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Genetic Diversity and Population Structure in Upland Rice (*Oryza sativa* L.) of Mizoram, North East India as Revealed by Morphological, Biochemical and Molecular Markers

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Abstract

Upland rice landraces from different villages of Mizoram, Northeast India were analyzed for seed morphology, amylose content, aromatic characteristic, seed storage protein profiling and genetic diversity. Results revealed variation in grain length, width, weight and shape. Protein profiling showed polypeptide bands ranging from 7 to 10 with similarity coefficient from 0.556 to 1.000 in the studied populations. Population genetic analysis using simple sequence repeats markers revealed a total of 63 alleles with a high level of gene diversity at 0.6468. High values of Fst and PIC estimates were found at 0.7239 and 0.5984 respectively. The Biruchuk population was found to be the most genetically diverse cultivar and least gene diversity was found in Tuikuk buh. The UPGMA trees based on seed morphology, seed storage protein profiling and simple sequence repeats diversity showed the grouping of rice cultivars into three clusters which were further supported by model-based STRUCTURE analysis. This finding is the first-hand report in upland rice of the state and can be useful for selecting suitable rice lines for prebreeding and germplasm conservation of indigenous hill rice cultivars of Mizoram.

Introduction

Rice (*Oryza sativa* L.) is one of the most important staple food crop for Asia which has most diversified and adopted to a wide range of geographical, ecological and climatic regions (Yadav et al. 2013). The nutritional value of rice crops is determined by

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its types and quantities of metabolites content, which in turn is strongly influenced by environmental and genetic factors (Lemaux 2008).

Local farmers in many regions of the world favor landraces due to better adaptations to microclimate, therefore these varieties are cultivated though long duration as compared to the modern rice varieties (Parzies et al. 2004; Pusadee et al. 2009; Roy et al. 2016). India is very rich with regard to the genetic diversity of rice germplasm that includes indigenous rice varieties, wild rice species, natural hybrids between the cultivar and wild relatives, and in addition the germplasm resources generated from strong and robust breeding programs adopted by the Indian agricultural research system (Rai 1999). It has been documented that West Bengal and North Eastern (NE) States of India consisting Arunachal Pradesh, Assam, Manipur, Meghalaya, Mizoram, Nagaland and Tripura, are home to a large number of indigenous rice varieties and detailed examination of these land races with regard to morphology and genetics is very essential (Das et al. 2013; Choudhury et al. 2013).

The NE India, being the region of origin and one of the zones of domestication of rice, represents a valuable center for the conservation of genetic diversity of rice. It is well noted that rice landraces of this region possess unique traits which can be used for future crop improvement programs. However, information on the molecular characterization of this germplasm is very limited (Choudhury et al. 2014). In addition, it is known that genetic diversity plays a vital role in providing traits responsible for survival and adaptation of a species (Rao and Hodgkin 2002), detailed information and identification of superior varieties becomes essential. Some of the useful qualities identified in these landraces include unique adaptive traits for cold tolerance, flooding and salt tolerance etc. Once these traits are identified, accessed and catalogd, large number of these germplasm could be easily used as a source of genetically important traits for rice improvement programs (Pusadee et al. 2009; Roy et al. 2016).

The Indian state of Mizoram lies within the International boundary of Myanmar in the East and Bangladesh in the South West, and State boundaries of Manipur in the North East, Assam in the North and Tripura in the North West. It lies between $21°56'N$ to $24°31'N$ Latitude and $92°16'E$ to $93°26'E$ Longitude in an area span of 21,087 km^2. Rice, in Mizo language is called '*buh*' and is the main crop for people of Mizoram. The indigenous rice landraces or hill rice of the state are grown in upland areas such as *jhumland* and shifting cultivation sites where farmers directly seed the rice in these traditional farming areas.

The present study was undertaken to investigate seed morphological characters, amylose content, aromatic characteristic, seed protein profiles and genetic diversity using microsatellite or simple sequence repeats (SSR) markers of hill rice of Mizoram, to gain a better understanding on the diversity of indigenous rice cultivars and in turn device conservation strategies and facilitate their effective use for future breeding programs.

Materials and Methods

Collection and Planting

Seeds of indigenous rice cultivars were collected from local farmers from different villages of Mizoram (Fig. 1). *Indica* and *Japonica* varieties were kind gifts from ICAR Kolasib, ABF Hyderabad, and ICGEB New Delhi. A total of 30 indigenous, 5 *indica* and 5 *japonica* cultivars were analyzed in the current study. Seeds were planted on poly pots and grown at Department of Botany, Mizoram University (Table 1).

Estimation of Seed Morphology

Grain quality traits viz. grain length (mm), grain width (mm), 1000-grain weight (g) and grain length/width ratio were measured and recorded from all test entries. A total of five seeds per cultivar in triplicates were used for investigation. Grain quality data were used to construct a dendrogram for genotype diversity with the help of statistical computer software NTSYSpc 2.21 (Rohlf 2009).
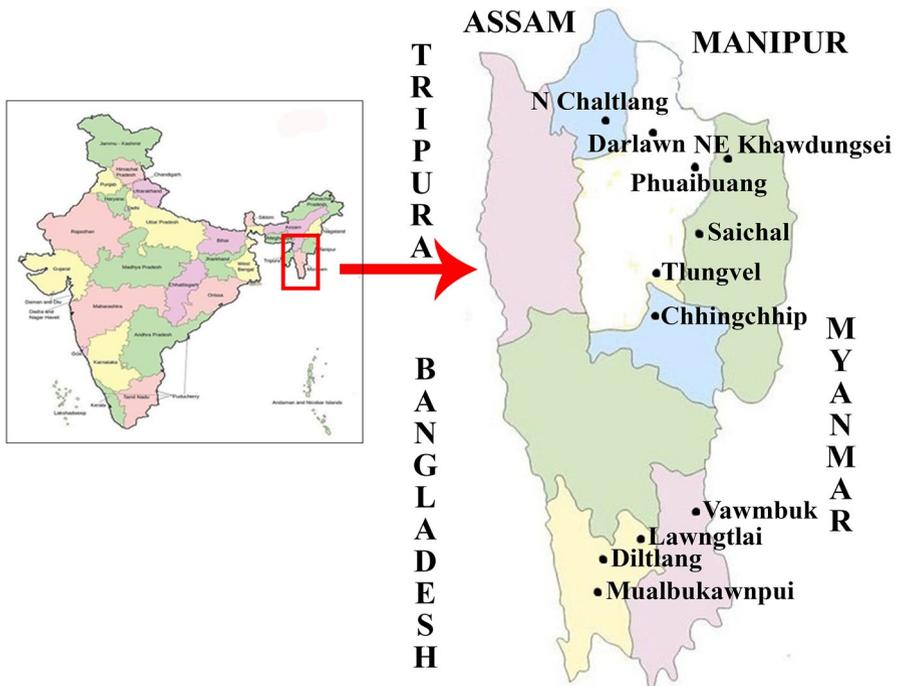

