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CHARACTERIZATION OF BANGLADESHI *AUS* RICE LANDRACES UNDER DROUGHT STRESS

MD.I. KHALIL, MD.R. HOSSAIN, A.K. CHOWDHURY and MD.M. HASSAN*

Department of Genetics and Plant Breeding, Patuakhali Science and Technology University, Dumki, Patuakhali-8602, Bangladesh
*Corresponding author email: mhassan@pstu.ac.bd
Email addresses of co-authors: ibrahimkhalil.ag15@gmail.com, kashem@pstu.ac.bd, reshadrk@gmail.com

SUMMARY

Genetic diversity is a prerequisite for crop improvement. This study, which was carried out at Patuakhali Science and Technology University, Bangladesh, explored the genetic diversity of 38 Bangladeshi *aus* rice (*Oryza sativa* L.) landraces under drought stress by using phenotypic and simple sequence repeat (SSR) markers. Nonhierarchal clustering analysis with Mahalanobis' D^2 statistic based on the data of morphological traits divided the studied landraces into four groups. High variability was found among the groups. Group 3 had the highest number of tillers per plant, spikelets per panicle, and panicle length. Group 2 had high 100-seed weight, and group 4 showed the highest yield per plant. Spikelets per panicle showed the maximum variation among all of the traits in the four groups. Principal component analysis showed that PC1 contributed 32.24% of the total variation, whereas PC2 accounted for 26.20%. Compared with the other traits, plant height, spikelet per panicle, and yield per plant exhibited a greater influence on the phenotypic variation observed in PC1. Compared with other traits, 100-grain weight, days to harvesting, and days to 50% flowering contributed highly to the variation found in PC2. In SSR analysis, the highest polymorphism information content (PIC) of 0.87 was observed for markers RM207 and RM256 and the lowest PIC of 0.64 was observed for markers RM212 and RM274. 'Madab jata' showed the highest similarity value (0.7) with 'BRRI dhan 42.' 'Lonka gora binni' exhibited a similarity value of 0.588, and 'Koba binni,' 'Parija', 'Gota irri,' 'Chitri', and 'Putiraj' presented a similarity value of 0.556 with 'BRRI dhan 42'. Among these genotypes, 'Madab jata,' 'Lonka gora binni,' and 'Koba binni' formed a cluster with 'BRRI dhan 42' with the coefficient of 0.53. Therefore, 'Madab jata,' 'Lonka gora binni,' and 'Koba binni' might contain drought-tolerant alleles and can be used for future research programs. The high genetic variability obtained in this work indicates that the studied rice genotypes contain drought-tolerant alleles and could be used for breeding drought-tolerant rice cultivars.

Keywords: *Aus* rice, genetic diversity, genetic variability, landraces, drought tolerant genotypes, principal component analysis, SSR markers

Key findings: Morphological and simple sequence marker data revealed that the *aus* rice landraces used in this study showed high variability. Through phenotypic and molecular marker data analyses, 'Madab jata', 'Lonka gora binni', and 'Koba binni' were identified as probable landraces containing drought-tolerant alleles.

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INTRODUCTION

Rice (*Oryza sativa* L.) is the primary food source of more than 50% of the world's population (Khush, 2005). It accounts for almost one-fifth of the total land area cultivated with cereals (Chakravarthi and Naraveneni, 2006). Approximately more than 90% of the world's rice is grown and consumed in Asia, where 60% of the world's people live (Khush, 2005). It is considered as a major crop in Bangladesh because it constitutes 91.79% of the total food grain production. It covers 77% of the total cropped area in Bangladesh (Bhuiyan *et al.,* 2002). In rain-fed and upland ecosystems, drought is recognized as the major abiotic stress that reduces productivity and adversely affects the grain quality of rice (Yang, 2008; Bimpong *et al*., 2011). In Asia, approximately at least 23 million ha of the rainfed rice area, which is 20% of the total rice area, is drought-prone (Pandey *et al.,* 2007).

Global rice yield loss due to drought is approximately 18 million tons, which is equivalent to 4% of the total world rice production, and has the estimated value of approximately US$ 3.6 billion (Kamoshita *et al.,* 2008; Singh *et al*., 2013). Every year, nearly 0.34 million ha of land of north-western Bangladesh becomes affected by severe drought (Habiba *et al.,* 2015). In Bangladesh, *aus* rice could suffer from drought at any stage from the seedling stage to the reproductive stage because it is direct-seeded and grown under rainfed upland conditions (Biswas, 2014). The identification of drought-tolerant genotypes is the first step to developing drought-tolerant rice cultivars. Landraces are an excellent source of genetic variation for adaptability, drought resistance, pests, and fungal diseases (Ganesh *et al*., 2004; Siddiq *et al.,* 2005; Prabakaran *et al.,* 2010; Sitaresmi *et al*., 2019). Bangladesh has approximately 10 000 rice landraces (Kaul *et al.,* 1982).

Simple sequence repeat (SSR) markers are powerful tools for the identification of the quantitative trait loci (QTLs) associated with drought-resistance traits and indirect selection by using the marker-assisted selection method (Kanagaraj *et al*., 2010; Akbar *et al*., 2018). Several QTLs with effects on grain yield under drought conditions have been identified in the past. Some of these QTLs include $qDTY_{1.1}$, $_qDTY_{2.1}$, $qDTY_{2.3}$, $qDTY_{3.1}$, $qDTY_{3.2}$, $qDTY_{6.1}$, $qDTY_{6.2}$, and $qDTY_{12.1}$ (Bernier *et al*., 2007; Venuprasad *et al*., 2009; Vikram *et al*., 2011; Ghimire *et al*., 2012; Mishra *et al*., 2013; Yadav *et al*., 2013; Dixit *et al*., 2014). $qDTY_{2.3}$, $qDTY_{3.2,}$ and $qDTY_{12.1}$ are considered as major QTLs for improving grain yield under drought for lowland and upland ecosystems (Mishra *et al*., 2013). Moreover, $qDTY_{6.1}$ and $qDTY_{6.2}$ have a large effect on grain yield under lowland and upland drought conditions (Dixit *et al*., 2014). $qDTY_{1.1}$, $_qDTY_{2.1}$, and $qDTY_{3.1}$ have an effect on rice yield under lowland drought stress (Venuprasad *et al*., 2009; Vikram *et al*., 2011).

Microsatellites, also known as simple sequence repeats (SSRs), have become a good marker system because of their abundance in genomes, hypervariability, codominance, and high reproducibility (Gao *et al.*, 2006). The availability of a large number of SSR markers makes them useful for many forms of high-throughput mapping, genetic analysis, and marker-assisted rice improvement. In Bangladesh, the protection of plant cultivars and the Farmer's Rights Act require the registration and protection of new and notified/extant plant cultivars on the basis of the criteria of distinctness, uniformity, and stability (DUS) of morphological characteristics. However, these morphological traits have not been able to resolve closely related genotypes. Molecular markers can very well support DUS testing in such cases. In this study, the genetic diversity of 38 rice genotypes was analyzed by assessing the variation of eight morphological traits and nine SSR markers linked to the major QTLs of grain yield under drought stress conditions.

