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Genetic Diversity and Population Structure in Wild Rice Germplasm

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ABSTRACT

Wild rice species are rich sources of useful allelic variation for genetic improvement of cultivated rice for improvement of yield and yield-related traits and biotic and abiotic stress tolerance. The study of genetic diversity and population structure is a crucial measure for the proper utilization of available genetic resources in a plant breeding program. Seventy-seven wild rice and relatives collected from International Rice Research Institute and different regions of Bangladesh were investigated using 42 simple sequence repeat (SSR) markers to unveil the population structure and to estimate genetic diversity. A total of 200 alleles were detected, with an average of 4.76 alleles per locus. Besides, 19 unique alleles were found with 16 SSR markers, which can be used as diagnostic tools for germplasm identification. A comparative assessment of STRUCTURE and UPGMA showing a high level of concordance in grouping the wild rice accessions suggested wide divergence among the genotypes and could be utilized for the future rice genomic studies especially for development of stress tolerant rice varieties. The results suggest that the SSR markers are effective for assessing the genetic diversity of wild rice and its related species.

1. Introduction

Rice (*Oryza sativa* L.) is one of the world's most significant cereal crops, serving as a staple food for more than half of the global population including Bangladesh. In the context of climate change, abiotic stress and emergence of new strains, races or biotypes of biotic factors are endangering food security through yield loss of rice crop. Wild rice species serve as rich source of useful genes (Song et al., 2005) for biotic and abiotic stresses. *O. rufipogon* Griff. is a perennial species, which is widely distributed from southern part of China through south and south-east Asia down to Papua New Guinea and northern Australia (Vaughan, 1994), is a progenitor of Asian cultivated rice (Khush, 1997). As an ancestor of cultivated rice, wild species docks valuable genes for resistance to blast (Ram et al., 2007); brown plant hopper (Rongbai et al., 2001) and grassy stunt virus (Khush et al., 1977). Improved Sambha Mahsuri and improved Pusa Basmati1 were released in India introgressing Xa21 gene for bacterial blight (BB) resistance from *O. longistaminata*. Similarly, modern rice varieties BRRI dhan55, BRRI dhan87, BRRI dhan89 and BRRI dhan96 were developed and released in Bangladesh by hybridizing with *Oryza rufipogon* (BRRI, 2025). Wild rice *O. officinalis* contains the valuable genes for early morning flowering (EMF) trait to mitigate high temperature-induced sterility at anthesis (Ishimaru et al., 2010). Moreover, *O. minuta*, a tetraploid wild relative of cultivated rice, shows the potential resistance against blast, bacterial blight, white backed plant hopper (WBPH) and brown plant hopper (BPH). Again, various resistance genes have been transferred effectively to cultivated rice from

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O. minuta (Rahman et al., 2009). It is well recognized that wild rice species carries considerably higher genetic diversity than the cultivated rice (Sun et al., 2001).

Species with greater genetic diversity offer more variations to choose for the most appropriate alleles of a given trait. Species that have lesser genetic variation are at risk of extinction. The genetic diversity within the crop species essentially determines yield and a number of other economically advantageous traits in that crop species. The cultivated rice varieties available are the end product of human selection from the actual and available genetic diversity in the various wild types. Modern breeding programs have led to developed varieties that are more uniform, stable, and improved for the controlled and limited environments of traditional fields. As a result, the crop has become more susceptible to biotic and abiotic environmental stresses. However, natural populations of wild rice species are rapidly declining due to habitat loss, land-use changes, and climate impacts (Song et al., 2005; Gao, 2004), suggesting the urgency of conservation and characterization.

Molecular marker analysis is one of the most useful tools for evaluating the genetic variation of common wild rice germplasm (Liu et al., 2015; Wang et al., 2008; Zhou et al., 2003; Gao, 2004). With the initiation of PCR based molecular marker technology, SSR markers have been used for their high reproducibility, simplicity, easy scoring ability, reliability, co-dominant, multi-allelic nature and wide distribution across the genome (Siddique et al., 2014; Siddique et al., 2017; Islam et al., 2019; Islam et al., 2020; Vabna et al., 2021; Khalequzzaman et al., 2022; Siddique et al., 2023; Alkasim et al., 2024; Islam et al.,2025).

Bangladesh Rice Research Institute (BRRI) conserves more than 9000 rice germplasm accessions, including 90 wild rice and related species maintained under ex situ conservation (Siddique et al., 2025). Despite their importance, the genetic diversity and population structure of these wild rice accessions in Bangladesh have not been previously characterized. Therefore, the present study aimed to investigate the genetic diversity of wild rice using SSR markers and to analyze population structure using a model-based approach.

2. Materials and methods

2.1. Rice materials

A total 77 wild rice germplasm maintained in an ex-situ conservation in the net house under Genetic Resources and Seed Division (GRSD) of Bangladesh Rice Research Institute (BRRI) (Anonymous, 2025) were used in this study (Table 1).

Table 1
Name and origin of 77 wild rice and relatives.

