Genetic variability analysis of partially salt tolerant local and inbred rice (*Oryza sativa* L.) through molecular markers

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**A B S T R A C T**

Random Amplified Polymorphic DNA (RAPD) analysis was performed to assess the genetic variability in sixteen selected germplasms of rice, *Oryza sativa* L. using eight decamer RAPD primers. The data obtained from this investigation reveals a high level of polymorphism between cultivars. The primers produced a total of 255 bands of which all 255 bands were polymorphic indicating 100% polymorphism. The size of the amplified bands ranged from 220 bp to 2290 bp. The number of polymorphic fragments ranged from 24 to 49 with an average of 32 polymorphic bands for each primer. The primer OPX04 produced the maximum number (49) of polymorphic bands while the OPB04 and OPB17 produced the minimum number (24) of polymorphic bands. The polymorphic information content (PIC) values ranged from 0.6616 to 0.8845 with an average of 0.832. The highest PIC value (0.8845) was obtained for primer OPL03. The RAPD data was analyzed to determine the pair-wise genetic similarity coefficients which ranged from 0.00 to 0.83. The BRRIdhan 23 and the BRRIdhan 41 varieties were the closest genotypes with the highest similarity index of 83%. This was followed by 77% similarity between a pair of cultivars Kalamona and Horkuch. On the other hand, 100% dissimilarity was seen between BRRIdhan 53, BRRIdhan 50, BRRIdhan 10, BRRIdhan 70, BRRIdhan 54, BRRIdhan 40, BRRIdhan 23, BRRIdhan 47, BRRIdhan 41 and Dadsail respectively and between BRRIdhan 53 and Horkuch; indicating a high level of variability between paired genotypes. Cluster analysis was performed using Unweighted Paired Group of Arithmetic Means (UPGMA). The UPGMA dendrogram resolved the selected rice cultivars into four clusters.

1. Introduction

Rice (*Oryza sativa* L.) is considered one of the most important cereal food crops of the grass family Gramineae. It is a staple food crop of more than half of the world's population (Rabbani et al., 2008). Rice provides approximately 50–80% daily calorie intake among the world's poor population, its protein is around 88% and highly digestible (Ma et al., 2007). Bangladesh is the fourth producer of rice in the world and it is cultivated in Bangladesh throughout the year as Aush, Aman and Boro. Rice production contributes greatly to the economy of Bangladesh with 28 percent GDP. It accounts for rice covers about 75 percent of the total cropped area in Bangladesh (Chowdhury, 2009). It is very alarming that the amount of cultivable land is reducing day by day in Bangladesh due to effect of climate change which also affects yield potential of rice. The adverse effect resulted in various levels of abiotic stresses of drought, salinity, flood, extreme temperature stress and low soil fertility. Deliberate effort must be taken to develop high yielding, stable and tolerant rice varieties to meet food and nutritional requirements of this densely populated country (Mondal, 2010). Rice production has been increased every year to keep pace with the increasing population to meet the increasing food demand. By the next 15 years, more than 50% increase in rice production will be needed to meet the food demand of increased population in Bangladesh (Seraj et al., 2006). It is considered that rice is susceptible to saline water especially during early vegetative and later at the reproductive stages (Shannon et al., 1998). In case of salinity tolerance, there are differences among rice genotypes for which additive gene effects are responsible (Sahi et al., 2006). Soil salinity is a serious problem around the globe that affects approximately 20% of irrigated land and reduces crop yields significantly (Haque, 2018). Studies revealed that the salinity affected area has increased from 8,330 km² in 1973 to 10,560 km² in 2009 (Soil Resource Development Institute (SRDI, 2010) in Bangladesh. There are some noteworthy landraces that exhibit some degree of tolerance to salt stress, but the tolerance level is not high. Generation of additional genomic resources in the form of salt-responsive

