rice area accounts for 22.8 per cent of total cropped area, 35.6 per cent of the area under food crop and 43.9 per cent of the area under cereals. Rice production accounts for 31 per cent of food grain production and 45 per cent of cereal production, providing 20 to 25 per cent of agricultural income. The rice production has increased from 20 million tonnes in 1950-51 to 118.87 million tonnes in 2019, with a productivity increase from...
Investigation of population structure and molecular diversity and characterization of 88 rice genotypes differing in flowering time using SSR markers.

The present study was undertaken to assess the genetic diversity in rice (Powell et al., 2014). However, a study that was performed to comprehensively investigate genetic diversity and characterization of different duration has not been found. The present study was undertaken to assess the genetic diversity and characterization of 88 rice genotypes differing in flowering time using SSR markers.

MATERIALS AND METHODS

DNA from 88 rice genotypes (Table 1) was isolated by following the modified CTAB method (Murray and Thompson, 1980). Thirty nine molecular markers (Table 2) of the gene for the first flowering time were amplified with the isolated 88 DNA samples. The isolated DNA samples were amplified using a T100 thermal cycler (BIO-RAD, USA) with a total of 100 μl of 50 ng DNA, 1 μl of Tris-HCL 10 mM (pH 8.3), 0.5 μl of 1.5 mM MgCl2, 0.1 μl of 0.5 unit of Taq polymerase (New England Biolabs, U.K), 1 μl of 50 μM dNTP mixture and 0.2 μl each of forward and reverse primers (5 pico molar).

Conditions for carrying out DNA amplification were as follows: initial denaturation at 94°C for 5 min, followed by 35 repeated cycles of denaturation at 94°C for 45s, annealing for 45s (temperature specific to primer) and extension for the 60s at 72°C followed by a final extension for 8 min. at 72°C. Bromophenol blue was added to the samples and the molecular weight of the amplified DNA was estimated in 3.5% agarose gel with 50-bp ladder (New England Biolabs, UK) as standard in 1X Tris-Boric acid-EDTA (TBE) buffer. The resolved PCR bands were documented using the molecular Imager Gel Doc XR system (Bio-Rad).

Only clear and intense bands were recorded. The molecular size of the amplified fragments was determined by image lab software (Bio-Rad) using a 50 bp DNA ladder as standard. Amplification of DNA samples with primers was recorded as ‘1’ for the amplified and ‘0’ for the unamplified regions according to the molecular size of the marker. A data matrix with ‘O’ or ‘1’ against the molecular size was prepared for further analysis. Marker genetic diversity parameters viz., the number of alleles, allele frequency, genetic diversity index and heterozygosity were calculated by power marker software.

Polymorphism information content (PIC) for every polymorphic marker was computed using the formula

\[
PIC = 1 - \sum_{i=1}^{n} \frac{P_i^2}{2} - \sum_{i=1}^{n} \sum_{j=i+1}^{n} P_i P_j
\]

Where, ‘i’ is the sum of alleles detected ‘Pi’ is the frequency of the i\textsuperscript{th} allele and j=i+1. A neighbour-joining tree with bootstrap value (1000) was constructed using the unweighted pair group method with arithmetic average (UPGMA) algorithm with the help of DARwin version 6.0.

RESULTS AND DISCUSSION

Eighty- eight rice genotypes of different eco-geographic were genotyped using 39 SSR markers, which produced a total of 115 alleles (Table 3). Among these 115 alleles, 1% were considered as rare (showed an allele frequency of <1%). The number of alleles per loci varied from 1 to 5 with an average of 3 alleles per locus. The highest number of alleles (5) were detected...
### Table 1. List of genotypes used in the study