Sl. No.	Species	Code Name	Origin/Source	Sl. No.	Species	Code Name	Origin/Source
1	*Oryza alta*	WR1	IRRI	40	*Oryza rufipogon*	WR40	Gauranadi, Barishal
2	*Oryza alta*	WR2	IRRI	41	*Oryza officinalis*	WR41	Jhalokhati
3	*Oryza alta*	WR3	IRRI	42	Unknown	WR42	Jhalokhati
4	*Oryza alta*	WR4	IRRI	43	*Oryza rufipogon*	WR43	Shibaloy, Manikganj
5	*Oryza latifolia*	WR5	IRRI	44	*Oryza rufipogon*	WR44	Rangpur Sadar
6	*Oryza latifolia*	WR6	IRRI	45	*Oryza rufipogon*	WR45	Chitalmari, Bagerhat
7	*Oryza granular*	WR7	IRRI	46	*Oryza rufipogon*	WR46	Betagi, Barguna
8	*Oryza longistaminata*	WR8	IRRI	47	*Oryza rufipogon*	WR47	Madhupur, Tangail
9	*Oryza longistaminata*	WR9	IRRI	48	*Oryza rufipogon*	WR48	Cumilla Sadar
10	*Oryza minuta*	WR10	IRRI	49	*Oryza rufipogon*	WR49	Gazipur Sadar
11	*Oryza officinalis*	WR11	Sonagazi, Feni	50	*Oryza rufipogon*	WR50	Wazirpur, Barishal
12	*Oryza officinalis*	WR12	Betagi, Barguna	51	*Oryza rufipogon*	WR51	Shibaloy, Manikganj
13	*Oryza punctata*	WR13	IRRI	52	*Oryza rufipogon*	WR52	Fakirhat, Bagerhat
14	*Oryza rufipogon*	WR14	IRRI	53	*Oryza rufipogon*	WR53	Madhupur, Tangail
15	*Oryza rufipogon*	WR15	IRRI	54	*Oryza rufipogon*	WR54	Domar, Nilphamari
16	*Oryza rufipogon*	WR16	IRRI	55	*Oryza rufipogon*	WR55	Habiganj Sadar
17	*Oryza rufipogon*	WR17	IRRI	56	*Oryza rufipogon*	WR56	Cumilla Sadar
18	*Oryza rufipogon*	WR18	IRRI	57	*Oryza rufipogon*	WR57	Barguna Sadar
19	*Oryza rufipogon*	WR19	IRRI	58	*Oryza rufipogon*	WR58	Shibaloy, Manikganj
20	*Oryza rufipogon*	WR20	IRRI	59	*Oryza rufipogon*	WR59	Domar, Nilphamari
21	*Oryza rufipogon*	WR21	IRRI	60	Unknown	WR60	Gazipur Sadar
22	*Oryza rufipogon*	WR22	IRRI	61	*Oryza rufipogon*	WR61	Habiganj Sadar
23	*Oryza rufipogon*	WR23	IRRI	62	*Oryza rufipogon*	WR62	Saghata, Gaibandha
24	*Oryza rufipogon*	WR24	IRRI	63	*Porteresia coarctata*	WR63	Batiaghata, Khulna
25	*Oryza rufipogon*	WR25	IRRI	64	*Oryza rufipogon*	WR64	Gauranadi, Barishal
26	*Oryza rufipogon*	WR26	IRRI	65	*Oryza rufipogon*	WR65	Betagi, Barguna
27	*Oryza rufipogon*	WR27	IRRI	66	*Oryza rufipogon*	WR66	Madhupur, Tangail
28	*Oryza rufipogon*	WR28	IRRI	67	*Oryza rufipogon*	WR67	Fakirhat, Bagerhat
29	*Oryza rufipogon*	WR29	IRRI	68	*Oryza rufipogon*	WR68	Wazirpur, Barishal
30	*Oryza rufipogon*	WR30	IRRI	69	*Oryza rufipogon*	WR69	Chitalmari, Bagerhat
31	*Oryza rufipogon*	WR31	IRRI	70	*Oryza rufipogon*	WR70	Shibaloy, Manikganj
32	*Porteresia coarctata*	WR32	Shyamnagar, Shatkhira	71	*Oryza rufipogon*	WR71	Madhupur, Tangail
33	*Oryza rufipogon*	WR33	Wazirpur, Barishal	72	*Oryza rufipogon*	WR72	Rangpur Sadar
34	*Oryza rufipogon*	WR34	Betagi, Barguna	73	*Oryza rufipogon*	WR73	Domar, Nilphamari
35	*Oryza rufipogon*	WR35	Mollahat, Bagerhat	74	*Oryza rufipogon*	WR74	Cumilla Sadar
36	*Oryza rufipogon*	WR36	Madhupur, Tangail	75	*Oryza rufipogon*	WR75	Habiganj Sadar
37	*Oryza rufipogon*	WR37	Habiganj Sadar	76	*Oryza rufipogon*	WR76	Batiaghata, Khulna
38	*Oryza rufipogon*	WR38	Domar, Nilphamari	77	*Oryza rufipogon*	WR77	Fakirhat, Bagerhat
39	*Oryza rufipogon*	WR39	Saghata, Gaibandha				

WR = wild rice, IRRI = International Rice Research Institute, Unknown = Species not identified yet

2.2. SSR markers

Forty-two well distributed SSRs were used for diversity analysis. The chromosomal position (cM) and repeat motifs for these markers were retrieved from rice genome database (http://www.gramene.org). Marker position and motif of 42 SSRs are presented in Table 2.

2.3. Genomic DNA isolation and PCR amplification

DNA was extracted from the collected young leaves following the quick DNA extraction protocol of Ferdous et al. (2012). PCR analysis was performed in 10 µL reaction sample containing 3 µL of DNA template, 4.5 µL of Go Taq G2 Green Master Mix (Promega), 1.5 µL of Nuclease-Free Water, 0.5 µL each of 10 µM forward and reverse primers using a GeneAtlas G (Astec, Japan) 96-well thermal cycler. The mixture was overlaid with 10 µL of mineral oil to prevent evaporation. After initial denaturation for five minutes at 94°C, each cycle comprised 30 sec denaturation at 95°C, 30 sec annealing at 55°C, and 25 sec extensions with a final extension for 5 min at 72°C at the end of 32 cycles.

2.4. Electrophoresis and visualization of amplified products

The PCR products were mixed with bromophenol blue gel loading dye and were analyzed by electrophoresis on 8% polyacrylamide gel using a mini vertical polyacrylamide gels electrophoresis machine (CBS Scientific Co. Inc., CA, USA). 2.5 µL of amplification products were resolved by running gel in 0.5X TBE buffer for 1.5-2.5 hrs depending upon the allele size at around 100 volts and 500 mA current. The gels were stained in 5 µL SYBR Safe DNA gel stain (10,000X concentration in DMSO, USA) with 200 mL 0.5X TBE buffer for 15 min and exposed to UV light using a molecular imager gel documentation unit (XR System, Uvitec Cambridge, France) for visualization.