Fig. 1 Political map of India (inset) and Mizoram showing collection sites

Table 1 Details of rice cultivars used in the study

S. no.	Cultivar name	Place of collection	Location	Elevation (ft)	District	Type
1.	Kungtawi sen	Lawngtlai	22º31′42.40″ N 92º53′33.48″ E	2377	Lawngtlai	Landrace
2.	Vaiphei	Lawngtlai	22º31′42.40″ N 92º53′33.48″ E	2377	Lawngtlai	Landrace
3.	Kawnglawng	Diltlang South	22º29′33.48″ N 92º43′32.64″ E	2321	Lawngtlai	Landrace
4.	Biruchuk	Lawngtlai	22º31′42.40″ N 92º53′33.48″ E	2377	Lawngtlai	Landrace
5.	Tuikuk buh	Lawngtlai	22º31′42.40″ N 92º53′33.48″ E	2377	Lawngtlai	Landrace
6.	Fare	Diltlang South	22º29′33.48″ N 92º43′32.64″ E	2321	Lawngtlai	Landrace
7.	Kawnglawng tial	Mualbukawnpui	22º20′00.78″ N 92º42′43.41″ E	1601	Lawngtlai	Landrace
8.	Kawnglawng var	Mualbukawnpui	22º20′00.78″ N 92º42′43.41″ E	1601	Lawngtlai	Landrace
9.	Buhban Langak-thou	Vawmbuk	22º35′52.75″ N 93º04′35.06″ E	4195	Siaha	Landrace
10.	Buhbial	Vawmbuk	22º35′52.75″ N 93º04′35.06″ E	4195	Siaha	Landrace
11	Fazai	Vawmbuk	22º35′52.75″ N 93º04′35.06″ E	4195	Siaha	Landrace
12.	Laithangnu	Darlawn	24º00′51.63″ N 92º55′28.06″ E	3591	Aizawl	Landrace
13.	Tai sanghar	Darlawn	24º00′51.63″ N 92º55′28.06″ E	3591	Aizawl	Landrace
14.	Tai te	Darlawn	24º00′51.63″ N 92º55′28.06″ E	3591	Aizawl	Landrace
15.	Zawngin buh	Darlawn	24º00′51.63″ N 92º55′28.06″ E	3591	Aizawl	Landrace
16.	Baimasa	Phuaibuang	23º55′35.59″ N 93º07′17.46″ E	4571	Aizawl	Landrace
17.	Bialte	NE Khawdungsei	23º58′30.53″ N 93º12′51.77″ E	3802	Champhai	Landrace
18.	Buhban hmui	NE Khawdungsei	23º58′30.53″ N 93º12′51.77″ E	3802	Champhai	Landrace
19.	Buhngat	NE Khawdungsei	23º58′30.53″ N 93º12′51.77″ E	3802	Champhai	Landrace
20	Fazai ban	NE Khawdungsei	23º58′30.53″ N 93º12′51.77″ E	3802	Champhai	Landrace
21.	San	Saichal	23º43′07.92″ N 93º04′05.75″ E	3649	Champhai	Landrace
22.	Idaw	Tlungvel	23º36′17.20″ N 92º51′14.29″ E	3780	Aizawl	Landrace
23.	Mangbuh	Chhingchhip	23º28′15.32″ N 92º51′23.27″ E	3526	Serchhip	Landrace

Table 1 (continued)

S. no.	Cultivar name	Place of collection	Location	Elevation (ft)	District	Type
24.	Buhpui	N Chaltlang	24º01′20.21″ N 92º46′18.44″ E	2668	Kolasib	Landrace
25.	Naga	Tlungvel	23º36′17.20″ N 92º51′14.29″ E	3780	Aizawl	Landrace
26.	Fazu	Saichal	23º43′07.92″ N 93º04′05.75″ E	3649	Champhai	Landrace
27.	Phodum	NE Khawdungsei	23º58′30.53″ N 93º12′51.77″ E	3802	Champhai	Landrace
28.	Vaibuh	NE Khawdungsei	23º58′30.53″ N 93º12′51.77″ E	3802	Champhai	Landrace
29.	Varsiama	NE Khawdungsei	23º58′30.53″ N 93º12′51.77″ E	3802	Champhai	Landrace
30.	Dengchungnunga	NE Khawdungsei	23º58′30.53″ N 93º12′51.77″ E	3802	Champhai	Landrace
31.	BM71[a]	ABF, Hyderabad	17º23′06.16″ N 78º29′12.02″ E	–	Hyderabad	Improved
32.	IR71033–121-15B[a]	ABF, Hyderabad	17º23′06.16″ N 78º29′12.02″ E	–	Hyderabad	Improved
33.	MO1[a]	ABF, Hyderabad	17º23′06.16″ N 78º29′12.02″ E	–	Hyderabad	Improved
34.	PTB33[a]	ABF, Hyderabad	17º23′06.16″ N 78º29′12.02″ E	–	Hyderabad	Improved
35.	TN1[a]	ICGEB, New Delhi	28º31′47.21″ N 77º10′05.37″ E	–	New Delhi	Improved
36.	CAUR1[b]	ICAR, Kolasib	24º12′44.00″ N 92º40′32.69″ E	2057	Kolasib	Improved
37.	Gomati[b]	ICAR, Kolasib	24º12′44.00″ N 92º40′32.69″ E	2057	Kolasib	Improved
38.	RCM9[b]	ICAR, Kolasib	24º12′44.00″ N 92º40′32.69″ E	2057	Kolasib	Improved
39.	RCM10[b]	ICAR, Kolasib	24º12′44.00″ N 92º40′32.69″ E	2057	Kolasib	Improved
40.	RCM13[b]	ICAR, Kolasib	24º12′44.00″ N 92º40′32.69″ E	2057	Kolasib	Improved

[a]Represents *Indica* varieties, [b]Represents *Japonica* varieties.