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QTLS and molecular markers and characterization of the genes and their upstream regulatory regions are equally essential for developing tolerant varieties (Ganie et al., 2019). Several salt-responsive QTLS and their linked markers have been discovered in rice and characterization of salt responsive miRNA-SSR markers have been done to understand the genetic diversity of salt responsive-miRNA genes in rice (Mondal and Ganie, 2014). Genetic variation is a major factor for consideration in any crop improvement program to develop hybrid vigor. The analysis of genetic diversity is becoming quite useful for estimation and development of genetic relatedness among germplasms and detection of diverse parental combinations for breeding program (Smith, 1984; Cox et al., 1986; Barrett and Kidwell, 1998; Thompson et al., 1998). This also allows for introduction of novel genes from elite genotypes to the local genotypes and proper selection of parental plants to produce superior progenies (Islam et al., 2012). The conventional approaches involving morphological and physiological differences were previously employed for screening rice genotypes. But nowadays their use has been replaced by molecular techniques owing to their several limitations such as cost, space, time required, and labor-intensive, and also requires large sample size. The utilization of molecular markers for assessing and evaluating genetic diversity or relatedness among rice germplasms is becoming a potent molecular technique allowing more accuracy and overcoming the limitation of conventional approaches (Sebbenn et al., 2005). Random amplified polymorphic DNA (RAPD) marker, developed by Williams et al. (1990) is a simple and reproducible PCR amplification technique that is commonly used in the evaluation of genetic diversity. It is well established that RAPD is a potent marker for determining genetic diversity in rice (Ko et al., 1994; Mackill, 1995; Virk et al., 1995; Fuentes et al., 1999) and a variety of RAPD markers have been utilized to identify, protect, and determine parents for further crop improvement (Wang et al., 1994). Numerous researchers have used RAPD marker technique to determine genetic diversity in a variety of cultivated rice varieties, local rice landraces and aromatic rice cultivars (Rekha et al., 2011; Hasan and Raihan, 2014; Xie et al., 2000). The aim of this study was to establish genetic variability among 16 different local landraces and inbred rice varieties employing RAPD fingerprinting technique.

2. Results

2.1. Polymorphism analysis of RAPD markers

The result of this current experiment confers a meaningful evaluation that a considerable level of genetic variability exists among the selected varieties and local landraces. All the eight RAPD markers used for the PCR amplification of the selected cultivars produced a variety of bands with a total number of 255 bands (Table 1). These markers produced varied bands in the range of 220 bp to 2290 bp. Among the 255 bands all the bands were polymorphic, producing no monomorphic bands. All the markers produced polymorphic bands with OPX04 marker producing maximum number (49) and OPB04 and OPB17 markers producing minimum number (24) of polymorphic bands. All the primers showed a 100% polymorphism. Polymorphism information content (PIC) value reflects allelic diversity and frequency among varieties. The highest PIC value (0.8845) was obtained in OPL03 primer and the lowest PIC value (0.6616) was seen in OPA01 primer with an average of 0.832.

2.2. Within and between variety similarity index

Genetic distance is calculated by measuring similarity or differences between two genotypes in relation to the frequency of a particular trait. A similarity matrix (Table 2) based on the proportion of shared RAPD fragments was used to establish the level of relatedness between the traditional and improved cultivars. A pair-wise estimate of similarity within variety is 1. The Pair-wise estimates of similarity between varieties ranged from 0.00 to 0.83. The BRRIdhan 23 and the BRRIdhan 41 varieties were the closest genotypes with the highest similarity index of 89%. This was followed by 77% similarity between a pair of cultivars Kalamona and Horkuch. On the other hand, 100% dissimilarity was seen between BRRIdhan 53, BRRIdhan 50, BRRIdhan 10, BRRIdhan 70, BRRIdhan 54, BRRIdhan 40, BRRIdhan 23, BRRIdhan 47, BRRIdhan 41 and Dadsail respectively and between BRRIdhan 53 and Horkuch.