| S. No. | Name             | Duration | S. No. | Name             | Duration |
|--------|------------------|----------|--------|------------------|----------|
| 1      | Jayanthidhan     | Long     | 45     | CR dhan 101      | Medium   |
| 2      | CR 1014          | Long     | 46     | CR dhaan 202     | Medium   |
| 3      | Reeta            | Long     | 47     | CR dhan 310      | Medium   |
| 4      | CR dhan 601      | Long     | 48     | Khitish          | Medium   |
| 5      | Jalamani         | Long     | 49     | Pyari            | Medium   |
| 6      | Lunishru         | Long     | 50     | Naveen           | Medium   |
| 7      | CR dhan 408      | Long     | 51     | IC-206447        | Medium   |
| 8      | CR dhan 307      | Long     | 52     | IC-125757        | Medium   |
| 9      | Varshadhan       | Long     | 53     | IC-0514489       | Medium   |
| 10     | Tapaswini        | Long     | 54     | IC-135318        | Medium   |
| 11     | Hanswari         | Long     | 55     | IC-124436        | Medium   |
| 12     | Improve lalat    | Long     | 56     | IC-0627835       | Medium   |
| 13     | CR dhan 500      | Long     | 57     | IC-114312        | Medium   |
| 14     | Swarna Sub 1     | Long     | 58     | IC-0627836       | Medium   |
| 15     | IC-ARC-7220      | Long     | 59     | IC-0623213       | Medium   |
| 16     | IC-ARC-7078      | Long     | 60     | IC-114188        | Medium   |
| 17     | IC-ARC-11547     | Long     | 61     | IC-0517840       | Medium   |
| 18     | IC-299694        | Long     | 62     | IC-214312        | Medium   |
| 19     | IC-ARC-7408      | Long     | 63     | IC-135191        | Medium   |
| 20     | IC-ARC-13300     | Long     | 64     | IC-114971        | Medium   |
| 21     | IC-379792        | Long     | 65     | ADT 38           | Medium   |
| 22     | IC-215370        | Long     | 66     | ADT 39           | Medium   |
| 23     | IC-377869        | Long     | 67     | CO 50            | Medium   |
| 24     | IC-ARC-1119      | Long     | 68     | Vandana          | Early    |
| 25     | IC-379136        | Long     | 69     | IC-0098989       | Early    |
| 26     | IC-611162        | Long     | 70     | IC-0124198       | Early    |
| 27     | IC-386231        | Long     | 71     | IC-0203398       | Early    |
| 28     | IC-ARC-11203     | Long     | 72     | IC-0135769       | Early    |
| 29     | IC-300981        | Long     | 73     | IC-0123756       | Early    |
| 30     | IC-67725         | Long     | 74     | IC-0207960       | Early    |
| 31     | IC-264987        | Long     | 75     | IC-0135529       | Early    |
| 32     | IC-518987        | Long     | 76     | IC-0134873       | Early    |
| 33     | IC-ARC-7432      | Long     | 77     | IC-0209056       | Early    |
| 34     | IC-ARC-10595     | Long     | 78     | IC-0207992       | Early    |
| 35     | ADT 44           | Long     | 79     | IC-0135063       | Early    |
| 36     | ADT 50           | Long     | 80     | IC-0207955       | Early    |
| 37     | CR 1009 Sub 1    | Long     | 81     | ADT-36           | Early    |
| 38     | ADT 52           | Long     | 82     | ADT-37           | Early    |
| 39     | Annanda          | Medium   | 83     | ADT-42           | Early    |
| 40     | Satyabhama       | Medium   | 84     | ADT-43           | Early    |
| 41     | Phalguni         | Medium   | 85     | ADT-45           | Early    |
| 42     | CR dhan 203      | Medium   | 86     | ADT-48           | Early    |
| 43     | CR dhan 305      | Medium   | 87     | ASD-16           | Early    |
| 44     | Sahabhagidhan    | Medium   | 88     | PTB-15           | Early    |
Table 2. List of SSR markers used in the study

