Delineation of Bangladeshi coastal rice germplasm based on qualitative phenotypic traits

Susmita Banik[1], Md. Golam Rasul[1], Nasrin Akter Ivy[1], M. Moynul Haque[2] and Mehfuz Hasan[1] ●

[1]Department of Genetics and Plant Breeding, Bangabandhu Sheikh Mujibur Rahman Agricultural University, Gazipur 1706, Bangladesh and [2]Department of Agronomy, Bangabandhu Sheikh Mujibur Rahman Agricultural University, Gazipur 1706, Bangladesh

Abstract

A detailed study of rice genetic resources in Bangladesh's coastal areas is necessary. This understanding is a necessary requirement for its utilization in selective breeding. The study reports on the qualitative morphological trait-based assessment of 150 local rice samples collected from Bangladesh's coastal zone, including 50 advanced lines developed from coastal germplasm. Six of the thirteen analysed characters had a substantial gene contribution, whereas the average was 0.694. The most impressive diversity was in leaf blade intensity of green colour (LBIGC: 0.705). The total morpho-qualitative diversity was calculated to be 0.412. The character efficiency content ranged from 0.655 (LBIGC) to 0.136 (Leaf Sheath: Anthocyanin colouration, Leaf Blade: Presence/Absence, and Leaf Blade: Anthocyanin. Colouration). As per the morphological variance study, 93% of morphological changes were detected within individuals, whereas 7% were found in populations. The 150 germplasm samples were divided into four subpopulations using STRUCTURE-based population analysis. A moderate genotypic difference was detected amongst all groups, with an F_{st} value of 0.111. The G statistic backed up the record as well. The Shannon mutual information index reached a value of 1.252 between populations 2 and 3. In terms of gene exchange, the highest value was found between populations 3 and 4. Our data indicate a high degree of diversity in Bangladesh's coastline rice germplasm. The findings will aid in conferring the farmers' Intellectual Property Rights on the investigated rice germplasm.

Introduction

Rice is without a doubt the most significant grain on a worldwide scale. South Asia has been dubbed Asia's 'meal cart' due to its prominence in rice domestication (Pachauri *et al.*, 2013). Bangladesh is one of the leading rice-growing countries in Asia. Bangladesh ranks fourth in the world (Bandumula, 2018). Apart from rice variations, Bangladesh cultivates a diverse range of cultivars and landraces. For several centuries, farmers have kept several thousands of distinct landraces (Gao, 2003). These cultivars were selected for favourable characteristics, including crop yields, seed size and resistance to environmental stress. Rice landraces have been critical in ensuring regional sustainable development and agriculture's long-term viability, and their value as a genetic reservoir for selective breeding cannot be overstated (Ali *et al.*, 2020).

However, rice landraces are being displaced by the widespread cultivation of modern varieties and numerous interventions in rice habitats throughout Bangladesh (Jahan *et al.*, 2020). For centuries, the majority of aboriginal crops have been referred to by their native creole names. As a result, records on the genetic nature and history of such genotypes are scarce or non-existent. As an outcome, their characterization could show their phylogeny, which would be extremely useful for the genetic improvement of existing rice varieties using this germplasm. The characterization of accessions enables the quantification and organization of genotypic variability and its conservation, preservation and use in crop improvement projects (Li *et al.*, 2010; Aravind *et al.*, 2019).