ABF Agri Biotech Foundation, *ICGEB* International Centre for Genetic Engineering and Biotechnology, *ICAR* Indian Council of Agricultural Research

Amylose Content Analysis

Amylose content was measured by following the method described by Juliano (1971). Rice seeds were ground into a fine powder and 100 mg was placed into a 100 ml volumetric flask. Then, 1 ml of 95% ethanol and 9 ml of 1 M sodium hydroxide were added. The contents were boiled for 10 min. After cooling down to room temperature, the volume was made up to 100 ml with distilled water. A 5 ml solution

was taken into a fresh 100 ml volumetric flask, and 1 ml of 1 M acetic acid and 2 ml of 2% I_2KI solutions were added. The final volume was made up to 100 ml with distilled water. The absorbance was measured at 620 nm. Amylose contents of the rice samples were determined in reference to standard curve and expressed on percent basis.

Aroma Test

Aromatic characteristic of rice cultivars were identified by following the method described by Sood and Siddiq (1978). One gram of rice seed powder was taken and placed in petri dishes with 5 ml of 1.7% KOH solution. After 30 min, the dishes were opened and smelled. The presence (+) or absence (−) of aroma was scored.

Extraction of Seed Storage Protein and SDS-PAGE

Seed storage protein was estimated by following the protocol suggested by Jugran et al. (2010). Where 500 µl of extraction buffer (0.5 M Tris pH 6.8, 20% glycerol, 10% SDS, 0.1% bromophenol blue and 2-mercaptoethanol) was added to 0.5 g of powdered seed and mixed by vortexing for 2 min. The sample was denatured at 100ºC for 5 min and centrifuged at 7000 rpm for 12 min. The resulting supernatant was used to run on a 10% denaturing SDS-PAGE. Sizes of the bands were estimated with reference to Protein Molecular Weight Marker (Genei, India).

SDS-PAGE Data Analysis

Polypeptide bands were scored as present (1) or absent (0) for all the samples analyzed using Image Lab 5.0 (Bio-Rad Laboratories, USA). Based on these scores, Jaccard's similarity coefficient between rice cultivars was calculated and UPGMA (The unweighted pair group method with an arithmetic mean) tree was constructed using open access online software, DendroUPGMA (https://genomes.urv.cat/UPGMA) (Garcia-Vallve and Puigbo 2002).

Genomic DNA Isolation and PCR Amplification

Genomic DNA of indigenous rice cultivars were isolated as per protocol suggested by Edwards et al. 1991. Where, leaflet from a 15-day-old seedlings after sowing was macerated using micro-pestle in a 2 ml centrifuge tube and 400 µl of extraction buffer (200 mM Tris–HCl (pH 7.5), 250 mM NaCl, 25 mM EDTA, 0.5% SDS) was added. The sample was vortex vigorously for 1 min and centrifuged at 13,000 rpm for 5 min. Then, 300 µl of the supernatant was transferred to a fresh tube with equal volume of isopropanol and then centrifuged at 13,000 rpm for 5 min. The resulting pellet was air-dried and dissolved in 100 µl TE (10 mM Tris, 1 mM EDTA) buffer.

Twelve SSR primers (Table 2), which were chosen according to their location on the rice chromosomes, were used to amplify genomic DNA (Panaud et al. 1996; Temnykh et al. 2000). Amplification was performed in an ABI Veriti 96 well

Table 2 Details of SSR primers used in the present study

S. no.	Primer name	Sequences (forward primer/reverse primer)	Chr. no.	Expected size	T_a (ºC)
1.	RM1	Fp: 5′-GCGAAAACACAATGCAAAAA-3' Rp: 5′-GCGTTGGTTGGACCTGAC-3'	1	113	55
2.	RM154	Fp: 5′-ACCCTCTCCGCCTCGCCTCCTC-3' Rp: 5′-CTCCTCCTCCTGCGACCGCTCC-3'	2	183	61
3.	RM131	Fp: 5′-TCCTCCCTCCCTTCGCCCACTG-3' Rp: 5′-CGATGTTCGCCATGGCGTCTCC-3'	4	215	61
4.	RM135	Fp: 5′-CTCTGTCTCCTCCCCCGCGTCG-3' Rp: 5′-TCAGCTTCTGGCCGGCCTCCTC-3'	3	131	55
5.	RM153	Fp: 5′-GCCTCGAGCATCATCATCAG-3' Rp: 5′-ATCAACCTGCACTTGCCTGG-3'	5	201	55
6.	RM190	Fp: 5′-CTTTGTCTATCTCAAGACAC-3' Rp: 5′-TTGCAGATGTTCCTGATG-3'	6	124	55
7.	RM125	Fp: 5′-ATCAGCAGCCATGGCAGCGACC-3' Rp: 5′-AGGGGATCATGTGCCGAAGGCC-3'	7	127	55
8.	RM72	Fp: 5′-CCGGCGATAAAACAATGAG-3' Rp: 5′-GCATCGGTACTAACTAAGGG-3'	8	166	55
9.	RM278	Fp: 5′-GTAGTGAGCCTATCAATAATC-3' Rp: 5′-TCAACTCAGCATCTCTGTCC-3'	9	141	55
10.	RM171	Fp: 5′-CGATCCATTCCTGCTGCTCGCG-3' Rp: 5′-CGCCCCCATGCATGAGAAGACG-3'	10	328	55
11.	RM287	Fp: 5′-TTCCCTGTTAAGAGAGAAATC-3' Rp: 5′-GTGTATTTGGTGAAAGCAAC-3'	11	118	55
12.	RM117	Fp: 5′-CGCCCCCATGCATGAGAAGACG-3' Rp: 5′-CGATCCATTCCTGCTGCTCGCG-3'	12	208	55