MATERIALS AND METHODS

Plant material

The rice landraces used in this study were collected from different sources. Their details are given in Table 1. For the evaluation of morphological traits, the collected rice genotypes were raised during the *aus* growing season in the net-house of the Department of Genetics and Plant Breeding, Patuakhali Science and Technology University, Bangladesh.

Morphological characterization

All the experiments were conducted at Patuakhali Science and Technology University, Bangladesh. A total of 114 pots were prepared for 38 *aus* rice genotypes. The pots were filled with soils mixed with compost and the required amount of TSP and MoP fertilizer. The pots were then watered sufficiently, and pre-germinated seeds were transferred into the pots. A total of 5–6 seeds of each genotype

Table 1. List of rice germplasm used in this study.

No.	Genotypes	Types of genotypes	Source of Collection	Cluster Group
1	Aus patnai	Landrace	BRRI, Gazipur	2
2	Veri	-do-	-do-	4
3	Chaplo	-do-	-do-	2
4	IR 9	-do-	-do-	4
5	Dhalai kachari	-do-	-do-	1
6	Boalia	-do-	-do-	1
7	Goyal	-do-	-do-	4
8	Gutiari	-do-	-do-	1
9	Agali	-do-	-do-	1
10	Moishalema	-do-	-do-	1
11	Bogi	-do-	-do-	1
12	Begun bichi	-do-	-do-	1
13	Kolar thor	-do-	-do-	2
14	Aus Bogi	-do-	-do-	3
15	Chitri	-do-	-do-	3
16	Dharia Bogi	-do-	-do-	3
17	Ful chapala	-do-	-do-	3
18	aus gara binni	-do-	-do-	3
19	Alai	-do-	-do-	3
20	Khewali	-do-	-do-	3
21	Chapalo	-do-	-do-	3
22	Chaita mugur	-do-	-do-	4
23	Kajli	-do-	-do-	3
24	Goria	-do-	-do-	2
25	Pankhiraj	-do-	-do-	1
26	Kumri aus	-do-	-do-	2
27	Kali saita	-do-	-do-	2
28	Madab jata	-do-	-do-	1
29	Monsur irri	-do-	Dumki, Patuakhali	2
30	Gota irri	-do-	-do-	2
31	Parija	-do-	GPB lab, PSTU	1
32	Aus baku	-do-	-do-	2
33	Shoni aus	-do-	-do-	2
34	Putiraj	-do-	-do-	4
35	Poreengi	-do-	-do-	2
36	Koba binni	-do-	Rangamati	4
37	Lonka gora binni	-do-	-do-	2
38	BRRI dhan42	HYV	BRRI, Gazipur	4

were transferred into three pots that were leveled previously. Thinning was carried out after 25 days of sowing. Production practices, including water management, fertilizer application, intercultural operations, pest control, disease control, and grain harvesting, were conducted by following standard methods. Eight morphological traits, i.e., tillers per plant, plant height, days to 50% flowering, days to harvesting, panicle length, 100-grain weight, and yield per plant, were recorded. The data were analyzed by using Graphpad prism and the JMP program.

SSR analysis

Nine well-distributed SSRs were used on the basis of band intensity, consistency within an individual, presence of smearing, and potential for population discrimination. DNA was extracted from the leaves of 25-day-old seedlings by using a Promega A1120 DNA purification kit (Promega, USA) in accordance with the manufacturer's instructions. The amount of extracted DNA was estimated by using a Nano-100 microspectrophotometer (BOYN, China). The DNA samples were diluted to the desired concentration of 25–35 ng μl^{-1}.

Polymerase chain reaction (PCR) was performed in accordance with the standard procedure. A 15 µl PCR reaction volume was prepared by mixing 1 µl of forward primer, 1 µl of reverse primer, 1.0 µl of extracted rice DNA (25 ng μl^{-1}), 7.5 µl of Go Taq ® Green Master Mix (Promega, USA), and 4.5 µl of nuclease-free water. The PCR conditions were as follows: an initialization cycle at 94 °C for 5 min; 35 cycles of 94 °C for 45 s, 60 °C for 45 s, and 72 °C for 1 min; and a final extension cycle at 72 °C for 5 min. After PCR, electrophoresis was carried out on 2% agarose gel at 80 V for 40 min. Before the gels were poured into gel trays, they were stained with ethidium bromide to visualize the fine bands of each genotype. A 100 bp DNA ladder was also used to compare the molecular weight of each amplified PCR product. After electrophoresis, banding patterns were visualized with an ultraviolet gel documentation system.

Data analysis

Only unambiguous SSR markers were scored in accordance with the presence or absence of the corresponding band in the genotypes. All the genotypes were scored as "1" for the presence of SSR bands and scored as "0" for the absence of the SSR bands for different alleles. Polymorphism information content (PIC) was calculated for each SSR marker on the basis of the following formula:

$$PIC_i = 1 - \sum_{j=1}^{n} p2_{ij}$$

Whereas;
n = is the number of alleles for marker
i, P_{ij} is the frequency of the j^{th} allele for marker i. Gene diversity was also calculated for each marker.

The estimates of genetic similarity (F) were calculated between all pairs of the genotypes by using the Dice algorithm. An unweighted pair group method with arithmetic averages dendrogram based on these similarity coefficients was also constructed by using NTSYS-pc version 2.20.

RESULTS

Morphological diversity

In this study, 38 rice genotypes were evaluated for eight morphological traits (Table 1). Significant variation was found to exist among the genotypes evaluated for all the phenotypes assessed. Nonhierarchal clustering analysis with Mahalanobis' D^2 statistic based on the morphological traits divided the 38 genotypes into four groups (Figure 1, Table 1). Group 2 contained the highest number of genotypes, whereas group 4 contained the lowest number. Genotypes in group 4 appeared to be more distinct than those in the other groups.

The frequency distribution of the eight morphological traits in the four groups identified via cluster analysis is shown in Figure 2. Group 3 showed the highest tillers per plant, spikelets per panicle, and panicle length (Figure 2). Group 2 had high 100-seed weight, and group 4 showed the highest yield per plant. Among the studied traits, spikelets per panicle showed the maximum variation and was highest in group 4 followed by that in group 3. These results showed that significant variation existed among the four groups of rice landraces evaluated in this study. PCA also revealed large genetic variation for the eight morphological traits in the studied rice genotypes. PCA showed that the first five components with eigenvector value >1.0 accounted for 89.73% of the total variation (Figure 3A) of the eight characters. PC1 alone contributed 32.24% of the total variation, and PC2 accounted for 26.20% (Figure 3B). Plant height, spikelets per panicle, and yield per plant exhibited greater influences on the phenotypic variation observed in PC1, whereas 100-grain weight, days to harvesting, and days to 50% flowering contributed more to the variation found in PC2 (Figure 3C) than other traits.