Additionally, the world's population is growing, which increases climate vulnerability due to various activities and poses a massive threat to global food security (Nelson *et al.*, 2009; Mekonnen *et al.*, 2021). Salinity already impacts more than 20% of the world's agricultural soils, and it is becoming an increasingly severe danger to food production due to climate change (Haque, 2018; Mustafa *et al.*, 2019; Thorslund *et al.*, 2021). Around 2.8 million hectares of Bangladesh's south coastline region are afflicted by salt levels ranging from 3 to 23 ds/m. Traditional rainfed cultivars, which have a low production potential, are commonly grown in this area (Baten *et al.*, 2015; Haque, 2018).
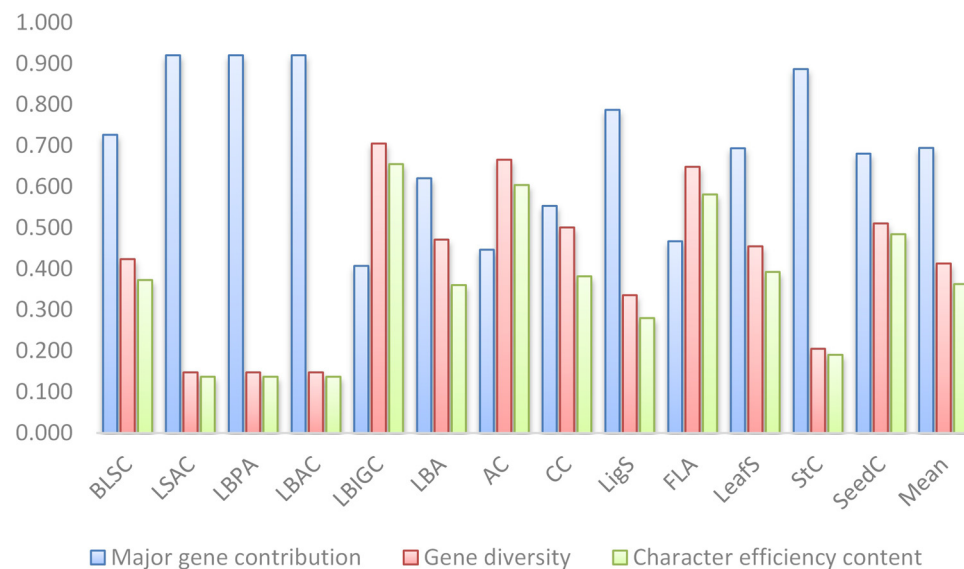
Fig. 1. Trait inputs to the diversity status of Bangladeshi coastal germplasm. The blue colour denotes significant gene contribution, while the maroon colour represents gene diversity. The colour green represents the Character efficiency content. BLSC: Basal Leaf Sheath Colour, LSAC: Leaf Sheath: Anthocyanin colouration, LBPA: Leaf Blade: P/A, LBAC: Leaf Blade: Anthocyanin. Colouration, LBIGC: Leaf Blade: Intensity of Green colour, LBA: Leaf Blade: Attitude, AC: auricle colour, CC: Collar colour, LigS: Ligule shape, FLA: Flag leaf: attitude, LeafS: Leaf: Senescence, StC: Stigma colour, SeedC: Seed colour.

Diversification is also critical for environmental adaptation (Mekonnen *et al.*, 2021). However, some rice varieties are becoming increasingly vulnerable to genetic erosion, or the loss of diversity, due to lower population sizes caused by land-use changes and degradation, as well as changes in local climate that may select against specific genotypes. As a result, there is an increasing urge to characterize and conserve the landraces of climate-vulnerable places. Numerous rice landraces are being planted throughout Bangladesh's saline-prone coastal regions (Lisa *et al.*, 2004; Seraj *et al.*, 2006). Rice from these locations is in high demand by customers due to its unique cultural customs. Thus, the region's notable landraces are well-suited to the soil conditions, such as salinity. As a result, they are pretty salt tolerant. Rice cultivars that are already adapted to stressful situations have several unique genes that help them survive in the location. Variety is a significant part of agriculture due to the broad array of crops available, which results in genotypic variation in farming (Modi and Bornman, 2004). However, numerous commercially significant characteristics inherent in landraces are underutilized (Roy *et al.*, 2016; Umakanth *et al.*, 2017). As a result, the focus should be given to analysing rice landrace diversity, which will provide valuable information for breeding efforts aimed at increasing rice production.

Although various researchers have characterized and analysed rice germplasm, research on the variability of common landraces and cultivars grown in Bangladesh's local regions is scarce. Given the widespread cultivation and importance of landraces, the research explored morpho-qualitative characteristics of 150 landraces collected from Bangladesh's coastal belt, including 50 advanced lines generated from coastal germplasm.

Materials and methods

Collection and characterization of rice germplasm

One hundred fifty rice cultivar samples were collected in Bangladesh's coastal zone, as indicated in online Supplementary Table S1. The samples were taken from local farmers in accordance with the ITPGRFA article no. 9. Farmers were recognized as the custodians and innovators of the material studied. Three replications of a randomized complete block design were used in the experiment. Data were collected following the recommendation of the Biodiversity International and the Africa Rice Center (AfricaRice), formerly known as the West Africa Rice Development Association (WARDA) (Imtiaz and Upadhyaya, 2010).