2.5. Allele scoring and diversity data analysis

The band size for each marker was measured by Alpha Ease FC 4.0 software. The summary statistics of the marker data such as the average number of alleles per locus, major allele frequency, gene diversity and polymorphism information content (PIC) were done by using Power Marker V3.25 (Liu and Muse, 2005). For the unrooted phylogenetic tree, genetic distance was calculated using the "C.S. Chord 1967" distance measure (Cavalli-Sforza and Edwards, 1967) followed by phylogeny reconstruction using neighbour joining as implemented in MEGA 5.1 software (Tamura et al., 2011). The allele frequency data were exported in binary format (allele presence = "1" and allele absence = "0") for analysis with NTSYS-pc version 2.2 (Rohlf, 2002). A similarity matrix was calculated with the Sim Qual subprogram using the Dice coefficient, followed by cluster analysis with the SAHN sub-program using the UPGMA (unweighted pair group method using the arithmetic mean) clustering method as implemented in NTSYS-pc. Principal coordinate analysis (PCoA) analysis is a form of multivariate analysis that forms groupings of germplasm based on either similarity co-efficient or variance-covariance values. It provides further information on the differentiation of major groups (Venkatesan and Bhat, 2015). Hence, the similarity matrix was also used PCoA with the Center, Eigen, Output, and MXPlot sub-programs in NTSYS-pc.

2.6. Population structure analysis

Population structure was examined using the Bayesian model-based approach implemented in STRUCTURE V2.3.4 (Pritchard et al., 2000). The number of subpopulations was classified based on the true K value. The number of clusters (K) ranged from 1 to 10. The analysis was performed using five replicate runs per K value, a burn-in period length of 5000, a run length of 50000, and the admixture model was followed. 'Structure harvester' program (http://taylor0.biology.ucla.edu) was used to determine the final K value (K = 3 was optimum for this analysis) based on both the LnP(D) and Evanno's Δ K (Evanno et al., 2005).

3. Results

3.1. SSR diversity of wild rice and relatives

In this research, genetic diversity of 77 wild rice and relatives were analyzed using 42 simple sequence repeat (SSR) markers located on all 12 chromosomes. The basic statistics of the 42 SSR markers are listed in Table 2. A total of 200 alleles were detected, which varied from 2 (RM454) to 9 (RM154), averaging 4.76 alleles per locus. The major alleles were sized between 66 bp (RM413) and 270 bp (RM496) and the frequency of the major alleles ranged from 33.00% to 94.00%. On average, 66.43% of the 77 wild rice and relatives had at least one major allele common across the loci. Gene diversity ranged between 0.12 (RM237) and 0.79 (RM154), with an average of 0.48, while PIC values ranged from 0.12 (RM237) to 0.76 (RM154), and averaging 0.44. Because of higher PIC value (0.76) and gene diversity (0.79), RM154 locus was classified as the best SSR marker for determining the 77 wild rice germplasm. DNA profile of the 77 wild rice and relatives is shown with an SSR marker RM20 in Fig. 1.

A total of 19 distinct alleles were amplified from 16 SSR markers (Table 3) with one to two unique alleles per locus. Of the 16 SSR markers, 13 SSR markers amplified only one unique allele, while three markers amplified two unique alleles across 77 wild rice accessions. Notably, four SSR markers, RM334 (chromosome 5, 155 bp), RM413 (chromosome 5, 70

bp), RM178 (chromosome 05, 122 bp), and RM237 (chromosome 01, 117 bp), amplified specific alleles only for WR60 (Unknown). Furthermore, there were two unique alleles amplified specific to WR4 (RM527, RM447), WR16 (RM334, RM338), WR21 (RM154, RM161), WR32 (RM118, RM507), and WR42 (RM224, RM320). The 232bp allele amplified by the SSR marker RM527 was specific to the wild rice WR4. Besides, two unique alleles 234bp and 185 bp were amplified by the marker RM20 from wild rice genotype WR25 and WR40, respectively. Likely, the marker RM334 amplified unique alleles of 208 bp and 155 bp in WR16 and WR60, respectively. The marker RM19 amplified a unique allele of 200 bp in WR41. RM282 also amplified a unique allele of 109bp in WR12. Likewise, the marker RM507 amplified unique alleles of 154 bp and 160 bp in WR32 and WR63 wild rice, respectively.

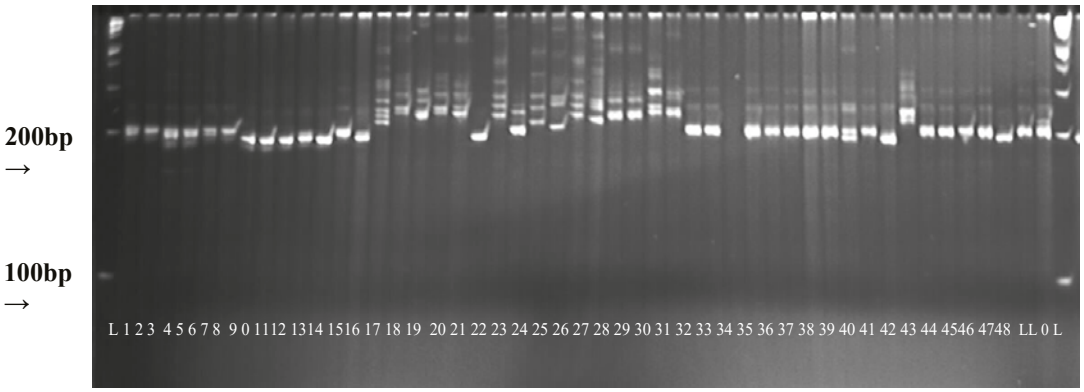
Table 2

Allele number, size and frequency, gene diversity and polymorphism information content of 42 SSR markers across 77 wild rice and relatives.