| S. No. | Marker | Chr. No. | Forward Primer | Reverse Primer | Motif Number of Repeats | Annealing Temperature |
|--------|--------|----------|----------------|----------------|-------------------------|----------------------|
| 1      | RM200  | 5        | CGCTAGGAATTTGGATGGA | CGATGAGCAGGTATCGATGAGAAG | GA | 16 | 55 |
| 2      | RM207  | 2        | ATGCTAGGATAGGACAGAAGACGTGAG | CCCCCTGCTCTTCCACCCTCCTCCGAGC | AG | 29 | 55 |
| 3      | RM214  | 7        | GACATGCTGTTCCAACCATGGAAGC | GATCCTCACTGATCGAGGACAGTGAC | AG | 32 | 55 |
| 4      | RM341  | 1        | GTTCTCGATCCTGATCAGTGGTACG | GGGATGATCAGATCTCCTCTGTTGAGC | AG | 16 | 55 |
| 5      | RM231  | 3        | CGAATATTTTTCTCAGGAGGAGAAGC | CTATTGCATAGTCTGAGTACGGCAG | CT | 16 | 55 |
| 6      | RM261  | 4        | CATCTCTCCCCCTTGTTGAGC | TGATCATCGCCCAAATCTCCGACG | C (CT) | 9(8) | 55 |
| 7      | RM282  | 3        | CTGTTGCAAGAGGCTGACGAGAAGC | CAGCTCCTGTTGAGGAAAGCAGAAG | GA | 15 | 55 |
| 8      | RM302  | 1        | TGAGGATCAAGAAGCTTGAGGAG | ATGTagGATGAGGGGTAGTTGAGAAGC | AT | 13 | 55 |
| 9      | RM319  | 1        | ATCAAGGTACCTAGACCACCGCAGG | TCCTGGTGCAGCTATGTCTGACG | GT | 10 | 55 |
| 10     | RM569  | 3        | CTGTTGCAAGAGGCTGACGAGAAGC | CAGCTCCTGTTGAGGAAAGCAGAAG | GA | 15 | 55 |
| 11     | RM115  | 7        | ATCTCGTGGTCTGAGGAGGACAG | CCACATTTCTCTTCCCTCTTCCGAAGC | AG | 15 | 55 |
| 12     | RM214  | 8        | GAGTGAGCAGCTGTCAGGAGGACAG | GAGGCAAGTCTCTGGACTGACG | AG | 16 | 55 |
| 13     | RM250  | 2        | CTACTTCTCCCTTCTGTTGAGGACAG | CTCCTGGCAGATTTCTCCTCTCGAGC | CT | 25 | 55 |
| 14     | RM207  | 1        | GCTTGCTTGTGATCTGATTGGAGGACAG | GGATGATCAGATCTCTCTTGGAGCAG | AG | 16 | 55 |
| 15     | RM231  | 3        | CGAATATTTTTCTCAGGAGGAGAAGC | CTATTGCATAGTCTGAGTACGGCAG | CT | 16 | 55 |
| 16     | RM261  | 4        | CATCTCTCCCCCTTGTTGAGC | TGATCATCGCCCAAATCTCCGACG | C (CT) | 9(8) | 55 |
| 17     | RM282  | 3        | CTGTTGCAAGAGGCTGACGAGAAGC | CAGCTCCTGTTGAGGAAAGCAGAAG | GA | 15 | 55 |
| 18     | RM212  | 2        | CATCTGCAGATCGCCACAGAGC | GGCCTCAGACTAGAATGAGAAGC | AG | 27 | 55 |
| 19     | RM258  | 10       | CTCCCCGTCCTTTTAAGGCTGTGCAAGG | GACGAACACGACAGAAGGAAAGGACAG | AG | 11 | 55 |
| 20     | RM259  | 1        | GACAGGTGTCTCTAAATCTGTTG | TTATGAGGATCTGAGGAGGACAG | AG | 22 | 55 |
| 21     | RM262  | 2        | CATCTCGTGTCGGCTCAGCAG | GAGAGCCAGGTTGCTGCTG | CT | 16 | 55 |
| 22     | RM266  | 11       | CTGGCTCTCTAGCTACAACCTTGCCACG | AAATCTCTGCCTGATGAGGACAG | AG | 21 | 55 |
| 23     | RM314  | 6        | CTAGCAGGAACTCCTTTCTCAGGAGGACAG | TTCCTCCACCTCCTTGATGCAGGACAG | AG | 24 | 55 |
| 24     | RM315  | 1        | AGGTCTATTTGAGGATGATCTGGAAGG | AGGGAAGAAGAAACTTGGAGGACAG | GT | 8 | 55 |
| 25     | RM404  | 8        | GGAGGAGCTAAGGGAGGAAAAGGAGGACAG | GCTCTGTGTCTCGAAGAGGACAG | AG | 29 | 55 |
| 26     | RM487  | 3        | TTTCTCGAAGGGCAGCAGGAGGACAG | GCTAGGAATCTCTGAGGACAG | AG | 10 | 55 |
| 27     | RM521  | 2        | ATGACATCGCAATTTCTGCTACAGCAG | CATGGGATGCTGAGATGGAGGACAG | AG | 14 | 55 |
| 28     | RM555  | 2        | TTGAGATCGGAAATTTGGAATAGGGAGGACAG | TTTGAGTACGCAAAAGGAGGACAG | AG | 11 | 55 |
| 29     | RM9    | 1        | GCTCCGACTACCTCTTGAGGACAG | GTCTCTGCTCTCCCTTCTCCCTTCC | AG | 14 | 55 |
| 30     | RM227  | 3        | ACCTTTCTGATCAAGGAGGACAG | GATTGGAGAGAAAGAAGGACAG | GT | 10 | 55 |
| 31     | RM246  | 11       | ATCGTATGCTCTCAGGAGGACAG | GTCTAATAAAAGGAGCTG | AAG, A | 8, 13 | 55 |
| 32     | RM206  | 11       | ATCGATCGTATGTTGCTTCAGGAGGACAG | GTCTAGTGAACAATCTTATGTTGAGGACAG | AC | 15 | 55 |
| 33     | RM168  | 3        | TGTCGCAAGGGGAGTGGAGGAGGACAG | GAATCTACCGAGCGACAG | AG | 33 | 55 |
| 34     | RM81   | 3        | GAGTGCTTGTGCAAGATCCAAGAGGACAG | GCCTTTCTCTTCCCTTCCCTTCCCTTCC | AG | 10 | 55 |
| 35     | RM237  | 1        | CAAATTTCTGACTGCTTGCCACGAG | GCTGAGGAGAGAAAGGAGGACAG | AG | 18 | 55 |
| 36     | RM324  | 2        | TGCTCAGGCTAGGAGGAGGACAG | GCTAGGAGGCTGAGGAGGACAG | ATC | 9 | 55 |
| 37     | RM263  | 2        | ATGCTATGCTAGGAGGAGGACAG | TGAAGGAGATGGTCAGTGGTGGAGGACAG | AG | 14 | 55 |
| 38     | RM163  | 5        | CGCCTTTATGAGGAGGAGGAGGACAG | AAATCTTCTGACAGCTGCTG | AG | 15 | 55 |
| 39     | RM248  | 7        | AGAGAGAAGTTTGGAGGAGGACAG | ACCAGAGGGTGGACTG | AG | 15 | 55 |
### Table 3. Genetic diversity parameters and PIC values of the 39 markers