Chr. no. chromosome number, T_a annealing temperature

Thermal cycler (ABI, USA) in 25 μl reaction containing 1× PCR buffer, 200 μM dNTP mixture, 3 mM $MgCl_2$, 0.5 U Taq polymerase (Genei, India), 50 ng of each primer and 30 ng template DNA. The amplification conditions were set as, initial denaturation at 94 °C for 5 min, 35 cycles of denaturation at 94 °C for 30 s, annealing temperature (depending on primer) for 30 s, extension at 72 °C for 1 min followed by final extension at 72 °C for 7 min. The amplified products were electrophoresed on 2.5% agarose gel and visualized by standard ethidium bromide staining (Sambrook et al. 2001) in an AlphaImager Mini (Protein Simple, USA). Sizes of amplified bands were ascertained by comparing with molecular weight marker (Genei, India) using Alpha View software (Protein Simple, USA).

Analysis of SSR Data

The total number of alleles, number of polymorphic loci, expected heterozygosity, Nei's gene diversity, Fst and population-wise diversity index were calculated using genetic analysis package POPGENE 1.31 (Yeh et al. 1999). Population-wise diversity index was calculated using Arlequin 3.5 (Excoffier and Lischer 2010). Analysis

of molecular variance (AMOVA) and principal coordinate analysis (PCoA) were performed in GenAlEx 6.5 (Peakall and Smouse 2012). Major allele frequency (MAF) and polymorphism information content (PIC) were calculated using PowerMarker 3.25 (Liu and Muse 2005) and UPGMA tree based on Nei's genetic distance was constructed using MEGA 6 (Tamura et al. 2013). The possible population structure was detected using a Bayesian model-based STRUCTURE 2.3.4 (Pritchard et al. 2000). The parameter was set at 100,000 burn-in periods and 100,000 Markov Chain Monte Carlo (MCMC) repeats after burn-in. A possible number of clusters (K) was determined by setting $K = 1$ to $K = 10$ with 10 replicate runs per K value (Evanno et al. 2005). Online program Structure Harvester (Earl and von Holdt 2012) was used to identify final K value.

Results

Seed Morphology, Amylose Content and Aroma Test

Seeds of the studied rice cultivars showed distinguished variation in its grain quality traits (Table 3). The longest grain length was recorded for Kawnglawng (11.4 mm), whereas the shortest grain length for Zawngin buh (7.16 mm) with mean population grain length of 9.32 mm. On the other hand, grain width was ranged from 2.66 mm (Biruchuk) to 3.82 mm (Buhban langakthou) with an average of 3.17 mm. The highest length/width ratio was observed in Biruchuk (3.77), while the least value was recorded in Buhbial (2.08). The average length/width ratio was found to be 2.96. A 1000-grain weight ranged from Dengchungnunga (22.87g) to Kawnglawng (38.84 g) with an average of 28.33 g. Dendrogram constructed based on grain quality traits using NTSYSpc grouped indigenous rice cultivars into three clusters (Fig. 2). Cluster I comprising 9 cultivars, cluster II was represented by 9 populations and cluster III comprising 12 cultivars.

Amylose content of studied rice cultivars ranged from 9.72% (Fazai ban) to 47.31% (Vaibuh) with an average of 25.97% (Table 3). The amylose content of rice can be classified as waxy (0–2%), very low (3–9%), low (10–19%), intermediate (20–24%), high (25–29%) and very high (> 30%) (Juliano 1971). According to this classification, one cultivar (Fazai ban) possessed very low amylose content, 14 cultivars (Vaiphei, Kawnglawng, Tuikuk buh, Kawnglawng tial, Buhban langakthou, Laithangnu, Tai sanghar, Tai te, Buhban hmui, Idaw, Mangbuh, Naga, Fazu and Dengchungnunga) possessed low amylose content. Three cultivars (Kungtawi sen, Buhngat and San) contained intermediate amylose. Kawnglawng var possessed high amylose content and 10 cultivars (Biruchuk, Fare, Buhbial, Fazai, Zawngin buh, Baimasa, Bialte, Buhpui, Phodum, Vaibuh and Varsiama possessed very high amylose content.

Aroma test was performed for indigenous rice cultivars using 1.7% KOH solution. Eleven out of thirty rice cultivars viz., Kungtawi sen, Kawnglawng, Tuikuk buh, Kawnglawng tial, Kawnglawng var, Laithangnu, Tai sanghar, Tai te, Fazu and Phodum possessed aromatic characteristics (Table 3).