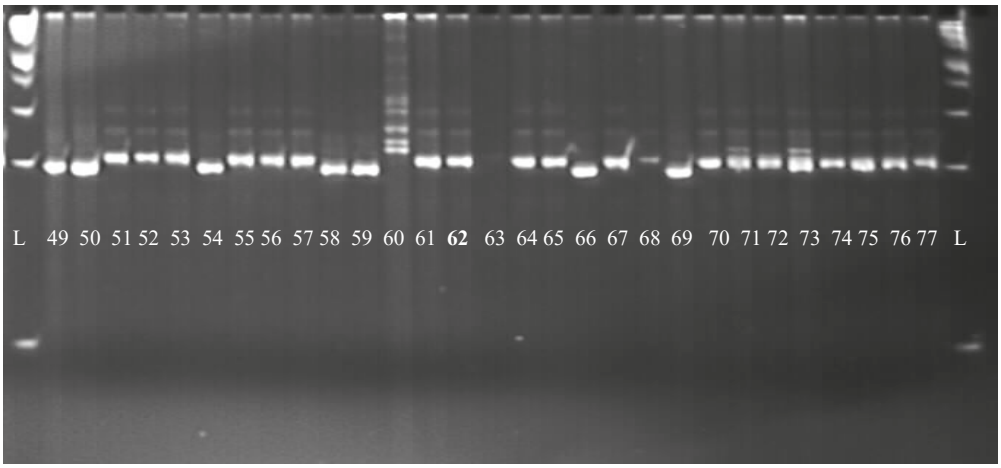
Marker	Chro. No.	Position (cM)	Motif*	No. of Allele	Size range (bp)	Size (bp)	Freq (%)	Gene diversity	PIC
RM20	12	0	(ATT)14	6	182-240	182	60.00	0.59	0.54
RM224	11	120.1	(AAG)8(AG)13	6	111-142	142	42.00	0.72	0.68
RM320	7	62.4	(AT)11GTATGT)13	4	122-184	184	78.00	0.37	0.33
RM334	5	141.8	(CTT)20	7	130-208	208	74.00	0.43	0.40
RM413	5	26.7	(AG)11	6	66-100	66	64.00	0.56	0.53
RM496	10	113	(TC)14	5	219-270	270	68.00	0.50	0.46
RM527	6	61.2	(GA)17	4	202-232	232	71.00	0.45	0.38
RM151	1	36.2	(TA)23	4	170-225	225	83.00	0.30	0.29
RM487	3	127.9	(AC)10	3	180-186	180	87.00	0.23	0.21
RM567	4	153.6	(GA)21	3	240-260	260	79.00	0.34	0.30
RM14643	3	53.1	(GA)n	4	97-136	136	53.00	0.63	0.57
RM222	10	11.3	(CT)18	4	85-238	238	51.00	0.65	0.60
RM338	3	108.4	(CTT)6	3	179-190	190	91.00	0.17	0.16
RM412	6	142.4	(GA)22	5	178-202	202	55.00	0.60	0.53
RM432	7	43.5	(CATC)9	3	178-225	178	86.00	0.25	0.23
RM442	3	224.2	(AAG)10	5	241-266	266	77.00	0.40	0.38
RM6	2	154.7	(AG)16	3	155-214	155	62.00	0.54	0.48
RM11	7	47	(GA)17	5	118-140	140	77.00	0.40	0.38
RM19	12	20.9	(ATC)10	6	200-260	200	66.00	0.55	0.52
RM44	8	60.9	(GA)16	3	96-114	96	78.00	0.36	0.33
RM105	9	32.1	(CCT)6	4	105-148	148	55.00	0.61	0.56
RM118	7	96.9	(GA)8	6	156-178	156	66.00	0.52	0.48
RM125	7	24.8	(GCT)8	4	114-128	128	65.00	0.50	0.43
RM134	7	-	(CCA)7	5	80-96	96	51.00	0.62	0.55
RM154	2	4.8	(GA)21	9	138-220	220	33.00	0.79	0.76
RM161	5	96.9	(AG)20	7	120-196	196	44.00	0.69	0.65
RM165	1	-	(CT)13	5	164-195	185	43.00	0.67	0.61
RM178	5	118.8	(GA)5(AG)8	5	90-122	122	48.00	0.65	0.59
RM215	9	99.4	(CT)16	4	135-150	148	44.00	0.61	0.53
RM282	3	100.6	(GA)15	7	100-142	142	56.00	0.63	0.60
RM284	8	83.7	(GA)8	4	80-92	92	83.00	0.30	0.28
RM316	9	1.8	(GT)8(TG)9(TTTG)4 (TG)4	8	175-235	175	66.00	0.54	0.52
RM408	8	1.1	(CT)13	4	111-125	125	70.00	0.46	0.40
RM447	8	124.6	(CTT)8	7	79-132	132	69.00	0.49	0.46
RM454	6	99.3	(GCT)8	2	267-290	267	73.00	0.40	0.32
RM507	5	0	(AAGA)7	5	154-253	253	75.00	0.40	0.37
RM515	8	80.5	(GA)11	6	204-230	204	52.00	0.64	0.59
RM566	9	47.7	(AG)15	3	230-245	245	82.00	0.31	0.28
RM18	7	90.4	(GA)4AA(GA)(AG)16	6	139-178	139	52.00	0.66	0.62
RM237	1	115.2	(CT)18	3	117-132	132	94.00	0.12	0.12
RM133	6	0	(CT)8	4	215-228	228	92.00	0.15	0.14
RM26243	11	-	(CTG)8	3	172-220	220	75.00	0.39	0.34
Total				200		7503	2790.00	20.17	18.49
Minimum				2		66	33.00	0.12	0.12
Mean				4.76		178.64	66.43	0.48	0.44
Maximum				9		270	94.00	0.79	0.76

Table 3
Genotype-specific alleles identified for 11 wild rice and relatives.