The morphological data obtained for the various genotypes used in this study are given in Table 2. Among the assessed genotypes, 'Kajli' showed the highest number of tillers per plant (7.55), maximum plant height (140.54 cm), minimum days to harvest (106.67), and longest panicle length (28.49

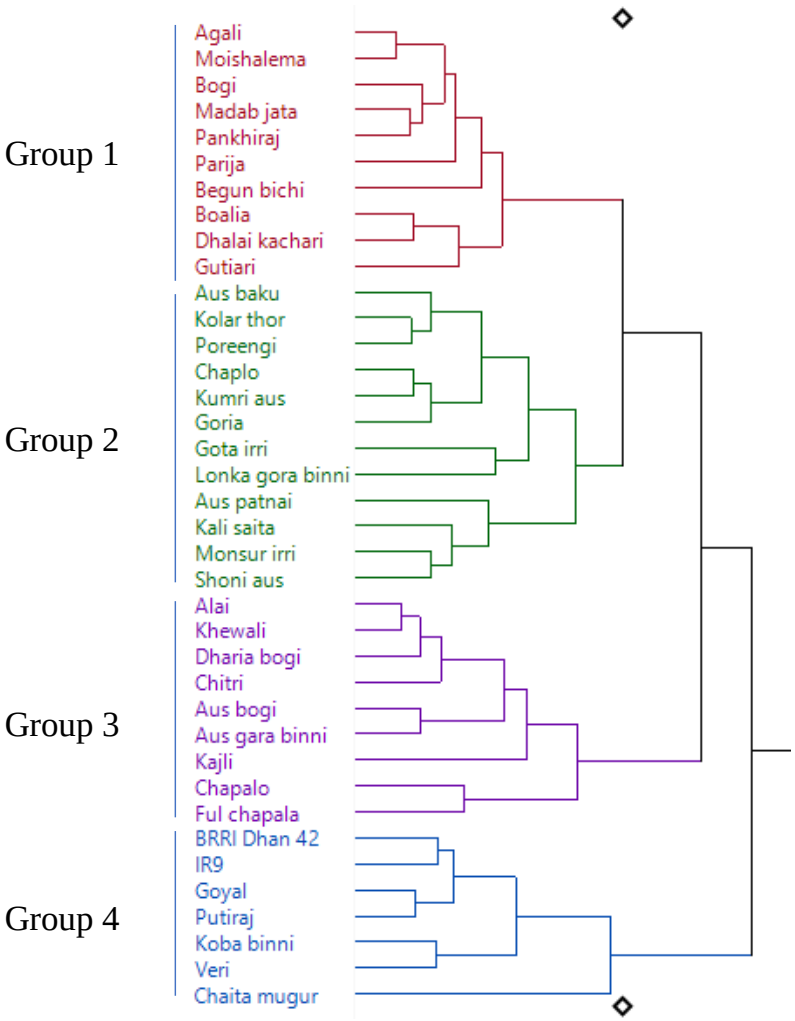


Figure 1. Dendogram depicting the genetic relationship among 38 rice genotypes used in this study.

cm) but had a low yield per plant (15.47 g) (Table 2). 'Goria' showed the second-highest number of tillers per plant (6.44) with the lowest number of spikelets per panicle (68.73) and yield per plant (10.51 g) (Table 2). 'Monsur irri' had the shortest plant height (85.1 cm) followed by 'Gota irri' (90.39 cm). 'IR9' showed the highest number of spikelets per panicle (178.80) along with the second-highest yield (18.74 g). However, the said genotype had an average panicle length (27.12 cm). 'Lonka gora binni' collected from Rangmati District showed the highest 100-grain weight (3.104 g) but a low number of spikelets per panicle (73.93) and yield per plant (11.01 g).

'Koba binni,' a landrace collected from Rangmati District, required 73 days to reach 50% flowering. It also required the maximum days for harvesting (121.67), and its yield per plant was only 16.16 g. However, its panicle length (26.78 cm), number of spikelets per panicle (146.2), and 100-grain weight (2.655g) were satisfactory (Table 2). 'Chaita mugur' had the second-highest number of tillers (6.67) per plant, plant height (137.24 cm), spikelets (173.33) per panicle, 100-grain weight (3.038 g), and yield per plant (28.02 g) (Table 2). 'Kali saita' showed the minimum panicle length (16.76 cm) with the minimum number of spikelets (80.67) per panicle and low yield per plant (7.47 g). The landraces 'Gutiari' and 'Goyal' had the lowest number of tillers (3.55) per plant. 'Chapalo' showed the minimum days (56.67) for 50% flowering but had unsatisfactory performance for other parameters.