Table 3 Seed morphological features of rice cultivars of Mizoram

S. no.	Name	Grain length (mm)	Grain width (mm)	1000-grain weight (g)	L/W ratio	State[a]	Amylose content (%)	Aroma test[b]
1.	Kungtawi sen	10.14	3	23.08	3.38	ELS	20.38	+
2.	Vaiphei	9.3	3.36	30.12	2.77	LB	16.08	−
3.	Kawnglawng	**11.4**	3.36	**38.84**	3.40	ELS	15.17	+
4.	Biruchuk	10.02	*2.66*	26.92	**3.77**	ELS	36.97	−
5.	Tuikuk buh	10	3.16	26.44	3.16	ELS	13.94	+
6.	Fare	9.14	3.04	27.32	3	ELS	34.91	−
7.	Kawnglawng tial	10.22	3.36	33.09	3.04	ELS	13.77	+
8.	Kawnglawng var	9.12	3.62	30	2.52	LB	28.96	+
9.	Buhban Lan-gakthou	10.6	**3.82**	37.23	2.77	LB	17.24	−
10.	Buhbial	7.28	3.5	26.05	*2.08*	LB	40.28	−
11.	Fazai	9.2	3	27.12	3.06	ELS	30.03	−
12.	Laithangnu	9.68	3.38	28.57	2.86	LB	17.57	+
13.	Tai sanghar	10.46	3.02	27.16	3.46	ELS	13.19	+
14.	Tai te	8.92	2.7	23.25	3.3	ELS	18.56	+
15.	Zawngin buh	*7.16*	3.08	24.91	2.32	LB	41.93	−
16.	Baimasa	9	2.98	26.7	3.02	ELS	37.22	−
17.	Bialte	7.72	3.46	25.42	2.23	LB	36.48	−
18.	Buhban hmui	10.92	2.96	31.23	3.69	ELS	10.96	+
19.	Buhngat	9.1	3.12	29.15	2.92	LB	24.25	−
20.	Fazai ban	10.74	3.06	30.88	3.51	ELS	*9.72*	−
21.	San	9.02	3.04	26.98	2.97	LB	24.01	−
22.	Idaw	10.24	2.98	30.04	3.44	ELS	12.94	−
23.	Mangbuh	8.28	3.02	24.61	2.74	LB	17.32	−
24.	Buhpui	8.3	3.06	25.25	2.71	LB	31.11	−
25.	Naga	9.18	3.16	30.42	2.9	LB	11.71	−
26.	Fazu	9.16	3.56	30.89	2.58	LB	10.05	+
27.	Phodum	9.26	3.12	29.17	2.96	LB	30.37	+
28.	Vaibuh	8.08	3.28	25.98	2.46	LB	**47.30**	−
29.	Varsiama	8.9	3.5	30.11	2.54	LB	30.62	−
30.	Dengchung-nunga	9.06	2.76	*22.87*	3.28	ELS	19.38	−
	Mean	9.32	3.17	28.33	2.96		23.75	
	SE	1.03	0.27	0.68	0.42		1.98	

[a]As referred in Rice Research in India: ICAR Publication, 1985. *ELS* Extra long slender, *LB* Long bold.
[b] + represents presence of aroma and − represents absence of aroma. For each grain quality trait, highest value cell was indicated in bold and lowest value cell was indicated in italics
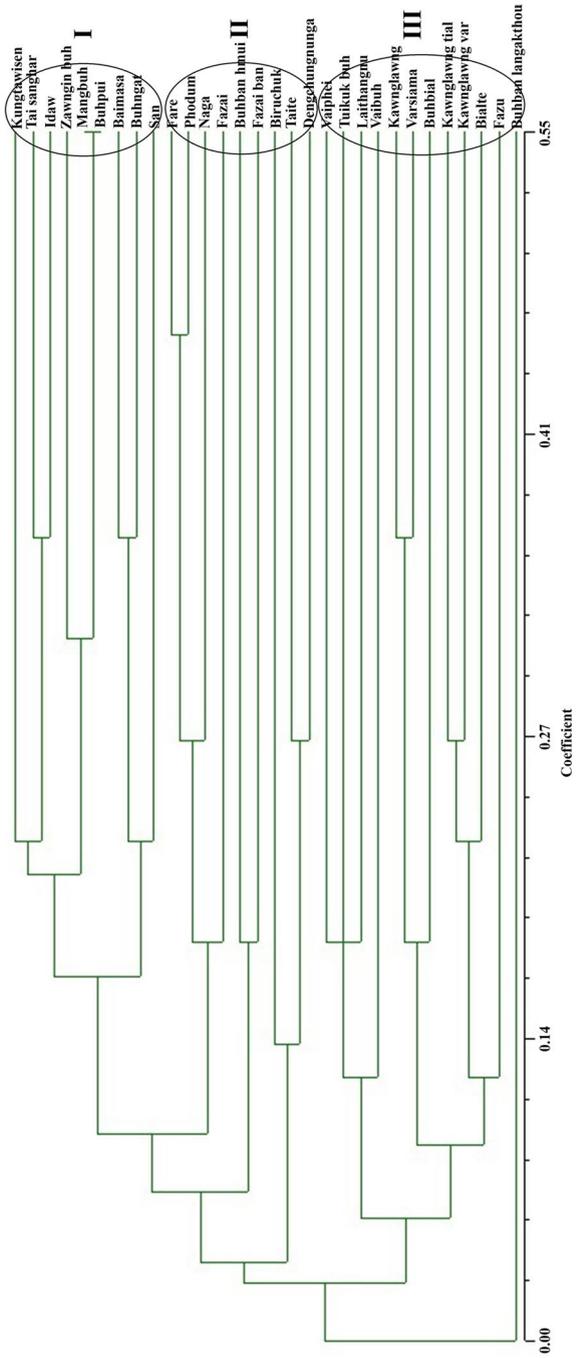
Fig. 2 Dendrogram based on seed morphology of indigenous rice cultivars of Mizoram

Protein Profiling

The SDS-PAGE analysis of seed storage proteins of hill rice cultivars of Mizoram showed little variation among different populations. The number of polypeptide bands per cultivar ranged from 7 to 10. Fourteen cultivars viz., Kawnglawng, Tuikuk buh, Fare, Kawnglawng tial, Buhban Langakthou, Buhbial, Fazai, Laithangnu, Tai sanghar, Zawngin buh, Bialte, Fazai ban, Idaw and Fazu exhibited seven bands, ten cultivars viz., Kungtawi sen, Vaiphei, Kawnglawng var, Tai te, Baimasa, Buhban hmui, San, Buhngat, Vaibuh and Varsiama exhibited 8 bands, five cultivars viz., Buhngat, Mangbuh, Naga, Phodum and Dengchungnunga exhibited 9 bands and only one population, Biruchuk possessed 10 bands. A UPGMA tree constructed based on Jaccard's similarity coefficient using DendroUPGMA showed three clusters. Cluster I was represented by only one cultivar (Biruchuk), cluster II by 9 cultivars and Cluster III comprise of 20 cultivars (Figs. 3, 4).