2.3. Cluster analysis

Genetic similarities obtained from RAPD data were used to create a cluster diagram. A cluster analysis based on linkage distance using UPGMA (Unweighted Pair Group Method of Arithmetic Means) was done to evaluate the phylogenetic relationship among the different salt tolerant rice genotypes considered for the present study. The UPGMA clustering system grouped sixteen rice germplasms into four clusters (Figure 1). Cluster 1 contains a total of 5 varieties that is subdivided into two subclusters: subcluster 1 and subcluster 2. Subcluster 1 includes BRRIdhan 53, BRRIdhan 54, BRRIdhan 23 and BRRIdhan 41 and the subcluster 2 includes BRRIdhan 47 alone. Cluster 2 contains a total of 4 varieties with subcluster 1 containing BRRIdhan 50, BRRIdhan 10 and BRRIdhan 70 and subcluster 2 containing BRRIdhan 40 alone. Cluster 3 includes a total of 5 varieties with subcluster 1 containing Chotalonakuchi, Gabura, Kalamona and Horkuch and subcluster 2 containing Dadsail alone. Cluster 4 includes two varieties namely baralonakuchi and Luta. From this analysis it was observed that BRRIdhan 23, BRRIdhan 41, Chotalonakuchi and Gabura are very closely related to each other at the linkage distance of 2.7.

Table 1. RAPD primers with corresponding bands and their size range together with polymorphic bands and PIC values.

| Primer name | Size range of bands (bp) | Total number of bands | Polymorphic Bands | Percentage of polymorphism (%) | PIC Value |
|-------------|--------------------------|-----------------------|-------------------|--------------------------------|-----------|
| OPA01       | 570–1400                 | 29                    | 29                | 100                            | 0.6616    |
| OPA02       | 290–960                  | 45                    | 45                | 100                            | 0.8724    |
| OPB04       | 320–1090                 | 24                    | 24                | 100                            | 0.8739    |
| OPB17       | 350–1010                 | 24                    | 24                | 100                            | 0.8323    |
| OPL03       | 220–2290                 | 41                    | 41                | 100                            | 0.8845    |
| OPX04       | 350–1250                 | 49                    | 49                | 100                            | 0.8303    |
| OPZ04       | 270–1350                 | 41                    | 41                | 100                            | 0.8499    |
| OPA17       | 350–1220                 | 26                    | 26                | 100                            | 0.8484    |
| **Total**   |                          |                       | 255               | 255                            |           |
| **Average** |                          |                       | 31.875            | 31.875                         | 0.832     |

**Note:** PIC: Polymorphic Information Content.
3. Discussion

Scope of crop improvement depends on the conserved use of genetic variability and diversity in plant breeding programmes and use of new biotechnological tools. Molecular characterization can reveal the maximum genetic variation or genetic relatedness found in a population (Xu et al., 2000). The outcomes of this present experiment indicated the effectiveness and usefulness of utilization of RAPD markers in determining variability among different rice cultivars. In this study, eight RAPD markers were employed to amplify the DNA of 16 rice genotypes and it revealed a good polymorphic data. Each of the eight primers revealed a 100% polymorphism, without producing any monomorphic bands. The size of amplified fragments ranges between 220 bp to 2290 bp. It was observed that the number of polymorphic bands differed with the primers and cultivars. A total of 255 polymorphic bands were produced with an average of 32 bands per primer. The OPX04 primer generated the highest number (49) of polymorphic bands while the OPB04 and the OPB17 primers generated the lowest. The success of RAPD marker analysis in rice genotypes for evaluating the variability were also carried out previously in different studies by other researchers (Davierwala et al., 2000; Kanawapee et al., 2011; Choudhury et al., 2001). The level of polymorphism observed in this investigation was different from that observed in other studies using RAPD markers. Kanawapee et al. (2011) reported 68.94% polymorphism with 20 RAPD primers among 30 salinity tolerant rice cultivars and a total of 161 bands were produced. Moreover, a variety of other researchers observed different level of polymorphism, 80% polymorphism among 42 Indian elite varieties was revealed by using 40 RAPD primers (Davierwala et al., 2000).