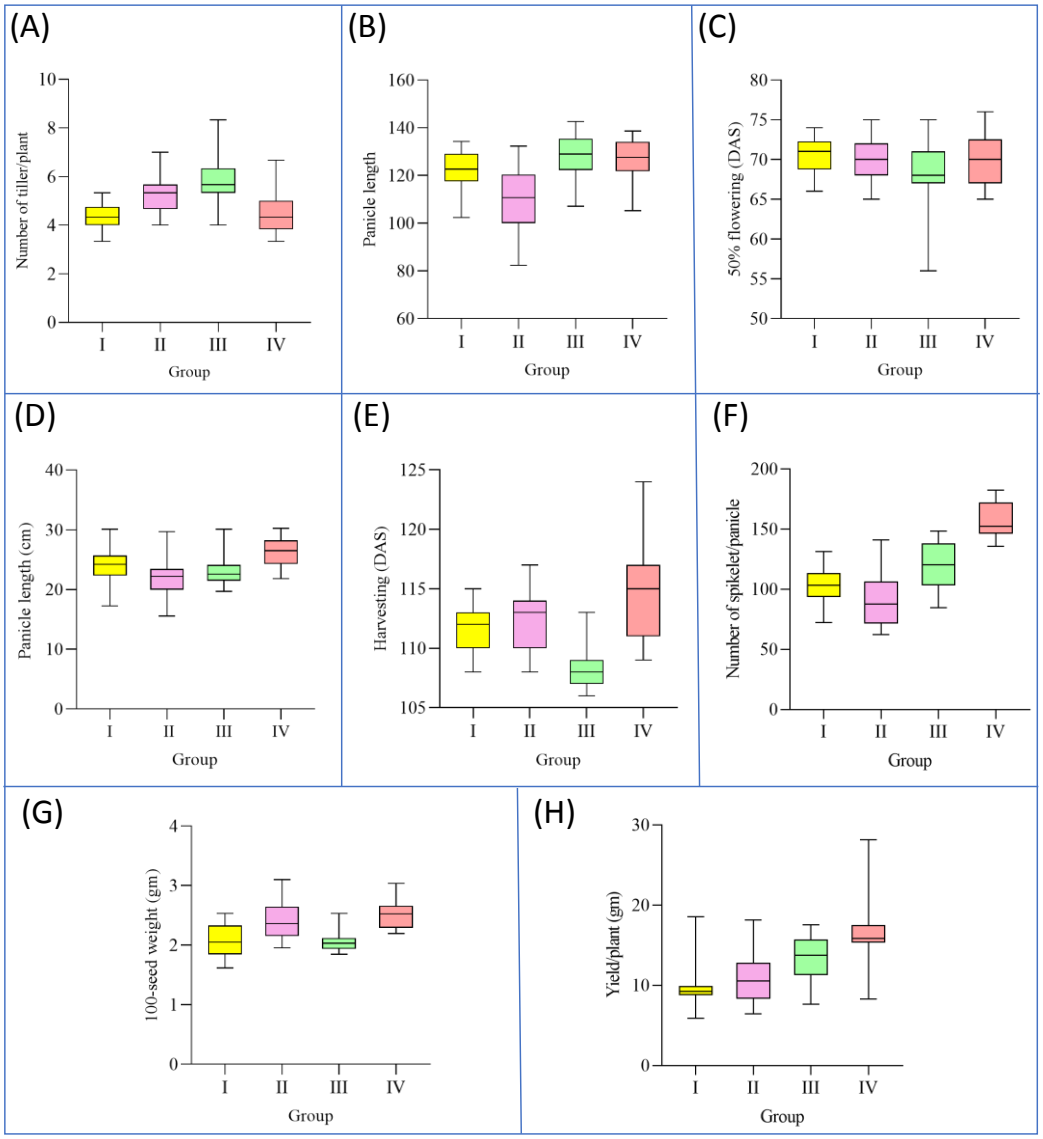


Figure 2. Distribution of eight quantitative traits in four groups of population dervied from cluster analysis.

Table 2. Mean performance of the rice genotypes for morphological traits.

Rice genotypes	Tiller per plant	Plant height (cm)	Days to 50% flowering	Days to harvesting	Panicle length (cm)	Spikelet per panicle	100-grain weight (g)	Yield per plant (g)
Agali	4.44 g–l	122.41 a–f	73.67 b–c	112.33 e–i	25.33 a–d	93.60 l–m	2.094 n	9.72 l–p
Alai	5.44 c–g	125.08 a–e	69.67 e–j	107.67 l–m	21.47 c–e	108.33 h–j	1.938 p–q	11.31 h–i
Aus baku	5.00 e–i	110.90 a–g	69.33 f–k	112.00 e–j	24.08 a–d	87.87 m–n	2.746 c	11.56 g–h
Aus Bogi	4.67 f–l	121.70 a–f	72.67 b–d	108.67 k–m	25.58 a–d	139.07 c–e	2.533 e	15.69 d
aus gara binni	5.22 d–h	132.61 a–c	72.67 b–d	108.67 k–m	23.22 a–e	142.33 c–d	2.208 k	17.26 c
Aus patnai	4.00 i–l	97.27 d–g	78.33 a	119.67 a	21.68 b–e	87.73 m–n	2.252 i–j	7.78 s
Begun bichi	5.11 d–i	104.53 b–g	72.67 b–d	108.33 k–m	25.30 a–d	118.80 g–h	1.615 t	9.92 k–n
Boalia	3.89 j–l	132.91 a–c	68.67 g–k	113.67 c–g	24.47 a–d	100.40 j–l	2.534 e	9.78 l–o
Bogi	4.00 i–l	112.96 a–g	69.67 e–j	110.67 h–k	22.18 a–e	106.80 i–k	2.019 o	8.93 o–q
BRRI dhan 42	5.11 d–i	122.81 a–f	70.33 d–i	115.33 b–d	28.35 a	136.80 d–f	2.203 k	15.75 d
Chaita mugur	6.67 a–b	137.24 a–b	71.33 c–g	109.33 j–m	22.66 a–e	173.33 a	3.038 b	28.02 a
Chapalo	6.22 b–d	136.86 a–b	56.67 m	107.67 l–m	21.15 c–e	139.40 c–d	1.850 r	15.58 d
Chaplo	5.45 c–g	120.04 a–f	68.67 g–k	113.33 d–h	22.80 a–e	88.60 m–n	2.322 h	10.83 h–k
Chitri	6.44 a–c	125.91 a–e	67.33 j–l	110.67 h–k	23.19 a–e	118.00 g–i	2.032 o	13.73 e
Dhalai kachari	3.89 j–l	130.49 a–d	66.67 k–l	111.67 f–j	25.38 a–d	109.13 h–j	2.157 l	8.35 q–s
Dharia Bogi	5.00 e–j	132.81 a–c	68.67 g–k	106.67 m	22.63 a–e	123.93 g	2.120 l–n	12.40 f–g
Ful chapala	6.00 b–e	110.10 a–g	57.00 m	107.67 l–m	22.33 a–e	92.40 l–m	2.150 l–m	11.24 h–j
Goria	6.44 a–c	126.62 a–e	70.33 d–i	114.33 b–f	20.89 c–e	68.73 p	2.439 f	10.51 i–l
Gota irri	5.44 c–g	90.39 f–g	70.67 d–h	113.00 d–i	19.31 d–e	127.80 e–g	2.678 d	17.97 b–c
Goyal	3.55 l	134.28 a–c	67.67 i–l	113.33 d–h	28.11 a–b	161.60 b	2.532 e	15.45 d
Gutiari	3.55 l	126.18 a–e	71.00 c–h	112.67 d–i	28.42 a	127.27 f–g	1.845 r	8.81 p–r
IR9	4.55 f–l	130.24 a–d	65.67 l	116.33 b–c	27.12 a–c	178.80 a	2.440 f	18.74 b–c
Kajli	7.55 a	140.54 a	67.67 i–l	106.67 m	28.49 a	117.47 g–i	1.930 p–q	15.47 d
Kali saita	4.22 h–l	102.46 c–g	69.67 e–j	112.33 e–i	16.76 e	80.67 n–o	2.111 m–n	7.47 s–t
Khewali	5.45 c–g	126.97 a–e	68.33 h–l	108.67 k–m	21.89 a–e	86.87 m–n	2.009 o	7.98 r–s
Koba binni	4.56 f–l	110.48 a–g	73.00 b–d	121.67 a	26.78 a–c	146.20 c–d	2.655 d	16.16 d
Kolar thor	5.33 c–h	107.79 a–g	69.67 e–j	108.67 k–m	22.47 a–e	109.33 h–j	2.402 f–g	13.25 e–f
Kumri aus	5.67 b–f	111.78 a–g	65.67 l	113.33 d–h	21.01 c–e	64.60 p	2.228 i–k	7.85 s
Lonka gora binni	4.89 e–j	101.87 c–g	72.33 b–e	116.67 b	24.63 a–d	73.93 o–p	3.104 a	11.01 h–j
Madab jata	4.22 h–l	128.06 a–e	71.67 c–f	110.33 i–l	22.99 a–e	106.80 i–k	2.391 g	9.98 k–m
Moishalema	4.78 f–k	120.59 a–f	71.67 c–f	113.33 d–h	23.92 a–d	102.07 j–l	1.901 q	9.12 m–q
Monsur irri	4.78 f–k	85.71 g	71.67 c–f	114.67 b–e	20.75 c–e	100.47 j–l	2.126 l–n	9.35 m–p
Pankhiraj	4.78 f–k	119.58 a–g	71.33 c–g	111.00 g–k	20.57 c–e	88.33 m–n	2.328 h	8.98 n–q
Parija	4.44 g–l	121.54 a–f	67.33 j–l	112.33 e–i	22.24 a–e	73.87 o–p	1.739 s	5.94 u
Poreengi	5.11 d–i	113.78 a–g	67.33 j–l	109.33 j–m	24.45 a–d	96.80 k–m	2.205 k	10.31 j–l
Putiraj	3.67 k–l	127.08 a–e	67.67 i–l	110.67 h–k	27.16 a–c	150.07 c	2.293 h–i	11.56 g–h
Shoni aus	5.56 b–g	95.27 e–g	72.67 b–d	112.67 d–i	21.25 c–e	62.93 p	1.954 p–q	6.59 t–u
Veri	4.44 g–l	123.73 a–f	74.67 b	116.67 b	23.79 a–d	149.07 c	2.527 e	15.83 d
P value	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Significance	**	**	**	**	**	**	**	**
C.V.	18.327543	11.431481	5.7654842	3.09745073	11.472840	26.8252354	14.671497	35.67471
Standard error	0.1483065	2.2065599	0.6501871	0.56232884	0.4382528	4.85572953	0.5364590	0.401134