Analysis of SSR Diversity

Out of 12 simple sequence repeats primers used in the present study, 11 were found to be polymorphic (91.67% polymorphism). A total of 63 bands were detected using these 12 primers and the maximum band (10) was amplified by primer RM135 while the minimum band (1) was generated by RM278 across all the cultivars screened (Table 4). Major allele frequency (MAF) varied from 0.2475 (RM72) to 0.9463 (RM153) with an average of 0.4674 while expected heterozygosity ranged from 0.1210 (RM153) to 0.8320 (RM135) with averages of 0.6468, respectively.
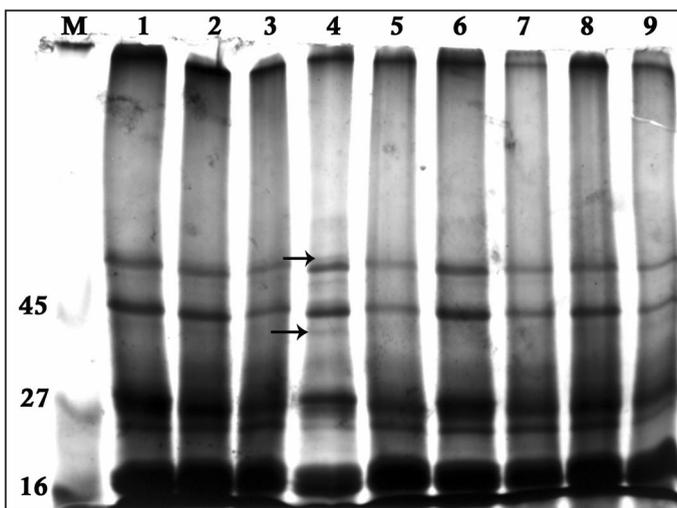

Fig. 3 Protein profile of rice cultivars of Mizoram. (Lanes 1–9 indicated individuals from Kungtawi sen, Vaiphei, Kawnglawng, Biruchuk, Tuikuk buh, Fare, Kawnglawng tial, Kawnglawng var and Buhban langakthou.) Lane M represents protein marker. Arrows indicated polymorphic bands
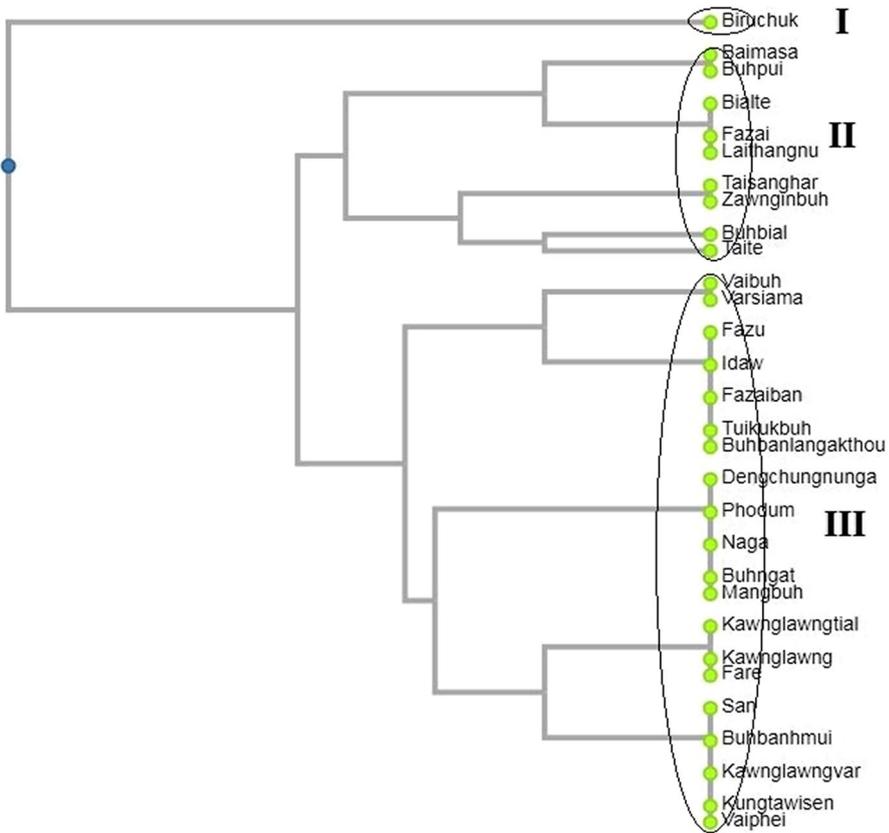
Fig. 4 Dendrogram of indigenous rice cultivars of Mizoram based on seed storage protein

Nei's gene diversity varied from 0.1208 (RM153) to 0.8310 (RM135) with an average of 0.6460. Genetic differentiation (Fst) varied from 0.4400 (RM117) to 0.9344 (RM287) with an average of 0.7239. The polymorphism information content (PIC) value ranged from 0.0973 (RM153) to 0.8134 (RM135) with an average of 0.5984 (Table 5; Fig. 5).

Analysis of Population Structure Based on SSR Data

Model-based grouping method, STRUCTURE, supported genetic structure of the population into three groups with the highest ΔK at $K = 3$ (Fig. 6 and 7). An UPGMA clustering based on genetic distance also grouped the studied cultivars into three clusters. Cluster I represents all *Japonica* check varieties used in the present study and 9 indigenous cultivars—Bialte, Buhngat, Fazai ban, Vaibuh, Varsiama, Buhban hmui, Phodum, Dengchungnunga, and Naga. Cluster II consists of *Indica* varieties and cluster III comprising 21 indigenous cultivars viz., Biruchuk, Kawnglawng, Laithangnu, Fare, Buhban Langakthou, Kawnglawng tial, Tai sanghar, Baimasa,