Data analysis

For three consecutive years, data were collected on ten qualitative morphological characters in 25 randomly picked plants (online Supplementary Table S2). Major gene contribution (MGC), character efficiency content (CEC) and gene diversity were calculated using PowerMarker 3.23 (Liu and Muse, 2005). GenAlEx V6.5 (Peakall and Smouse, 2006) was used to examine the analysis of morphological variance (AMOVA), F, and G statistic. STRUCTURE 2.3.3 was used to explore the population structure model-based application (Pritchard *et al.*, 2000). For each K, there were three replications. For each run with 100,000 Monte Carlo Markov Chain repetitions, a burn-in duration of 100,000 iterations was used. For each genotype, a spectrum of genetic groups ranging from 1 to 10 was employed to assess its membership. The associated allele frequency and the admixture model were taken into consideration. To ascertain the threshold of K, the graph of LnPD computed for each K was employed. It was constructed using the Structure Harvester application.

Results

Importance of characters to the variability of the collected landraces

The prevalence of the MGC to every locus was determined using Fig. 1. The frequency ranged between 0.407 (leaf blade intensity of green colour, LBIGC) and 0.920 (LSAC, LBPA and LBAC). Six of
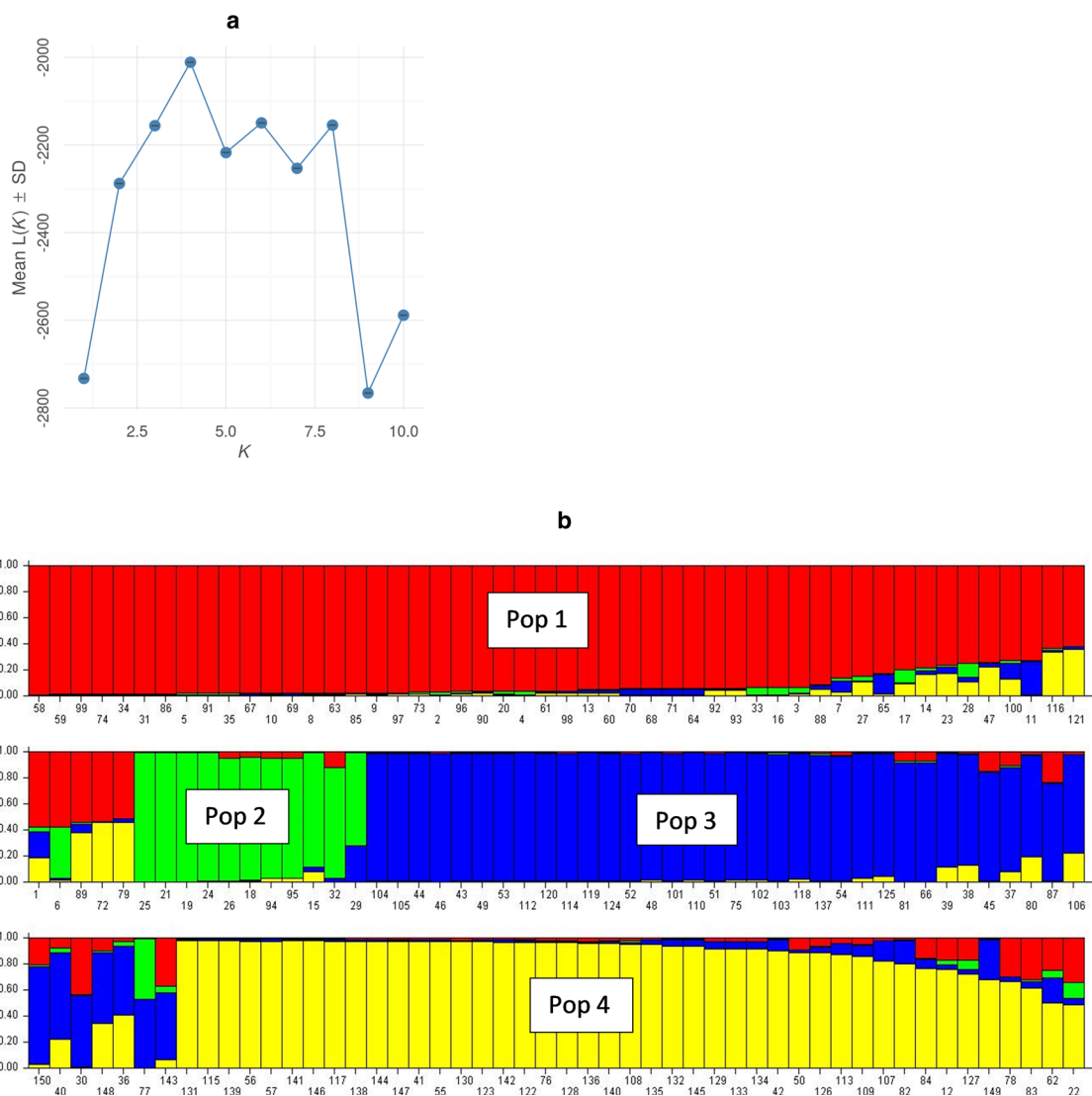
Fig. 2. (a) *K* value for the purpose of the total of subpopulations. The *K* values ranged from 1 to 10. (b) Population differentiation pattern of coastal rice germplasm represented by a bar plot. Each vertical coloured column denotes a different genotype. The *X*-axis indicates the types of coastal rice, while the *Y*-axis represents the subpopulations.