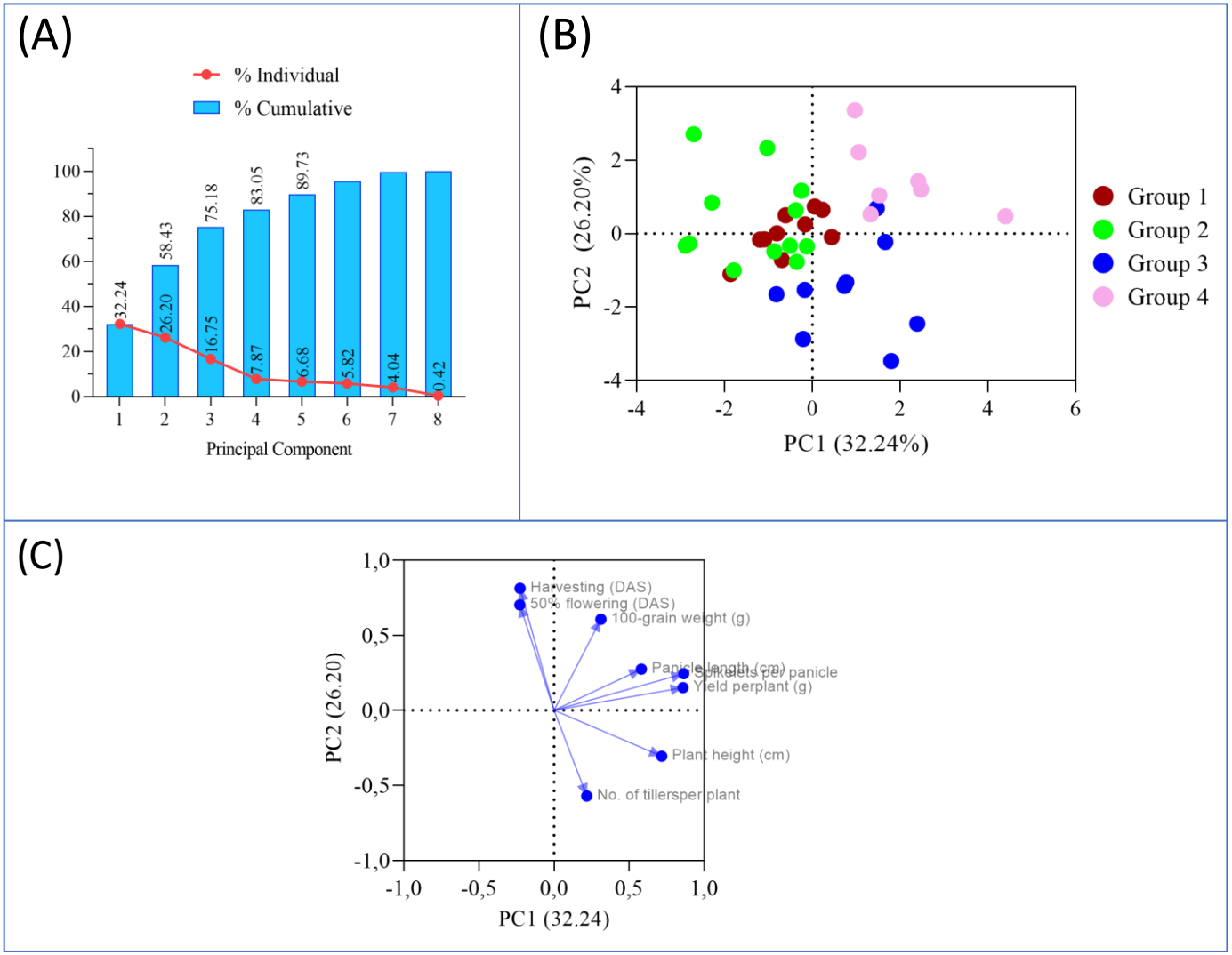
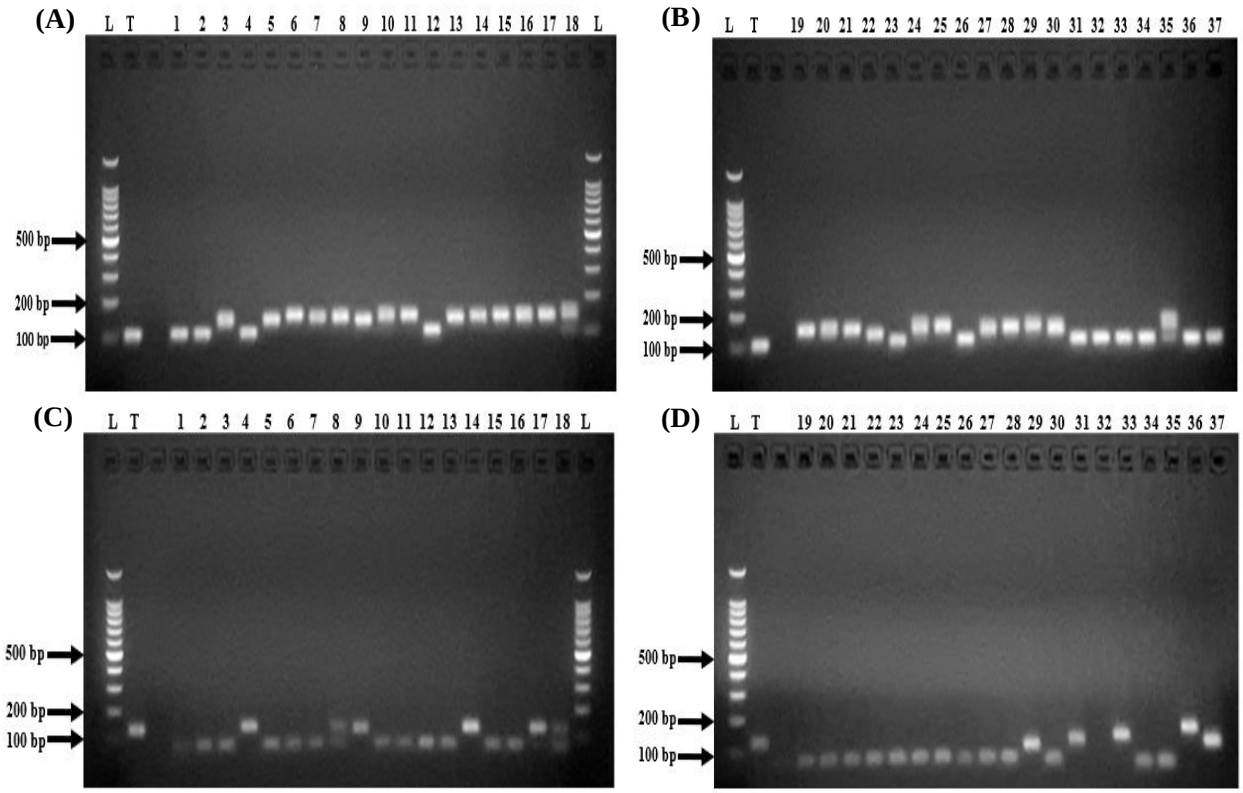