| S. No. | Marker | Allele Number | Major Allele Frequency | Genetic Diversity | Heterozygosity | PIC |
|--------|--------|---------------|------------------------|-------------------|----------------|-----|
| 1      | RM200  | 3             | 0.8011                 | 0.3337            | 0.1591         | 0.3023 |
| 2      | RM207  | 3             | 0.8011                 | 0.3299            | 0.0341         | 0.2935 |
| 3      | RM214  | 4             | 0.9148                 | 0.1590            | 0.0114         | 0.1521 |
| 4      | RM431  | 1             | 1.0000                 | 0.0000            | 0.0000         | 0.0000 |
| 5      | RM231  | 3             | 0.6932                 | 0.4321            | 0.0227         | 0.3480 |
| 6      | RM261  | 2             | 0.9091                 | 0.1653            | 0.0455         | 0.1516 |
| 7      | RM282  | 2             | 0.9886                 | 0.0225            | 0.0000         | 0.0222 |
| 8      | RM302  | 3             | 0.5227                 | 0.6126            | 0.0455         | 0.5438 |
| 9      | RM319  | 1             | 1.0000                 | 0.0000            | 0.0000         | 0.0000 |
| 10     | RM569  | 4             | 0.9375                 | 0.1195            | 0.0114         | 0.1167 |
| 11     | RM11   | 3             | 0.7841                 | 0.3603            | 0.0227         | 0.3294 |
| 12     | RM223  | 3             | 0.8239                 | 0.3049            | 0.0341         | 0.2827 |
| 13     | RM250  | 3             | 0.7614                 | 0.3840            | 0.0000         | 0.3418 |
| 14     | RM21   | 4             | 0.4716                 | 0.6562            | 0.0568         | 0.5968 |
| 15     | RM118  | 3             | 0.9659                 | 0.0664            | 0.0000         | 0.0652 |
| 16     | RM153  | 3             | 0.6932                 | 0.4693            | 0.0000         | 0.4200 |
| 17     | RM218  | 3             | 0.5511                 | 0.5897            | 0.0114         | 0.5204 |
| 18     | RM221  | 1             | 1.0000                 | 0.0000            | 0.0000         | 0.0000 |
| 19     | RM258  | 3             | 0.7330                 | 0.4026            | 0.0114         | 0.3378 |
| 20     | RM259  | 3             | 0.9545                 | 0.0878            | 0.0000         | 0.0859 |
| 21     | RM262  | 1             | 1.0000                 | 0.0000            | 0.0000         | 0.0000 |
| 22     | RM286  | 4             | 0.8636                 | 0.2459            | 0.0568         | 0.2337 |
| 23     | RM314  | 3             | 0.4773                 | 0.5850            | 0.0000         | 0.4960 |
| 24     | RM315  | 2             | 0.9545                 | 0.0868            | 0.0000         | 0.0830 |
| 25     | RM404  | 3             | 0.8693                 | 0.2350            | 0.0114         | 0.2208 |
| 26     | RM487  | 3             | 0.9489                 | 0.0982            | 0.0341         | 0.0956 |
| 27     | RM521  | 3             | 0.8807                 | 0.2136            | 0.1250         | 0.1970 |
| 28     | RM555  | 3             | 0.6648                 | 0.4773            | 0.0795         | 0.4055 |
| 29     | RM9    | 3             | 0.5284                 | 0.5188            | 0.7614         | 0.4058 |
| 30     | RM227  | 2             | 0.9602                 | 0.0764            | 0.0795         | 0.0735 |
| 31     | RM224  | 4             | 0.6818                 | 0.4949            | 0.0455         | 0.4567 |
| 32     | RM206  | 5             | 0.5227                 | 0.6020            | 0.1705         | 0.5318 |
| 33     | RM168  | 3             | 0.6250                 | 0.4848            | 0.0000         | 0.3873 |
| 34     | RM81   | 3             | 0.8523                 | 0.2621            | 0.0455         | 0.2454 |
| 35     | RM237  | 2             | 0.9659                 | 0.0659            | 0.0000         | 0.0637 |
| 36     | RM324  | 5             | 0.3580                 | 0.7033            | 0.0795         | 0.6476 |
| 37     | RM263  | 3             | 0.8864                 | 0.2069            | 0.0227         | 0.1951 |
| 38     | RM163  | 4             | 0.5625                 | 0.6105            | 0.1136         | 0.5615 |
| 39     | RM248  | 5             | 0.3239                 | 0.7492            | 0.0682         | 0.7055 |
| **Mean** | 2.9744 | 0.7752 | 0.3131 | 0.0554 | 0.2799 |