the thirteen features had a significant gene contribution greater than 0.7, whereas the average was 0.694. LBIGC exhibited the most remarkable morpho-qualitative diversity (0.705), whereas LSAC, LBPA and LBAC exhibited the least (0.147). The overall diversity was determined to be 0.412. The amount of character efficiency in 150 rice germplasm was determined by calculating the CEC levels for each gene encoding a character. The character's genes were utilized as the ground for the computation. CEC values varied from 0.655 (LBIGC) to 0.136 (LSAC, LBPA and LBAC), with an average of 0.362 (Fig. 1).

Morpho-quantitative trait diversity and population structure of rice germplasm

The population formation and relationships of 150 rice germplasm samples from coastal Bangladesh were studied using STRUCTURE v2.3.4 (Pritchard *et al.*, 2000). *K*-value was applied to identify the population structures of germplasm based on the qualitative data of 13 characteristics. A total of 41, 10, 33 and

36 pure genotypes were found in Pops 1, 2, 3 and 4 (Fig. 2). Subpopulations 1, 2, 3 and 4 had admixtures of 14, 1, 8 and 7 lines, respectively.

Morpho-qualitative divergence and analysis of morphological variance

GenAlEx was used to determine the morphological variation in 150 rice genotypes' qualitative attributes. Seven per cent of the morphological variance was observed between populations, while ninety-three per cent was shown within individuals (Table 1).

The F_{st} is a method for determining population differences due to genetic structure. When comparing populations, a F_{st} value greater than 0.15 can be considered significant (Frankham *et al.*, 2002). Thus, when the F_{st} values from the STRUCTURE are investigated, a considerable divergence was identified within each of the four subpopulations (Table 2). However, a F_{st} value of 0.111 was observed among the four subpopulations. This

Table 1. Summary of analysis of morphological variance (AMOVA)

Source	df	SS	MS	Est. Var.	%
Among populations	3	116.908	38.969	0.526	7%
Among individuals	146	285.412	1.955	0.000	0%
Within individuals	150	974.500	6.497	6.497	93%
Total	299	1376.820		7.023	100%

Table 2. Wright's *F* statistics

F-statistics	Value	P (r and $\geq$ data)
F_{st}	0.111	0.001
F_{is}	−0.537	1.000
F_{it}	−0.367	1.000
F_{st} max	0.350	
F'_{st}	0.317	
N_m	2.007	

score shows a degree of phenotypic difference between the subpopulations as a whole (Hartl and Clark, 1997). The finding is consistent with the AMOVA result (Table 2), which indicated that 93% of the overall variations were contained within the individual variants. The remaining 7% was revealed as a result of demographic variation. A N_m value less than one indicates that gene exchange between subpopulations is limited (Wright, 1965). In this study, the N_m value (2.007) indicates that gene flow between groups is continuous (Perdereau *et al.*, 2014). According to Eltaher *et al.* (2018), this shows the possibility of adequate gene flow or genetic interchange. When this occurs, it is possible for considerable genetic differentiation to occur between subpopulations. The F_{st} (0.317) value, which was less than 50%, offered information about homozygosity.