| Genotype | A   | B   | C   | D   | E   | F   | G   | H   | I   | J   | K   | L   | M   | N   | O   | P   |
|----------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| A        | 1.00|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| B        | 0.58| 1.00|     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| C        | 0.48| 0.73| 1.00|     |     |     |     |     |     |     |     |     |     |     |     |     |
| D        | 0.5 | 0.61| 0.52| 1.00|     |     |     |     |     |     |     |     |     |     |     |     |
| E        | 0.62| 0.41| 0.28| 0.25| 1.00|     |     |     |     |     |     |     |     |     |     |     |
| F        | 0.21| 0.59| 0.56| 0.37| 0.24| 1.00|     |     |     |     |     |     |     |     |     |     |
| G        | 0.65| 0.71| 0.54| 0.5  | 0.6  | 0.36| 1.00|     |     |     |     |     |     |     |     |     |
| H        | 0.46| 0.64| 0.62| 0.38| 0.4  | 0.26| 0.65| 1.00|     |     |     |     |     |     |     |     |
| I        | 0.70| 0.67| 0.6  | 0.46| 0.55| 0.22| 0.83| 0.69| 1.00|     |     |     |     |     |     |     |
| J        | 0.14| 0.27| 0.25| 0.15| 0.24| 0.29| 0.30| 0.21| 0.22| 1.00|     |     |     |     |     |     |
| K        | 0.06| 0.14| 0.15| 0.06| 0.13| 0.17| 0.15| 0.13| 0.14| 0.63| 1.00|     |     |     |     |     |
| L        | 0.00| 0.00| 0.00| 0.00| 0.00| 0.00| 0.00| 0.00| 0.00| 0.29| 1.00|     |     |     |     |     |
| M        | 0.00| 0.15| 0.17| 0.06| 0.07| 0.19| 0.16| 0.09| 0.1  | 0.77| 0.5 | 1.00|     |     |     |     |
| N        | 0.13| 0.26| 0.24| 0.14| 0.23| 0.28| 0.29| 0.21| 0.22| 0.76| 0.56| 0.38| 1.00|     |     |     |
| O        | 0.17| 0.23| 0.21| 0.06| 0.32| 0.12| 0.26| 0.32| 0.29| 0.41| 0.49| 0.15| 0.32| 0.4 | 1.00|
| P        | 0.15| 0.16| 0.09| 0.05| 0.22| 0.2  | 0.18| 0.2  | 0.17| 0.5 | 0.64| 0.19| 0.55| 0.54| 0.69| 1.00|

A = BRRIdhan 53, B = BRRIdhan 50, C = BRRIdhan 10, D = BRRIdhan 70, E = BRRIdhan 54, F = BRRIdhan 40, G = BRRIdhan 23, H = BRRIdhan 47, I = BRRIdhan 41, J = Chotalonakuchi, K = Kalamaona, L = Dadail, M = Horkuch, N = Gabura, O = Baralonakuchi, P = Luta.

Figure 1. Dendrogram showing clustering pattern of sixteen rice germplasms based on 8 RAPD primers using UPGMA method.
2000), 96.2% polymorphism was reported among 27 selected Indian rice genotypes using 30 decamer RAPD primers (Roopa and Chikkaswamy, 2016), RAPD analysis in 10 traditional, 28 improved and 2 Japanese cultivars of rice using 25 random primers revealed 89.4% polymorphism (Rabbani et al., 2008) and 80% polymorphism was observed among 9 upland and 4 lowland indica and japonica rice cultivars using 42 RAPD primers (Yu and Nguyen, 1994). Choudhury et al. (2001) found slightly lower level (67.5%) of polymorphism among the tested rice varieties. Moreover, RAPD analysis between hybrid rice parental lines in Iran revealed only 35% polymorphism using 15 RAPD primers (Kiani and Katalani, 2018).