Figure 3. Principal component analysis of thirty-eight genotypes based on morphological data. (A) Proportion of variance, (B) PC score plot showing the distribution of the studied genotypes, (C) Loading plot showing the distribution of the studied characters. PC1: Principal component 1, PC2: Principal component 2.



Here, L= 100bp ladder, T= BRRI dhan42, 1= Aus patnai, 2= Veri, 3= Chaplo, 4= IR 9, 5= Dhalai kachari, 6= Boalia, 7= Goyal, 8= Gutiari, 9= Putiraj, 10= Agali, 11= Moishalema, 12= Poreengi, 13= Bogi, 14= Begun bichi, 15= Kolar thor, 16= Aus bogi, 17= Chitri, 18= Madab jata, 19= Dharia bogi, 20= Ful chapala, 21= Aus gara binni, 22= Alai, 23= Khewali, 24= Chapalo, 25= Chaita mugur, 26= Kajli, 27= Goria, 28= Pankhiraj, 29= Kumri aus, 30= Kali saita, 31= Parija, 32= Monsur irri, 33= Gota irri, 34= Aus baku, 35= Shoni aus, 36= Koba binni, 37= Lonka gora binni

Figure 4. SSR profile of rice genotypes using primer RM256 (A&B) and RM207 (C&D).

Genetic diversity

Nine SSR primer pairs were used for the genetic diversity analysis of 38 rice genotypes. Detailed information on these primers is shown in Table 3. A total of 337 alleles were detected in the 38 rice genotypes. The average number of alleles per single marker was 37.44 and ranged from 35 (RM212 and RM231) to 40 (RM256) (Table 4). Representative amplification profiles (RM256) are shown in Figure 4. Rare alleles were observed at all of the SSR loci in one or more of the 38 genotypes with an average of 0.33 rare alleles per locus across all of the loci (Table 4). An allele that was observed in less than 5% of the 38 accessions was considered to be rare (Hassan *et al.*, 2012). The highest level of genetic diversity (0.5) was observed for the RM242 locus and the lowest (0.1892) was observed for the RM207 locus with the mean diversity of 0.3321 (Table 4). The level of polymorphism among the 38 genotypes was evaluated by calculating the PIC values of each of the nine SSR loci. The PIC values varied widely among loci and ranged from a low of 0.64 (RM212) to a high of 0.87 (RM207 and RM256) per marker with an average of 0.76 (Table 4). The average genetic diversity across all the evaluated loci was 0.3321 with the highest (0.4875) for RM234 and the lowest (0.1892) for RM207 (Table 4).

Table 4. PIC and genetic diversity found in the present study by using SSR markers.

Locus	Allele size range (bp)	Difference	Allele number	Rare allele[a]	PIC[b]	Gene diversity
RM207	85–210	125	39	2	0.87	0.1892
RM212	125–150	25	35	0	0.64	0.2057
RM231	190–226	36	35	0	0.80	0.2165
RM234	156–180	24	38	0	0.74	0.4875
RM242	208–246	38	38	1	0.79	0.2484
RM247	150–176	26	38	0	0.75	0.5000
RM252	216–225	09	37	0	0.74	0.4411
RM256	120–196	76	40	0	0.87	0.4534
RM274	150–165	15	37	0	0.64	0.2472
Means	-	-	37.44	0.333	0.76	0.3321

[a]Rare alleles are defined as alleles with frequencies less than 5%.
[b]PIC: Polymorphism information content.

Genetic similarity analysis based on SSR marker analysis

The values of pair-wise similarity among the 38 genotypes calculated from the binary data of the nine primers are shown in Table 5. The highest similarity value was found between 'Veri' and 'Aus patnai;' 'Bogi' and 'Dhalai kachari;' 'Chitri' and 'Putiraj'; 'Khewali' and 'Poreengi'; 'Kajli' and 'Poreengi'; 'Goria' and 'Dhalai kachari;' 'Goria' and 'Bogi'; 'Kali saita' and 'Dhalai kachari;' 'Kali saita' and 'Bogi'; 'Aus bogi' and 'Kolar thor;' 'Kajli' and 'Khewali'; 'Kali saita' and 'Goria'; and 'Chapalo' and 'Chaita mugur.' The lowest similarity was found between 'Gota irri' and 'Begun bichi;' 'Gota irri' and 'Kolarthor'; 'Gota irri' and 'Aus bogi;' 'Parija' and 'Ful chapala;' 'Parija' and 'Aus gara binni;' 'Parija' and 'Alai'; 'Gota irri' and 'Chapalo'; 'Gota irri' and 'Chaita mugur;' and 'Gota irri' and 'Aus gara binni' (Table 5).

In this study, 'BRRI dhan 42' was used as the check cultivar. This genotype was released by Bangladesh Rice Research Institute. 'BRRI dhan 42' is a well-known drought-tolerant *aus* rice cultivar in Bangladesh. The genetic similarity values of the 37 landraces with the check cultivar ranged from 0.111 to 0.7. The highest similarity value (0.7) with 'BRRI dhan 42' was obtained by 'Madab jata'. The second-highest similarity value (0.588) was shown by 'Lonka gora binni.' The third-highest similarity value (0.556) was provided by 'Koba binni,' 'Parija', 'Gota irri,' 'Chitri.' and 'Putiraj'. Among the genotypes with high similarity values, 'Koba binni' and 'Lonka gora binni' were collected from Rangamati District. 'Dharia bogi' had the lowest similarity value (0.111) with 'BRRI dhan 42.' Through SSR marker analysis in rice, Panaud *et al.* (1996) found high genetic similarity among landraces with a common geographic origin and low similarity among landraces with diverse geographic origins. A dendrogram based on genetic similarity was generated and is presented in Figure 5.