Mean values are calculated across all markers.
for the loci RM 206, RM 324 and RM 248 and the lowest was detected for a group of markers viz., RM431, RM319, RM221 and RM262. A similar number of alleles (2 to 5) for SSR markers were reported in 141 basmati rice accessions of North Western Himalaya (Salgotra et al., 2005). The PIC value represents the relative informativeness of each marker and in the present study, the average PIC value was found to be 0.5615. The PIC values ranged from zero for RM431, RM319 and RM 262 to 0.6476 for RM324. Singh et al. (2016) reported a mean PIC value of 0.29, in different sets of rice varieties which were in agreement with the present study. Heterozygosity was found to be very low which may be due to the autogamous nature of rice. Expected heterozygosity or gene diversity (He) computed according to Nei (1973) varied from 0.00 to 0.7614 (RM9) with an average of 0.0554 (Table 3). The low level of heterozygosity was reported in other studies on rice (Choudhury et al., 2014) and Nachimuthu et al., 2015) and this could be attributed itself pollination behaviour. The genetic diversity ranged from 0.000 to 0.7033 (RM324) with an average of 0.6105. Gene diversity obtained in the present study was quite low (0.52).

\[
\text{DeltaK} = \frac{\text{mean}([L''(K)])}{\text{sd}(L(K))}
\]
The population structure of the 88 germplasm line was analysed by Bayesian based approach. The log likelihood revealed by structure showed the optimum value as 3 (K=3). Similarly, the maximum of adhoc measures AK was found to be KM (Fig. 1) which indicated that the entire population can be grouped into three subgroups. Based on the membership fractions, the accessions with the probability of ≥ 80% were assigned to corresponding subgroups with others categorized as admixture. Earlier studies on population structure have reported two to eight sub population using different rice collections (Roy et al., 2015 and Upadhyay et al. 2012).