The polymorphism information content (PIC) values ranged from 0.6616 (OPA01) to 0.8845 (OPL03) with an average of 0.832 (Table 1). The PIC values that was detected in present study were much higher than previous study by Kanawapee et al. (2011) (ranged from 0.08 to 0.73, average = 0.38). These PIC values obtained in this investigation reveal that all these primers employed were highly informative and efficient in distinguishing genotypes.

Pair-wise estimates of similarity ranged from 0.00 to 0.83. The highest genetic similarity (83%) was reported between BRRIdhan 23 and BRRIdhan 41. The lowest genetic similarity (0%) was observed between Dadsail and BRRIdhan 53, BRRIdhan 10, BRRIdhan 70, BRRIdhan 54, BRRIdhan 40, BRRIdhan 23, BRRIdhan47, BRRIdhan 41 respectively and between BRRIdhan 53 and Horkuch, meaning that they are distantly related to each other (Table 2). The level of genetic variability between 16 rice germplasms found in this study is quite high as revealed by the genetic similarity coefficients between lines. Kanawapee et al. (2011) found high genetic diversity among the 30 rice genotypes tested in which the pair-wise estimates of similarity ranged from 0.64 to 0.94, Roopa and Chikkaswamy (2016) and Rabbani et al. (2008) also found high genetic diversity among tested cultivars in which genetic similarity coefficients ranged from 0.46 to 0.91 and 0.50 to 0.96 respectively.

According to Aliyu et al. (2000), cluster analysis has efficacy and ability to identify crop accessions with highest level of similarity using the dendrogram. The linkage distance-based result seen in the UPGMA cluster reports variations among rice varieties. The genetic relationships among 16 rice varieties, shown in the dendrogram were constructed based on the alleles detected by eight RAPD primers. In this study, the UPGMA clustering system grouped 16 rice varieties into four clusters. From this analysis it is observed that BRRIdhan 23, BRRIdhan 41, Chotalonakuchi and Gabura are very closely related to each other.

Table 3. Selected germplasms used for evaluating genetic diversity.

| Serial number | Germplasm          |
|---------------|--------------------|
| 1             | BRRIdhan 53        |
| 2             | BRRIdhan 50        |
| 3             | BRRIdhan 10        |
| 4             | BRRIdhan 70        |
| 5             | BRRIdhan 54        |
| 6             | BRRIdhan 40        |
| 7             | BRRIdhan 23        |
| 8             | BRRIdhan 47        |
| 9             | BRRIdhan 41        |
| 10            | Chotalonakuchi     |
| 11            | Kalamona           |
| 12            | Dadsail            |
| 13            | Horkuch            |
| 14            | Gabura             |
| 15            | Baralonakuchi      |
| 16            | Luta               |

Table 4. Selected RAPD primers with their respective sequence, Tm value and GC content.

| RAPD primer | Sequence     | Tm value (°C) | GC content (%) |
|-------------|--------------|---------------|----------------|
| OPA01       | 5’-CAGGCCCTTC-3’ | 37            | 70.0           |
| OPA02       | 5’-TGCGAGCTG-3’  | 40.7          | 70.0           |
| OPA04       | 5’-GGACTGGAGT-3’ | 32.2          | 60.0           |
| OPA17       | 5’-AGGGACAGG-3’  | 33.1          | 60.0           |
| OPL03       | 5’-CCAGCAGCT-3’  | 35            | 60.0           |
| OPL04       | 5’-CGGCTACAGG-3’ | 39.1          | 70.0           |
| OPL04       | 5’-AGGCTGTGCT-3’ | 37.4          | 60.0           |
| OPL04       | 5’-GACGGTGTGT-3’ | 35.7          | 60.0           |

Tm value: Primer melting temperature; °C: Degree celsius; G: Guanine; C: Cytosine; %: Percentage.
respectively the linkage distance of 2.7 indicating with less similarity between them.