Sl. no.	Locus	Chromosome	Unique Allele	Size (bp)	Code no. of wild rice and relatives
1	RM20	12	1	234	WR25
2	RM20	12	1	185	WR40
3	RM224	11	1	111	WR42
4	RM320	7	1	184	WR42
5	RM334	5	1	208	WR16
6	RM334	5	1	155	WR60
7	RM413	5	1	70	WR60
8	RM527	6	1	232	WR4
9	RM338	3	1	179	WR16
10	RM19	12	1	200	WR41
11	RM118	7	1	178	WR32
12	RM154	2	1	138	WR21
13	RM161	5	1	132	WR21
14	RM178	5	1	122	WR60
15	RM282	3	1	109	WR12
16	RM447	8	1	94	WR4
17	RM507	5	1	154	WR32
18	RM507	5	1	160	WR63
19	RM237	1	1	117	WR60



Legend: 1.WR1, 2.WR2, 3.WR3, 4.WR4, 5.WR5, 6.WR6, 7.WR7, 8.WR8, 9.WR9, 10.WR10, 11.WR11, 12.WR12, 13.WR13, 14.WR14, 15.WR15, 16.WR16, 17.WR17, 18.WR18, 19.WR19, 20.WR20, 21.WR21, 22.WR22, 23.WR23, 24.WR24, 25.WR25, 26.WR26, 27.WR27, 28.WR28, 29.WR29, 30.WR30, 31.WR31, 32.WR32, 33.WR33, 34.WR34, 35.WR35, 36.WR36, 37.WR37, 38.WR38, 39.WR39, 40.WR40, 41.WR41, 42.WR42, 43.WR43, 44.WR44, 45.WR45, 46.WR46, 47.WR47, 48.WR48.



Legend: 49.WR49, 50.WR50, 51.WR51, 52.WR52, 53.WR53, 54.WR54, 55.WR55, 56.WR56, 57.WR57, 58.WR58, 59.WR59, 60.WR60, 61.WR61, 62.WR62, 63.WR63, 64.WR64, 65.WR65, 66.WR66, 67.WR67, 68.WR68, 69.WR69, 70.WR70, 71.WR71, 72.WR72, 73.WR73, 74.WR74, 75.WR75, 76.WR76, 77.WR77.

Fig. 1. DNA profile of 77 wild rice and relatives with RM20.

3.2. UPGMA cluster analysis

The UPGMA cluster analysis categorized 77 wild rice and relatives into five separate clusters at a coefficient of 0.49. The distance coefficient varied from 0.27 to 1.00 as illustrated in Fig. 2. Cluster I contained 58 wild rice and relatives, while clusters II contained 14, cluster III and IV each contained two and cluster V contained one wild rice germplasm (Table 4). Interestingly, *Oryza rufipogon* accession, WR36 collected from Tangail (central part of Bangladesh) and *Oryza rufipogon* accession, WR57 collected from Barguna district (southern region) were grouped into cluster III, while Cluster IV had two other wild rice accessions - WR24 which was collected from IRRI and WR60 from Gazipur district. Cluster V contained only one wild rice (WR14, *O. rufipogon*) accession, which was also obtained from IRRI.

Most of the wild rice accessions in Cluster I were collected from IRRI and from various locations in Bangladesh. The wild rice species classified in the cluster I were *Oryza alta* (04), *O. latifolia* (01), *O. officinalis* (02), *O. rufipogon* (49), *Porteresia coarctata* (02). Among these, two accessions of *Porteresia coarctata*, WR32 were collected from Shyamnagar, Satkhira, and WR63 were collected from Batiaghata, Khulna (South-west coastal region) of Bangladesh. Cluster II contained six different wild rice species, *Oryza latifolia, O. granular, O longistaminata, O. minuta, O. punctata* and *O. rufipogon*, all were collected from IRRI. Cluster II has the highest species diversity than all other four clusters.

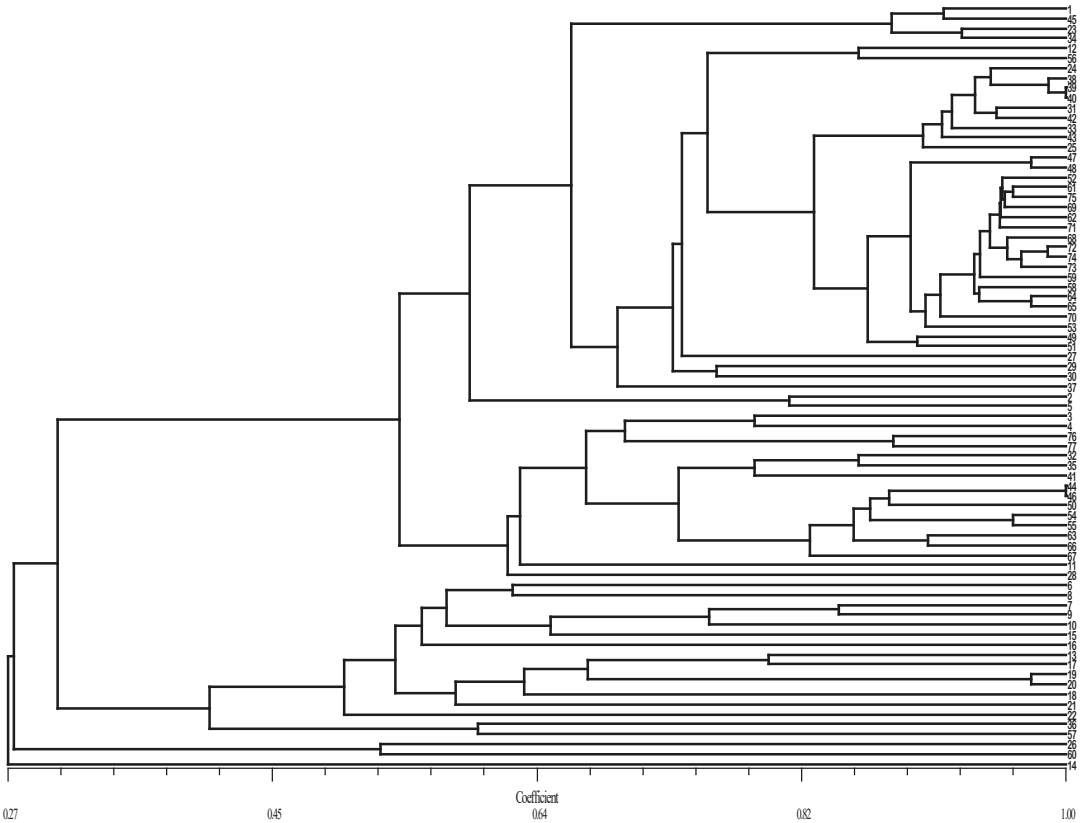


Fig. 2. Cluster analysis of 77 wild rice and relatives based on SSR markers polymorphism.