DISCUSSION

The *aus* rice genotypes examined in this study exhibited high phenotypic and genetic variability for all of the measured traits, particularly spikelets per panicle, 100-seed weight, and yield per plant. This result was also confirmed by mean performance, which also showed high variation among the accessions of *aus* rice studied here. In this study, we found that group 4 had high yield per plant. Group 4 included high-yielding cultivars, such as 'BRRI dhan 42' and 'IR9', and the high yield found in this group may be due to the contribution of these genotypes. However, we did not test this hypothesis. For all the traits measured, phenotypic variances were slightly higher than genotypic variances. This result suggested that the environment had little influence on the studied traits. The variability found in this study revealed that the investigated accessions can be improved by using their genetic potential in selection.

Little is known about the genetic diversity of Bangladeshi *aus* rice, particularly its SSR-based genetic diversity under drought stress. Here, nine SSR markers were used to assess the genetic diversity of 38 genotypes of rice. The number of alleles detected per SSR markers was high (ranging from 35 to 40 with an average of 37.44), and the results of this work were nearly similar to those of other

Table 5. Summary of the pairwise similarity values of rice genotypes.

Rice genotypes	BRRI dhan 42	Aus patnai	Veri	Chaplo	IR9	Dhalai kachari	Boalia	Goyal	Gutiari	Putiraj	Agali	Moisha lema	Poreengi	Bogi	Begun bichi	Kolar thor	Aus Bogi	Chitri	Madab jata
BRRI dhan 42	1																		
Aus patnai	0.444	1																	
Veri	0.444	1	1																
Chaplo	0.444	0.556	0.556	1															
IR9	0.444	0.556	0.556	0.222	1														
Dhalai kachari	0.333	0.667	0.667	0.889	0.333	1													
Boalia	0.333	0.667	0.667	0.778	0.333	0.889	1												
Goyal	0.222	0.556	0.556	0.667	0.444	0.778	0.889	1											
Gutiari	0.235	0.353	0.353	0.471	0.353	0.588	0.706	0.824	1										
Putiraj	0.556	0.444	0.444	0.889	0.333	0.778	0.667	0.556	0.471	1									
Agali	0.222	0.778	0.778	0.667	0.444	0.778	0.889	0.778	0.588	0.556	1								
Moishalema	0.444	0.556	0.556	0.889	0.222	0.778	0.889	0.778	0.588	0.778	0.778	1							
Poreengi	0.444	0.778	0.778	0.778	0.444	0.889	0.889	0.778	0.588	0.667	0.778	0.778	1						
Bogi	0.333	0.667	0.667	0.889	0.333	1	0.889	0.778	0.588	0.778	0.778	0.778	0.889	1					
Begun bichi	0.333	0.444	0.444	0.667	0.556	0.556	0.444	0.556	0.471	0.778	0.556	0.556	0.444	0.556	1				
Kolar thor	0.222	0.556	0.556	0.778	0.444	0.667	0.556	0.667	0.471	0.667	0.667	0.667	0.556	0.667	0.889	1			
Aus Bogi	0.222	0.556	0.556	0.778	0.444	0.667	0.556	0.667	0.471	0.667	0.667	0.667	0.556	0.667	0.889	1	1		
Chitri	0.556	0.444	0.444	0.889	0.333	0.778	0.667	0.556	0.471	1	0.556	0.778	0.667	0.778	0.778	0.667	0.667	1	
Madab jata	0.7	0.7	0.7	0.5	0.6	0.4	0.5	0.4	0.316	0.5	0.6	0.6	0.5	0.4	0.5	0.5	0.5	0.5	1
Dharia Bogi	0.111	0.667	0.667	0.556	0.333	0.667	0.778	0.667	0.588	0.444	0.889	0.667	0.667	0.667	0.444	0.556	0.556	0.444	0.5
Ful chapala	0.333	0.556	0.556	0.667	0.222	0.556	0.667	0.556	0.471	0.556	0.778	0.778	0.556	0.556	0.556	0.667	0.667	0.556	0.6
aus gara binni	0.25	0.5	0.5	0.625	0.25	0.5	0.625	0.625	0.533	0.5	0.75	0.75	0.5	0.5	0.625	0.75	0.75	0.5	0.556
Alai	0.235	0.588	0.588	0.824	0.235	0.706	0.588	0.471	0.375	0.706	0.706	0.706	0.588	0.706	0.706	0.824	0.824	0.706	0.526
Khewali	0.444	0.778	0.778	0.778	0.444	0.889	0.889	0.778	0.588	0.667	0.778	0.778	1	0.889	0.444	0.556	0.556	0.667	0.5
Chapalo	0.222	0.556	0.556	0.667	0.444	0.556	0.667	0.778	0.588	0.556	0.778	0.778	0.556	0.556	0.778	0.889	0.889	0.556	0.6
Chaita mugur	0.222	0.556	0.556	0.667	0.444	0.556	0.667	0.778	0.588	0.556	0.778	0.778	0.556	0.556	0.778	0.889	0.889	0.556	0.6
Kajli	0.444	0.778	0.778	0.778	0.444	0.889	0.889	0.778	0.588	0.667	0.778	0.778	1	0.889	0.444	0.556	0.556	0.667	0.5
Goria	0.333	0.667	0.667	0.889	0.333	1	0.889	0.778	0.588	0.778	0.778	0.778	0.889	1	0.556	0.667	0.667	0.778	0.4
Pankhiraj	0.222	0.778	0.778	0.778	0.444	0.889	0.778	0.667	0.471	0.667	0.889	0.667	0.778	0.889	0.667	0.778	0.778	0.667	0.5
Kumri aus	0.222	0.556	0.556	0.444	0.667	0.556	0.667	0.778	0.706	0.556	0.778	0.556	0.556	0.556	0.778	0.667	0.667	0.556	0.5
Kalisaita	0.333	0.667	0.667	0.889	0.333	1	0.889	0.778	0.588	0.778	0.778	0.778	0.889	1	0.556	0.667	0.667	0.778	0.4
Parija	0.556	0.333	0.333	0.111	0.444	0.222	0.222	0.333	0.353	0.111	0.111	0.111	0.333	0.222	0.111	0.111	0.111	0.111	0.3
Monsur irri	0.533	0.667	0.667	0.4	0.267	0.533	0.533	0.4	0.429	0.4	0.4	0.4	0.667	0.533	0.133	0.133	0.133	0.4	0.353
Gota irri	0.556	0.444	0.444	0.222	0.333	0.333	0.333	0.222	0.235	0.222	0.222	0.222	0.444	0.333	0	0	0	0.222	0.4
Aus baku	0.333	0.889	0.889	0.444	0.667	0.556	0.556	0.667	0.471	0.333	0.667	0.444	0.667	0.556	0.556	0.667	0.667	0.333	0.6
Shoni aus	0.316	0.632	0.632	0.632	0.526	0.737	0.842	0.947	0.778	0.526	0.737	0.737	0.842	0.737	0.526	0.632	0.632	0.526	0.476
Koba binni	0.556	0.556	0.556	0.556	0.333	0.444	0.444	0.333	0.235	0.556	0.556	0.556	0.556	0.444	0.556	0.556	0.556	0.556	0.5
Lonka gora binni	0.588	0.471	0.471	0.471	0.353	0.353	0.353	0.235	0.125	0.471	0.471	0.471	0.471	0.353	0.471	0.471	0.471	0.471	0.526

Table 5. (cont'd).