Overall this study reveals that the rice germplasms of Bangladesh has great genetic diversity. Apart from RAPD markers SSR markers have been developed and are being used in evaluating genetic diversity of rice around the world. A variety of studies with SSR markers have been conducted in Bangladesh. The results obtained from these studies are also comparable to this study. Shahriar et al. (2014) reported an average PIC value of 0.71 among 34 Bangladeshi Aman rice using 3 SSR markers. In another study, Siddique et al. (2016) found a PIC value of 0.90 among 96 Bangladesh Aman rice employing 8 SSR markers. Islam et al. (2017) studied genetic diversity in 120 Aus rice genotypes of Bangladesh and observed a PIC value of 0.8217. Other studies conducted using SSR markers revealed a polymorphism information content of 0.697 (Rashid et al., 2018), 0.6533 (Shakil et al., 2015), 0.36 (Syed et al., 2019) and 0.51 (Verma et al., 2019). All these results enumerated from a variety of researches employing RAPD and SSR markers revealed that Bangladesh has a high genetic diversity among rice germplasms.

4. Conclusion

High level of genetic diversity reported among selected genotypes reveals the ability of RAPD markers as a potential and efficient tool for diversity analysis studies. Maintenance of developmental stability and biological potential of an organism can be accomplished with the help of genetic variation. The present experiment revealed a great variability among the 16 tested salt tolerant rice germplasms that indicate very rich genetic resources of rice in Bangladesh. Information or data revealed from this investigation would be useful for researchers to take precise decisions for improvement of rice cultivars through selective breeding, crossbreeding and mutation breeding programs, which may lead to new rice varieties that may have high salinity tolerant capabilities, high yielding abilities and better growth. Major QTLs conferring salinity tolerance help to accelerate rice breeding programs, particularly focusing on marker-assisted selection for salinity tolerance and pyramiding of the tolerance and high yielding of rice. But further study is required to map and identify the QTLs for salinity tolerance in the rice genotypes used in this study.

5. Materials and methods

This experiment was conducted at Plant Genetic Engineering Laboratory, Department of Genetic Engineering and Biotechnology, Shahjalal University of Science and Technology, Sylhet-3114.

A total of 16 genotypes have been used as plant materials including both local landraces and inbred rice varieties (Table 3). The rice genotypes were selected based on their tolerance to salinity. The seeds of these rice genotypes were collected from Bangladesh Rice Research Institute (BRRI) and confirmed as partially salt tolerant by Senior Scientific Officer of BRRI, Gazipur, Bangladesh. The seeds were germinated separately on petri dishes by labeling the names of individual variety. About 25 seeds of each variety were placed on petri dish containing Whatman filter paper soaked by normal water and were kept at dark under room temperature (28 °C) for germination. After 2–3 days, the seeds of all varieties were germinated.

5.1. Plant materials

5.2. Genomic DNA extraction

Genomic DNA was isolated from young healthy leaves (0.2 g) collected from 21 days old rice plants. DNA extraction was conducted by employing a mini preparation of modified CTAB method (Zheng et al., 1995). This method does not require liquid nitrogen and only a small amount (less than 1 g) of rice leaf sample is enough to generate sufficient amount of DNA (Shamim, 2016; Tan et al., 2013; Dubey et al., 2017). After extraction the genomic DNA was stored at -20 °C. Extracted genomic DNA samples were confirmed by agarose gel (0.7%) electrophoresis and visualized under UV light.