Table 4
Cluster group based on 42 SSR markers.

Cluster	Number of wild rice and relatives	Code name of wild rice and relatives	Frequency (%)
I	58	WR1, WR2, WR3, WR4, WR5, WR11, WR12, WR23, WR25, WR26, WR27, WR28, WR29, WR30, WR31, WR32, WR33, WR34, WR35, WR37, WR38, WR39, WR40, WR41, WR42, WR43, WR44, WR45, WR46, WR47, WR48, WR49, WR50, WR51, WR52, WR53, WR54, WR55, WR56, WR58, WR59, WR61, WR62, WR63, WR64, WR65,WR66, WR67, WR68, WR69, WR70,WR71, WR72, WR73, WR74, WR75, WR76, WR77	75.32
II	14	WR6, WR7, WR8, WR9, WR10, WR13,WR15, WR16, WR17, WR18, WR19, WR20, WR21, WR22	18.18
III	2	WR57, WR36	2.6
IV	2	WR60, WR24	2.6
V	1	WR14	1.3

3.3. Genetic distance-based analysis

The unrooted neighbor-joining tree of genetic distance-based results showed that the 77 wild rice and relatives were clustered into main three clusters (Fig. 3). The 77 wild rice and relatives were also clustered into several sub-clusters. Cluster I contained the largest number of wild rice (74) followed by II (2) and III (1), which contained the lowest number of wild rice accessions.

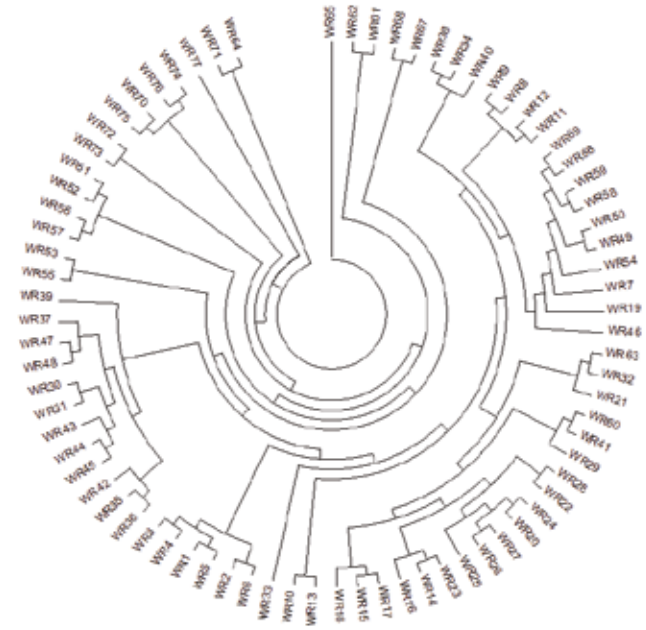


Fig. 3. An unrooted neighbor-joining tree showing the genetic relationships among 77 wild rice and relatives.

3.4. Population structure analysis

A Bayesian STRUCTURE based method was used to investigate the population structure of the 77 wild rice and relatives. The structure analysis was carried out with K set at 1 to 10 with 5 iterations which demonstrated a maximum log likelihood of K = 3 (Fig. 4) which was indicative of three distinct groups representing three main clusters, namely, GI (red color), GII (green color) and GIII (blue color) (Fig. 5). The first group consisted of 35 genotypes (45.45%), the second group consisted of 23 genotypes (29.87%), and the third group consisted of 19 genotypes (24.68%). Overall, 24 (31.16%) of wild rice exhibited evidence of admixture when K = 3.

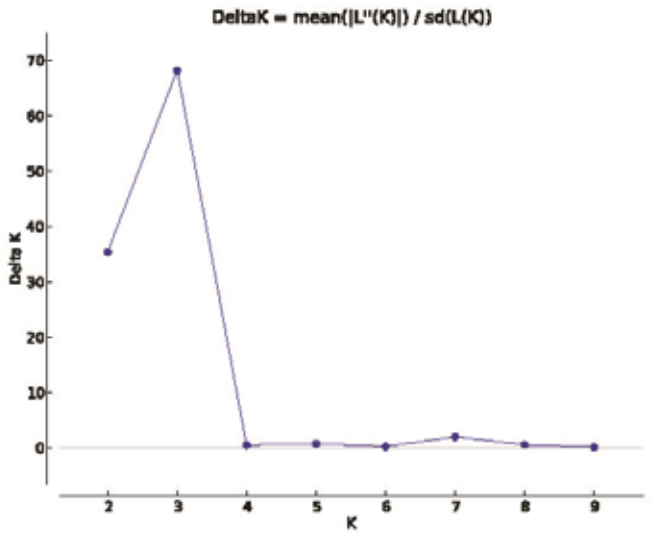


Fig. 4. STRUCTURE estimation of the number of groups for the K values ranging from 1 to 10, by delta K values.