We demonstrated that all 13 traits have an effect on population differentiation using *G* statistics and Wright's *F* statistics (Table 3).

Between Pop 2 and Pop 3, the Shannon mutual information index was revealed to be the greatest when all subpopulations were included (1.252). While the lowest value (1.091) was found between Pops 3 and 4, as illustrated in Table 4. The highest significant gene exchange (1.552) occurred between Pop 3 and Pop 4. The smallest value (0.232) was observed between Pop 2 and Pop 3.

Discussion

Overemphasis cannot be placed on how important it is to examine genotypic variability in rice landraces in the saline region of Bangladesh. As a result of this examination, the abandoned heritage resource will be better conserved, and genes responsible for desirable features will be discovered. Achieving this will allow rice breeders to use these qualities for rice improvement. En outre, in the places afflicted by salt, there exist many rice

Table 3. *F*- and *G*-statistics for the studied traits

F and *G* values	BLSC	LBPA	LBIGC	AC	LigS	LeafS	SeedC	LSAC	LBAC	LBA	CC	FLA	StC	Tot
F_{is}	−0.651	−0.976	−0.435	−0.374	−0.484	−0.560	−0.438	−0.976	−0.647	−0.346	−0.534	−0.460	−0.697	−0.561
F_{st}	0.160	0.267	0.125	0.078	0.115	0.043	0.047	0.267	0.213	0.056	0.093	0.115	0.021	0.123
G_{is}	−0.640	−0.976	−0.418	−0.357	−0.469	−0.544	−0.422	−0.976	−0.635	−0.328	−0.520	−0.444	−0.685	−0.547
G_{st}	0.155	0.267	0.117	0.069	0.108	0.036	0.039	0.267	0.208	0.047	0.087	0.107	0.016	0.117

BLSC, Basal Leaf Sheath Colour; LSAC, Leaf Sheath: Anthocyanin colouration; LBPA, Leaf Blade: P/A; LBAC, Leaf Blade: Anthocyanin. Colouration; LBIGC, Leaf Blade: Intensity of Green colour; LBA, Leaf Blade: Attitude; AC, auricle colour; CC, Collar colour; LigS, Ligule shape; FLA, Flag leaf: attitude; LeafS, Leaf: Senescence; StC, Stigma colour; SeedC, Seed colour.

Table 4. Pairwise population studies of Shannon mutual information (sHua) and degree of gene exchange (N_m)

Population		sHua	N_m
Pop1	Pop2	1.142	0.664
Pop1	Pop3	1.160	0.532
Pop2	Pop3	1.252	0.232
Pop1	Pop4	1.106	1.142
Pop2	Pop4	1.224	0.286
Pop3	Pop4	1.091	1.552

landraces. A systematic characterization of the genotypes is therefore essential. There is now parental material for crop improvement from the qualitative data gained for the 150 germplasm taken from saline-affected coastal Bangladesh.

Impact of the traits on the rice diversity

Regardless of the type of crop, significant genes are vital to the level of diversity. An average value of 0.69 was found in this investigation. If the phenotypic expression of the evaluated traits was pronounced, then the value is high. It is known that significant genes play a vital role in determining the qualitative attributes of coastal germplasm in Bangladesh (Kinoshita *et al.*, 2017; Bazrkar-Khatibani *et al.*, 2019; Rahman *et al.*, 2019). Scientists have identified several genes that determine the quality of rice grain (Zhao *et al.*, 2015; Wang *et al.*, 2017; Park *et al.*, 2019).