5.3. RAPD primer selection

Eight decamer RAPD primers were selected to be utilized in assessing the genetic diversity among these germplasms (Table 4). These primers were obtained from Integrated DNA Technologies, Inc. (Coralville, IA, U.S.). These primers were selected based on their highly polymorphic nature that has been observed in previous study of various groups of researchers although the polymorphism content of RAPD primers vary with the cultivars (Davierwala et al., 2000; Kanawapee et al., 2011; Choudhury et al., 2001; Roopa and Chikkaswamy, 2016; Kiani and Katalani, 2018; Ravi et al., 2003).

5.4. PCR amplification

PCR amplification of RAPD primers were accomplished using the method described by Williams et al. (1990) with some minor modifications of thermal cycles. PCR amplifications for each individual primer were performed in a reaction volume of 25 μl containing 12.5 μl Master Mix (Promega Go Taq® G2 Green Master Mix), 1.0 μl RAPD primer, 2.0 μl...
templates, 2 μl Dimethyl Sulfoxide (DMSO), and 7.5 μl Nuclease free water. PCR amplifications were executed in a standard AerisTM Thermal Cycler. The thermal cycler was programmed with an initial denaturation at 94 °C for 5 min followed by 40 cycles which contains denaturation at 94 °C for 1 min followed by annealing for 1 min. Annealing temperature was adjusted based on the Tm value of each primer and then extension at 72 °C for 2 min, with a final extension step at 72 °C for 10 min after the 40 cycles and then hold the reaction mixture at 4 °C for stabilization of the amplified products. The RAPD-PCR amplified products were analyzed electrophoretically on 1% agarose gel using EDVOTEK EVT 300 electrophoresis apparatus and visualized by staining with ethidium bromide and transilluminated under UV light and photographed by a digital camera (Nikon D5300).

5.5. Scoring of alleles

The stained gels were then analyzed by casting the banding patterns in the captured photographs. Figure 2 and Figure 3 shows gel images of PCR amplified products. The molecular weight of each amplicons was estimated in base pair by employing the Alpha Ease Fc 4.0 software. This software compares each of amplicons with known size of fragments of molecular weight marker (GenulerTM 1kb ladder). Then all the amplicons or generated bands were given identification numbers based on their position in the gel. Each DNA fragment amplified by a given primer was treated as a unit character and the RAPD fragments were scored using a binary system, as present (1) or absent (0) of the primer-cultivar combinations.

6. Data analysis

Values generated in matrix was implemented in the calculation of polymorphism information content (PIC). Polymorphism information content (PIC) value was calculated by using the following formula:

\[
PIC = 1 - \sum Pij^2
\]

Where, \(\sum\) is the summation symbol which denotes the sum of a particular primer’s frequency for multiple alleles. \(Pij\) is the frequency of the \(j^{th}\) allele for primer \(i\) and the summation extends over \(n^{th}\) alleles (Anderson et al., 1993). Pair-wise comparison of the cultivars based on the presence or absence of unique and shared amplification products were used to generate a similarity matrix. Estimation of genetic similarity (F) was calculated between all pairs of the cultivars according to Nei and Li (1979) based on following formula:

\[
Similarity (F) = \frac{2N_{ab}}{N_a + N_b}
\]

Where, \(N_a\) = the total number of fragments shown by individual ‘a’, \(N_b\) = the total number of fragments shown by individual ‘b’ and \(N_{ab}\) = the number of fragments shared by individuals ‘a’ and ‘b’. The resulting similarity coefficients were used to evaluate the relationships among the local landraces and improved varieties with a cluster analysis using an unweighted pair-group method with arithmetic averages (UPGMA). The analysis was plotted in the form of a dendrogram using Statistica 7 software.

Declarations

Author contribution statement

Sonia Rani Mazumder: Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Hammadul Hoque: Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data.

Beethi Sinha, Woasifur Rahman Chowdhury: Performed the experiments.

Md Nazmul Hasan: Analyzed and interpreted the data.

Shamsul H Prodhan: Conceived and designed the experiments; Contributed reagents, materials, analysis tools or data.

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Competing interest statement

The authors declare no conflict of interest.

Additional information

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