Rice genotypes	Dharia Bogi	Ful Chapalo	aus gara binni	Alai	Khewali	Chapalo	Chaita mugur	Kajli	Goria	Pankhiraj	Kumri aus	Kalisaita	Parija	Monsur irri	Gota irri	Aus baku	Shoni aus	Koba binni	Lonka gora binni
Dharia Bogi	*1*																		
Ful chapala	0.667	1																	
aus gara binni	0.625	0.875	1																
Alai	0.706	0.706	0.667	1															
Khewali	0.667	0.556	0.5	0.588	1														
Chapalo	0.667	0.778	0.875	0.706	0.556	1													
Chaita mugur	0.667	0.778	0.875	0.706	0.556	1	1												
Kajli	0.667	0.556	0.5	0.588	1	0.556	0.556	1											
Goria	0.667	0.556	0.5	0.706	0.889	0.556	0.556	0.889	1										
Pankhiraj	0.778	0.667	0.625	0.824	0.778	0.667	0.667	0.778	0.889	1									
Kumri aus	0.667	0.556	0.625	0.471	0.556	0.778	0.778	0.556	0.556	0.667	1								
Kalisaita	0.667	0.556	0.5	0.706	0.889	0.556	0.556	0.889	1	0.889	0.556	1							
Parija	0.222	0	0	0	0.333	0.111	0.111	0.333	0.222	0.111	0.222	0.222	1						
Monsur irri	0.4	0.267	0.154	0.286	0.667	0.133	0.133	0.667	0.533	0.4	0.267	0.533	0.533	1					
Gota irri	0.333	0.222	0	0.118	0.444	0	0	0.444	0.333	0.222	0.111	0.333	0.667	0.667	1				
Aus baku	0.556	0.444	0.5	0.471	0.667	0.667	0.667	0.667	0.556	0.667	0.667	0.556	0.444	0.533	0.333	1			
Shoni aus	0.632	0.526	0.588	0.444	0.842	0.737	0.737	0.842	0.737	0.632	0.737	0.737	0.421	0.5	0.316	0.737	1		
Koba binni	0.444	0.667	0.625	0.588	0.556	0.556	0.556	0.556	0.444	0.556	0.444	0.444	0.222	0.4	0.222	0.444	0.421	1	
Lonka gora binni	0.353	0.588	0.533	0.5	0.471	0.471	0.471	0.471	0.353	0.471	0.353	0.353	0.353	0.286	0.235	0.353	0.333	0.824	1

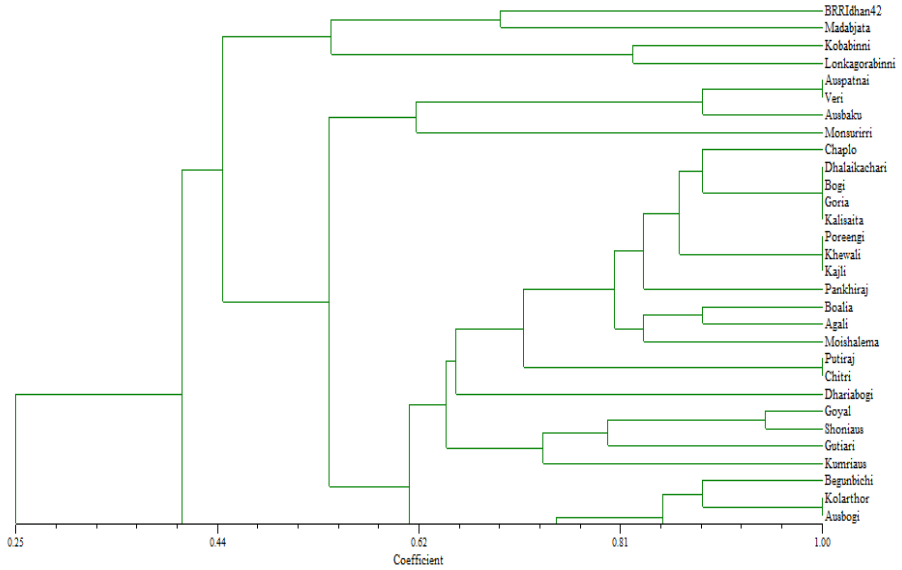


Figure 5. Dendogram derivedfrom cluster analysis of 38 rice genotypes based on the data revelead from simple sequence repeat (SSR) markers.

studies (Thomson *et al.*, 2007; Borba *et al*., 2009). However, the number of allele per locus was considerably larger than that previously reported (Rahman *et al.*, 2010; Pandy *et al.*, 2012) likely because of the inclusion of a high number of landraces. Nevertheless, the high level of SSR polymorphism that was detected here could be attributed primarily to the diverse origins of the accessions studied. The present study observed higher PIC values (varied from 0.64 to 0.87) than earlier works (Rahman *et al*., 2010; Islam *et al*., 2011). High PIC values were obtained likely because the rice accessions used in this study were collected from diverse regions of Bangladesh. Therefore, great variability might exist in such a population.

The higher average number of alleles and PIC values obtained in this work than those in other studies suggested that the germplasm used here possesses unique diversity. This considerable diversity could be readily used in broadening the genetic base of existing cultivars. Although almost all of the markers in the present work were highly polymorphic, RM256 was particularly robust in capturing diversity. It revealed the highest PIC value, number of alleles, and genetic diversity index (Table 4). These results indicated that this marker is well distributed throughout the rice genome and could be used routinely for the molecular characterization of rice germplasm. This study detected a low number of rare alleles (Table 4) likely due to the variable periods of genetic isolation during the evolutionary history of the studied accessions. Genotypes with rare alleles can be utilized in breeding programs as donor parents to develop highly adapted cultivars and broaden the genetic base of existing rice cultivars.

CONCLUSIONS

Phenotypic evaluation revealed significant differences among the investigated landraces. 'Chaita mugur' showed the most satisfactory results for the characters, particularly high yield per plant, assessed in this experiment. Therefore, the genotype 'Chaita mugur' was considered as the best landrace in this work. Pairwise similarity analysis revealed that genetic similarity was highest between 'Veri' and 'Aus patnai;' 'Chitri' and 'Putiraj'; 'Aus bogi' and 'Kolar thor;' 'Chapalo' and 'Chaita mugur;' 'Kajli' with 'Khewali' and 'Poreengi'; and 'Bogi' with 'Dhalai kachari,' 'Goria', and 'Kali saita.' 'Gota irri' showed the lowest similarity with 'Begun bichi,' 'Kolar thor,' 'Aus bogi,' 'Chapalo', 'Chaita mugur,' and 'Aus gara binni.' Parija showed the lowest similarity with 'Ful Chapala,' 'Aus gara binni,' and 'Alai'. 'Madab jata,' 'Koba binni,' and 'Lonka gora binni' clustered with 'BRRI dhan 42' with the clustering coefficient of 0.53. These three landraces showed the highest similarity with 'BRRI dhan 42.' Therefore, 'Madab jata,' 'Lonka gora binni,' and 'Koba binni' were identified as probable landraces containing drought-tolerant alleles.

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