Clustering analysis based on unweighted pair group method with arithmetic mean (UPGMA) method using DARwin separated the accessions into three main groups (Fig. 2), which showed similar results as structure analysis. This grouping was further supported by earlier studies of Upadhyay et al. (2012) and Das et al. (2013). Group II in the UPGMA tree consists of maximum accessions. In the UPGMA tree, the accessions within group I, II and III clustered into smaller subgroups based on their origin and types. Hence, the clustering analysis by two classification methods reveals a high level of similarity in the clustering of the genotypes. All these points to the accuracy and usefulness of the SSR markers in tracing the phylogency or pedigree of a germplasm or breeding materials. These observations, corresponds to the previous observations of other rice germplasm studies (Bonny et al., 2015 and Masuduzzaman et al., 2016). Similarly, a clustering pattern has also been reported by Pachauri et al. (2013) based on allelic and morphological data along with the location in rice varieties using SSR markers. Accessions that are found clustered together are assumed to have high genetic similarity, while those that are found far away from each other are considered to be divergent.

PCoA was used to characterize the sub-groups of the germplasm set. A two dimensional scatter plot involving all 88 genotypes showed that the first three PCA axes accounted for 8.33, 16.45 and 23.65 per cent of the
cumulative variation among populations (Table 4). In PCoA, rice varieties were labelled with three different colours which represent the three populations obtained from population structure. The POP 1 and POP 2 showed distinct grouping whereas the individuals of POP 3 were distributed over POP 1 and POP 2 (Fig. 3).

The hierarchical distribution of molecular variance by ANOVA and pair wise analysis revealed highly significant genetic differentiation among groups. It revealed that 5% of the total variation was among the population, while 78 per cent was among individuals within groups and within individuals it was 17 per cent (Table 5). Calculation of Wright’s F statistics at all SSR loci revealed that FIS was 0.819 and FIT was 0.828. Determination of FST for the polymorphic loci across all accessions showed FST as 0.047, which implies high genetic variation. The pair wise FST estimate among subgroups indicated that the two groups are significantly different from each other. Consequently, the differential between the overall groups and their geographical groups had really happened and resulted in high genetic diversity. Variation of similar pattern as observed in a previous study (Mazid et al., 2013). In one study, which involved 41 rice genotypes from three populations, 67 per cent of the total variation was attributed to variation within the genotype, while variation among the three populations represents the high level of genetic differentiation which will further strengthen the divergence of the population. High genetic differentiation is very important within the germplasm for creating a desirable heterotic group in base breeding populations (Alam et al., 2015). Thus, genetic diversity characterization is very important as it provides the basis for a planning conservation strategy, utilization, and establishment of breeding and improvement for rice plants (Li et al., 2011).

Table 4. Percentage of variation explained by the first 3 axes

| Axis | 1     | 2     | 3     |
|------|-------|-------|-------|
|      | 8.33  | 8.13  | 7.20  |
| Percent | 8.33 | 16.45 | 23.65 |

Fig. 3. Principal coordinates (PCoA)
Table 5. Summary of AMOVA

| Source          | df | SS     | MS    | Est. Var | %   |
|-----------------|----|--------|-------|----------|-----|
| Among pops      | 2  | 55.085 | 27.543| 0.296    | 5   |
| Among individual| 85 | 924.591| 10.878| 4.899    | 78  |
| Within individual| 8  | 95.000 | 1.080 | 1.080    | 17  |
| Total           | 175| 1074.676| 6.274 | 100      |     |

F- Statistics

| Value     | P (Rand >= data) |
|-----------|------------------|
| Fst       | 0.047            |
| Fis       | 0.819            |
| Fit       | 0.828            |
| Fst max   | 0.694            |
| F'st      | 0.068            |
| Nm        | 5.055            |

Genetic diversity is an important concept in any breeding program. It can be studied using SSR markers for the identification of potential parents in order to achieve heterosis in future rice breeding programs. SSR markers were exploited to provide an unbiased estimate of the diversity pattern in this rice germplasm. The current study found the existence of high levels of diversity among 88 rice accessions which are good for the introduction of new genes in the existing genotypes. The dendrogram, constructed to identify the genetic similarities among these genotypes showed that accessions from the same region were found to cluster together as well as in different clusters implying that genetic diversity is not related fully to geographical diversity. Clustering patterns on the basis of SSR markers provide ample information that is significantly crucial for the development of genetic manipulation through crop breeding.

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