The PCoA analysis clearly segregated wild rice and relatives based on their place of origin (Figs. 6 and 7). Wild rice was dispersed throughout the PCoA plot. The figures of PCoA provided two and three-dimensional representations of the spatial distributions of wild rice according to their place of origin/source. The wild rices namely *Oryza rufipogon* (WR69), *Oryza rufipogon* (WR59), *Oryza rufipogon* (WR66), *Oryza longistaminata* (WR8), *Oryza rufipogon* (WR19), *Oryza rufipogon* (WR18), *Oryza rufipogon* (WR38), *Oryza rufipogon* (WR28), *Oryza rufipogon* (WR21), *Porteresia coarctata* (WR32) and *Porteresia coarctata* (WR63) were found far away from the centroid of the cluster and the remaining wild rices were found more or less around the centroid (Figs. 6 and 7). The farther the distance of germplasm from the centroid, the greater is the genetic diversity and vice-versa.

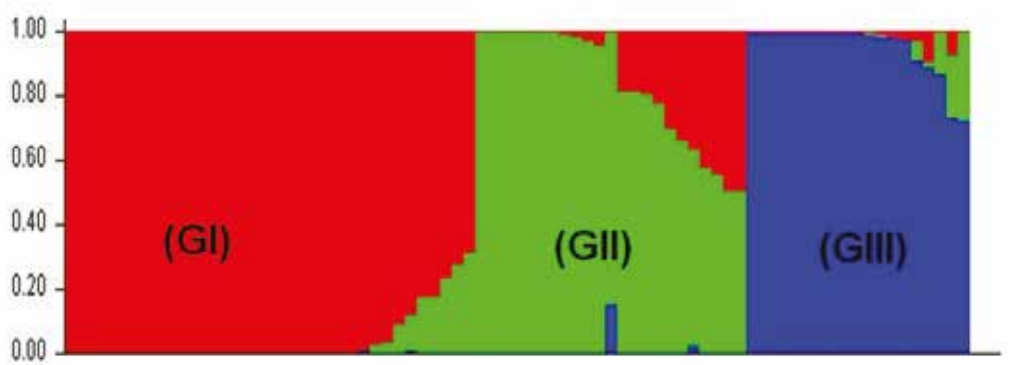


Fig. 5. Assignment of 77 wild rice and relatives to three groups (GI, GII and GIII) using STRUCTURE 2.3.4 software.

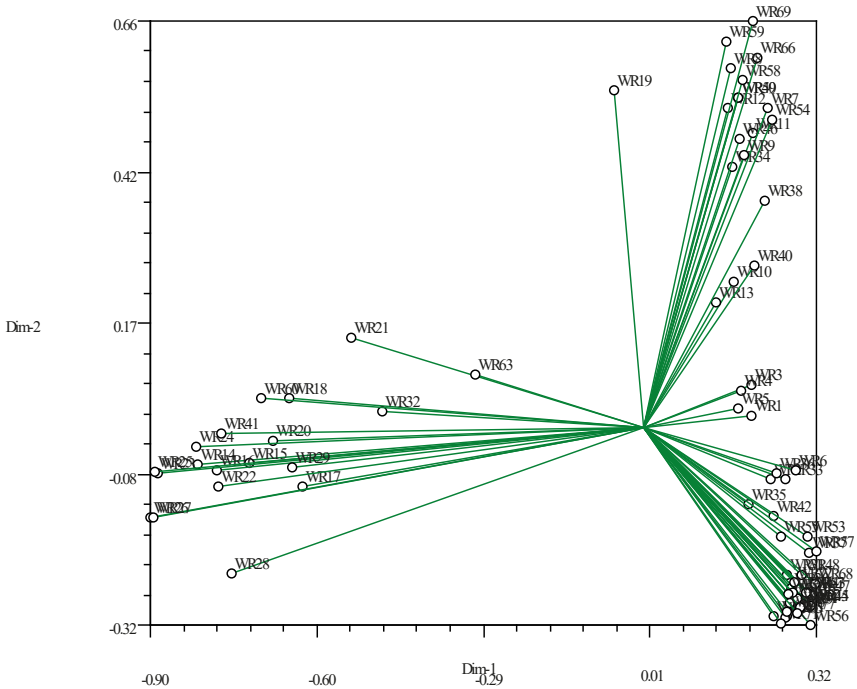


Fig. 6. Two-dimensional view of principal coordinate analysis (PCoA) with 42 SSR markers over 77 wild rice and relatives.

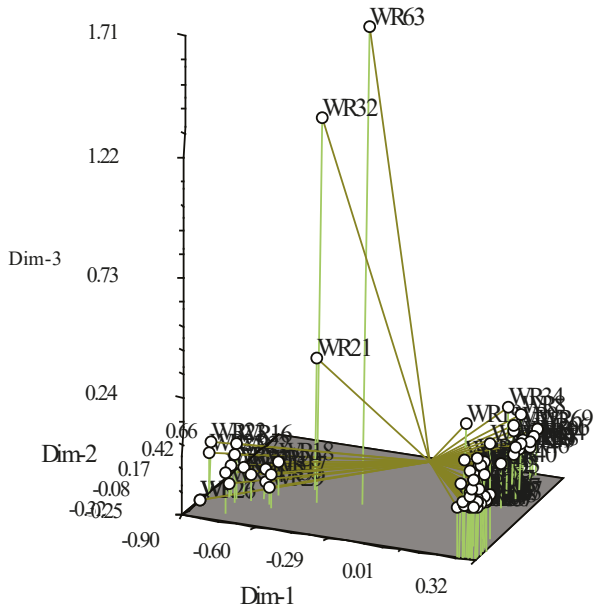


Fig. 7. Three-dimensional view of principal coordinate analysis (PCoA) with 42 SSR markers over 77 wild rice and relatives.

4. Discussion

Genetic divergence is important for the survival and adaptation of a species. Adapting to a changing environment often requires multiple resilience mechanisms in organism morphology to adapt and survive in extreme conditions. The improvement of rice varieties necessitates the detection of both highly diverse wild rice germplasm and highly polymorphic molecular markers that can be effectively used to map genes/QTLs for traits of economic importance followed by their application in molecular breeding. This study was undertaken to determine the genetic diversity of wild rice accessions collected from Bangladesh and IRRI Philippines.