According to a study conducted on the parameters of qualitative features, the rice germplasm sampled from coastal Bangladesh exhibited a low to moderate level of diversity. Diversity is used to measure the mean heterozygosity and genetic distances among individual plants (Shete *et al.*, 2000; Luo *et al.*, 2019). According to Chen *et al.* (2011), the average diversity of 300 rice samples gathered from various rice-growing regions worldwide was 0.358. The average diversity in our study was 0.238, which is equivalent to the 0.36 diversity reported for unique landraces gathered in India under farmer management (Kumar *et al.*, 2010). However, it is smaller than the 0.43 value for diversity found in the US accession panel (Agrama *et al.*, 2007). Similarly, the mean diversity of rice samples from China is 0.47 (Jin *et al.*, 2010), which is more than the average diversity observed in our experiment. Additionally, it is less diverse than the diversity observed (0.68) by Ali *et al.* (2011). Nachimuthu *et al.* (2015) estimate that most diversity panels with worldwide accessions have a diversity of between 0.5 and 0.7.

The CEC metric quantifies the gene diversity associated with each character. CEC values were generated for each trait to conduct the diversity analysis. Three character variables, including LBIGC, auricle colour (AC) and flag leaf attitude, were used to summarize the diversity status of the local rice germplasm (FLA). Any of the three characters may be used to differentiate landraces and choose landraces for crop development initiatives (Oladosu *et al.*, 2014; Sharifi and Ebadi, 2018).

Population structure of rice germplasm

The population genetic parameters are critical when estimating the average genotypic diversity of a region or field. Additionally,

they are valuable for determining the differences in allele frequencies between different populations and the disparities in polymorphism levels between populations (Kumar *et al.*, 2010). Understanding population genetic structure will aid in tracking the loss of variety through time and space. Additionally, it will aid in the development of a rational conversation approach for managing farmer landraces on-farm.

Numerous scholars frequently employ STRUCTURE's model-based technique to examine the population structure of rice (Huda *et al.*, 2019; Luo *et al.*, 2019; Mishra *et al.*, 2019; Hasibuzzaman *et al.*, 2020). Moderately constructed accessions revealed four subpopulations with F_{st} values of 0.398, 0.364, 0.206 and 0.281 among the 91 rice landraces from eastern and northeastern India (Das *et al.*, 2013). Islam *et al.* (2018) used a model-based approach to analyse the population structure of red rice germplasm from Bangladesh, resulting in four subpopulations. Additionally, a pairwise F_{st} analysis revealed significant divergence across all pairs of populations, ranging from 0.108 to 0.207. As a result of this finding, it is concluded that the four subpopulation structural groupings are highly distinct. The 150 germplasm samples in our study were divided into four subpopulations using structure-based population analysis. Each group had a small number of admixtures. Between Pop 3 and Pop 4, the highest number of gene exchange was discovered. Local farmers typically retain such germplasm in a traditional manner. For many years, local farmers have been preserving germplasm at the community level.

Our analysis of the germplasm's population structure indicates that farmers may sustain a major portion of its purity level satisfactorily. Outcrossing and natural mutations may play a significant role in the establishment of population admixtures. It is critical to define the rice landraces that indigenous consumers highly prize. According to our findings, natural selection, genetic migration, mutation and purity can explain four distinct subpopulations. Future research will find it beneficial to understand the rice landrace's diversity and population structure. With the understanding of genetic differentiation and key gene contributions, local rice germplasm can be preserved. Additionally, the data from this study will be beneficial to farmers in establishing intellectual property rights for the rice landraces from coastal Bangladesh that was studied, as some of the landraces demonstrated distinct traits. Certain landraces exhibited unique anthocyanin colouration on the leaf sheath and blade. Anthocyanins contribute to reproduction by enticing pollinators and seeds disperser and providing protection from abiotic and biotic stressors (Van den Ende and El-Esawe, 2014). Anthocyanins have been linked in the responses of plants to a range of concerns, including drought (An *et al.*, 2020), UV-B (Bai *et al.*, 2014) and heavy metals (Gao *et al.*, 2020) and also resilience to pests (Fan *et al.*, 2016) and diseases (Sivankalyani *et al.*, 2016). There are indications that anthocyanins are beneficial to health (Wallace and Giusti, 2019), rendering anthocyanin metabolism intriguing for plant breeders. Thus, the classification of the studied landraces will aid plant breeders and farmers identify and select favourable traits for crop development. However, little effort has been made to characterize Bangladeshi rice germplasm. As a result, deliberate efforts must be made to compile a comprehensive inventory of these important plant materials to estimate the presence of novel beneficial genes from this resource.

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