Table 4 Genetic parameters as revealed by polymorphic SSR primers

Locus	na	ne	MAF	H_E	Nei's	Fst	PIC
RM1	7.0000	4.6881	0.3263	0.7877	0.7867	0.8796	0.7564
RM154	7.0000	3.9412	0.3088	0.7472	0.7463	0.5630	0.7006
RM131	9.0000	4.8522	0.3838	0.7949	0.7939	0.5618	0.7673
RM135	10.0000	5.9157	0.3025	0.8320	0.8310	0.5258	0.8134
RM153	3.0000	1.1374	0.9463	0.1210	0.1208	0.6245	0.0973
RM190	4.0000	3.0936	0.4750	0.6776	0.6768	0.8201	0.6243
RM125	3.0000	1.8891	0.6350	0.4712	0.4707	0.8598	0.3689
RM72	7.0000	5.6286	0.2475	0.8234	0.8223	0.8524	0.7982
RM171	5.0000	3.4649	0.3625	0.7123	0.7114	0.9016	0.6617
RM287	3.0000	2.0420	0.6400	0.5109	0.5103	0.9344	0.4434
RM117	4.0000	2.7467	0.5138	0.6367	0.6359	0.4400	0.5611
Mean	5.6363	3.5817	0.4674	0.6468	0.6460	0.7239	0.5984
SD±	2.7345	1.6753	0.1973	0.2750	0.2747	0.1722	0.2097

na observed number of alleles, *ne* effective number of alleles, *MAF* major allele frequency, H_E expected heterozygosity, *Nei* Nei's gene diversity, *Fst* genetic differentiation, *PIC* polymorphism information content

Mangbuh, Buhpui, Idaw, Fazu, Kungtawi sen, Vaiphei, Tuikuk buh, Kawnglawng var, Taite, Zawngin buh, Buhbial, Fazai and San. G1 in barplot and cluster I in dendrogram were totally similar, G2 cultivars were located in cluster III except *Indica* varieties which formed cluster II, G3 cultivars were present together in cluster III. Analysis of molecular variance (AMOVA) showed that 67% of total variation was due to among-population differentiation and 33% was due to within-individual differentiation. Population-wise diversity indices (Table 4) showed that the expected heterozygosity (or gene diversity) of Biruchuk was highest at 0.3382, followed by Baimasa (0.3346) and Kawnglawng (0.3013) and the least expected heterozygosity was found in Tuikuk buh (0.0443). The average distance between individuals in the same cluster ranged from 0.4019 (cluster III), 0.4549 (cluster II) and 0.4867 (cluster I). The mean Fst value of cluster I was 0.3253 and that of cluster II and cluster III were 0.3357 and 0.4965, respectively. The mean alpha value was found to be 0.0341. Principal coordinates analysis showed three distinct groups among the studied populations which were further confirmed by the STRUCTURE results and UPGMA tree. Coordinate 1 extracted 26.58% of the variation while coordinate 2 extracted 16.46% of the variation (Tables 6, 7; Figs. 8, 9).

Discussion

Grain quality traits of upland rice genotypes of Mizoram were examined and the average grain length and width were found to be 9.32 mm and 3.17 mm, respectively. Seed length of Kawnglawng was the highest among the studied cultivars which is a similar value to an earlier report on Indian rice (Pachauri et al. 2013). As

Table 5 Population-wise diversity indices

| S. no.	Name	H_E	No. of polymorphic loci
1.	Kungtawi sen	0.1228	3
2.	Vaiphei	0.1890	4
3.	Kawnglawng	0.3013	7
4.	Biruchuk	0.3382	7
5.	Tuikuk buh	0.0443	2
6.	Fare	0.2114	4
7.	Kawnglawng tial	0.1987	5
8.	Kawnglawng var	0.0803	5
9.	Buhban Langakthou	0.2101	5
10.	Buhbial	0.0539	2
11	Fazai	0.1811	6
12.	Laithangnu	0.1838	5
13.	Tai sanghar	0.2105	5
14.	Tai te	0.0557	2
15.	Zawngin buh	0.1167	3
16.	Baimasa	0.3346	8
17.	Bialte	0.2487	7
18.	Buhban hmui	0.1627	5
19.	Buhngat	0.2092	6
20	Fazai ban	0.1228	3
21.	San	0.1531	4
22.	Idaw	0.2053	5
23.	Mangbuh	0.2500	6
24.	Buhpui	0.2329	6
25.	Naga	0.1167	4
26.	Fazu	0.1991	6
27.	Phodum	0.1754	5
28.	Vaibuh	0.1259	5
29.	Varsiama	0.1430	4
30.	Dengchungnunga	0.0895	3
31.	BM71	0.1825	4
32.	IR71033-121-15B	0.1846	4
33.	MO1	0.1386	3
34.	PTB33	0.1829	4
35.	TN1	0.2123	5
36.	CAUR1	0.1123	3
37.	Gomati	0.1386	3
38.	RCM9	0.1474	4
39.	RCM10	0.2127	6
40.	RCM13	0.1263	3