Molecular markers are better suited for identification of diverse germplasm than conventional methods (Thompson et al., 1998). This study used SSR markers data to analyze genetic diversity and to determine the level of genetic diversity among WR germplasms studied. The PIC value reflects the allelic variation, and frequency exists in the studied germplasm. In this study, the PIC ranged from 0.12 to 0.76, with an average of 0.44, which is consistent with previous PICs ranging from 0.40 to 0.88, averaging 0.71 (Prathepha., 2012) and PICs of 0.28 to 0.72, averaging 0.52 (Singh et al., 2016) which were both considerably higher than this study. Eighteen SSR markers exhibited PIC values above 0.5 with the highest PIC value (0.76) by RM154 and the least by RM237 (0.12), suggesting the highest polymorphism with RM154 and the least polymorphism with R237. Thus, high values of PIC are assumed to be representative of more informative or robustness of the markers (Islam et al., 2025) and lower values of PIC, generally represent a collection of germplasm based on a similar background or closely related types; high values indicate a greater diversity of germplasm (Islam et al., 2018a). The above results indicated that the 18 SSR markers with average PIC value of 0.44 can be a panel marker set for discriminating WR accessions as well as for detection of the potential duplicates among the studied WR accession and RM154 could be used reliably to distinguish wild germplasm accessions of rice.

Out of 68 markers evaluated in this study, 19 unique allelic profiles of 16 SSR marker were found in 11 wild rice accessions. These 19 unique alleles can be useful in accurately identifying these WR genotypes. The identification and use of unique alleles could have significant value in breeding programs (Behera et al., 2012; Thudi and Fakrudin., 2011). In addition, with a varietal improvement breeding program, germplasm with a higher number of unique alleles might represent a potential bank of unique and novel alleles (Singh et al., 2013). Unique alleles were also found in cultivated and wild rice (Wong et al., 2009 and Giarrocco et al., 2007), and the utility of unique alleles were also discussed for DNA fingerprinting by Nagaraju et al. (2002), Jain et al. (2004); Shivapriya and Hittalmani (2006); Das et al. (2013).

The dendrogram constructed based on hierarchical clustering categorized 77 wild rice and relatives' accessions into five main groups (Fig. 2), in which majority of the *Oryza rufipogon* germplasm accessions were grouped into cluster I. This implied shared ancestry of these accessions; however, cluster II demonstrated diversity even among the wild rice and relatives.

STRUCTURE analysis with the greatest log likelihood at K = 3 revealed three major sub-groups (GI, GII and GIII) among the studied wild rice accessions. A similar structure of three sub-groups was noted previously by Roy et al. (2015) in a collection of 107 aromatic rice landraces in India, and three groups were also classified by Islam et al. (2018b) in indica aromatic rice germplasm in Bangladesh. Population structure analyses of various rice diversity panels have reported differing numbers of sub-groups, ranged from 2 to 8 (Garris et al., 2005; Liakat-Ali et al., 2011; Das et al., 2013; Huang et al., 2015; Islam et al., 2021).

STRUCTURE analysis at K = 3 revealed three major genetic groups (GI, GII and GIII), with a substantial proportion of accessions displaying admixed ancestry (Fig. 5). Overall, 24 out of 77 accessions (31.16%) showed admixture (membership coefficient Q < 0.80), indicating historical or ongoing gene flow among wild rice populations. The observed admixture patterns correspond well with the geographic distribution of wild rice accessions across Bangladesh and IRRI collections. Many admixed accessions in GII (approximately 33–38% of accessions) and GIII (where ~25–30% of accessions) originated from ecologically heterogeneous and geographically connected regions, such as the central zone (Gazipur, Tangail), southern coastal belt (Barguna, Bagerhat, Khulna), and floodplain ecosystems, which are characterized by seasonal flooding, tidal influence, and frequent habitat disturbance; indicating weaker genetic differentiation in these groups.

In particular, coastal and lowland wild rice accessions (e.g., from Barguna, Khulna and Bagerhat) showed mixed ancestry between GI and GIII, suggesting secondary contact between genetically differentiated populations adapted to contrasting ecological niches. Similar geographic patterns of admixture have been reported in *Oryza rufipogon* populations from China and Southeast Asia, where river networks, monsoon-driven flooding, and human-mediated landscape changes enhanced gene flow among populations (Gao., 2004; Wang et al., 2008; Liu et al., 2015).

According to the results of this study, 14.28% of wild rice (WR69, WR59, WR66, WR8, WR19, WR18, WR38, WR28, WR21, WR32, WR63) clustered far away from the centroid of the cluster. The remaining 85.72 % of the wild rice accessions clustered around the centroid. Germplasm which clustered together are supposed to have high genetic similarity, whereas those that are found far away from each other are assumed to be divergent (Siddique et al., 2016a and 2016b; Khalequzzaman et al., 2017; Khalequzzaman et al., 2018; Islam et al., 2023; Siddique et al., 2023).

5. Conclusion

The creation and maintenance of genetic diversity in breeding germplasm and genetic stocks are essential to accelerate the response to selection. Wild rice and related taxa are considered as a potential source of resilient genes for genetic improvement of cultivated rice varieties. Genetic studies of wild rice are required to provide information for their proper utilization by hybridization, management and conservation programs. The present research investigated the genetic diversity and population structure of wild rice obtained from the IRRI and BRRI Genebank. SSR-based analysis revealed a high level of genetic diversity among the studied wild rice accessions and their relatives. The divergent genotypes (WR69, WR59, WR66, WR8, WR19, WR18, WR38, WR28, WR21, WR32, WR63) identified in this study can be used in the future rice improvement programs. The findings indicate that SSR markers are effective tools to estimate genetic diversity in wild rice. Owing to their enormous variability, these wild rice germplasms offer strong potential for allele mining and advanced rice breeding. It can be concluded that wild rice germplasm should be conserved both ex-situ and in-situ for future use against abiotic and biotic stresses.

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Declaration of interests

The authors declare that they have no conflicts of interest.

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