H_E expected heterozygosity (Nei 1978)
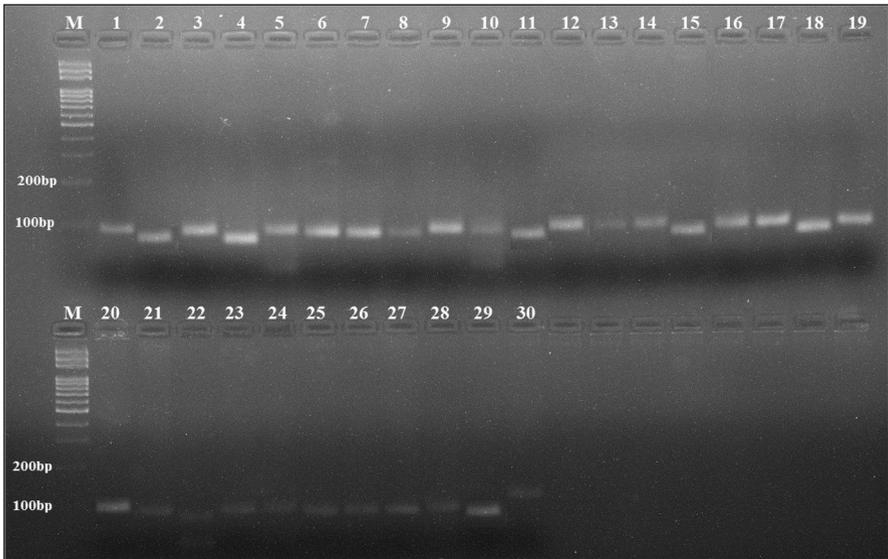
Fig. 5 A 2.5% agarose gel showing banding pattern of Mizoram rice cultivars generated by RM1. M represents 100bp DNA ladder
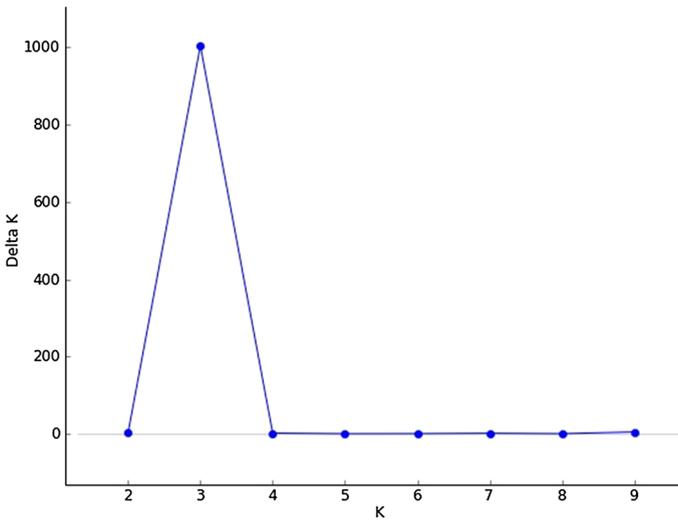

Fig. 6 Relationship between ΔK and K showing a true K for the three groups ($K = 3$)

referred in rice research in India, ICAR publication, rice cultivars can be categorized into short slender, short bold, medium slender, long slender, long bold, basmati type and extra long slender based on grain length and length/width ratio. In the present study, 16 cultivars can be categorized into long bold and 14 cultivars were extra
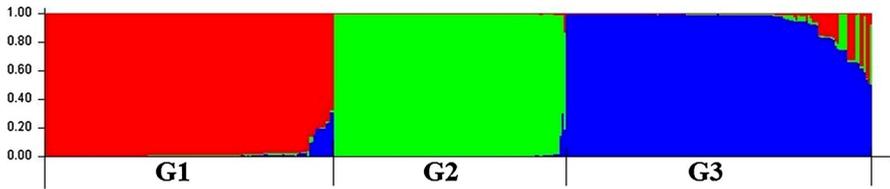
Fig. 7 Population structure of indigenous rice cultivars of Mizoram obtained from STRUCTURE. G1, G2, G3 = Group 1, group 2, group 3. Different groups were represented by different colors

long slender type. It is likely that slender grains have higher market values than the bold ones (Verma et al. 2012) and nearly 50% of the Mizoram rice cultivars fall in slender type. The average L/W ratio was slightly smaller as compared to the previous study (Pachauri et al. 2013). Amylose content is one of the most important grain quality trait (Avaro et al. 2009). Rice cultivars containing low amylose are soft and sticky when cooked, while rice cultivars with high amylose content are dry and less tender when cooked and become hard after cooling (Jain et al. 2012). Rice cultivars with low amylose content found in the present study are also locally known as sticky rice. Aroma characteristic is common in low amylose content cultivars in the present study.

SDS-PAGE analysis showed that the protein bands ranged from 7 to 10 among rice cultivars. Dendrogram constructed based on Jaccard's similarity coefficient using DendroUPGMA showed that more than half of the studied cultivars (66.6%) were grouped into the same cluster which may be indicative of genetic relatedness with respect to seed storage protein profile. While the number of clusters was more compared to earlier studies on rice of Uttarakhand State, India (Jugran et al. 2010) and rice accessions from International Rice Molecular Breeding Programme (Dhawale et al. 2015), Tahir (2014) has indicated that number of clusters might be due to high variation among the cultivars. Although the seed storage protein profiles of intra-species are very similar, the variations in numbers and positions of bands were also reported (Wei-dong et al. 2006; Vithyashini and Wickramasinghe 2015). The similarity banding pattern of seed storage protein made it a unique and powerful tool in evolutionary and diversity studies (Ladizinsky and Hymowitz 1979). Buhban langakthou showed distinct variation among the studied cultivars based on seed quality traits, while Biruchuk showed distinct variation from other cultivars based on protein profile.

Twelve SSR markers, each mapped to a chromosome of the rice genome, detected 63 alleles. The number of alleles per locus ranged from 1 to 10. According to Barker (1994), each locus should exhibit more than four alleles to reduce the standard error and to get a good result in the genetic diversity study. In our study, four loci viz., RM153, RM125, RM278, and RM287 generated less than four alleles but the average alleles per locus were found to be more than four. The mean expected heterozygosity for polymorphic loci found in the present study was a high value of heterozygosity index and showed similarity to a previous study (Roy et al. 2015). PIC determines the usefulness of the markers for linkage analysis (Elston 2005). The

Table 6 Grouping pattern of studied genotypes between barplot obtained from Structure and UPGMA tree constructed using Mega

S. no.	Name	Groups in barplot	Clusters in UPGMA
1.	Bialte	1	I
2.	Buhban hmui	1	I
3.	Buhngat	1	I
4.	Fazai ban	1	I
5.	Naga	1	I
6.	Phodum	1	I
7.	Vaibuh	1	I
8.	Varsiama	1	I
9.	Dengchungnunga	1	I
10.	CAUR1	1	I
11	Gomati	1	I
12.	RCM9	1	I
13.	RCM10	1	I
14.	RCM13	1	I
15.	BM71	2	II
16.	IR71033-121-15B	2	II
17.	MO1	2	II
18.	PTB33	2	II
19.	TN1	2	II
20	Kungtawi sen	2	III
21.	Vaiphei	2	III
22.	Tuikuk buh	2	III
23.	Kawnglawng var	2	III
24.	Buhbial	2	III
25.	Fazai	2	III
26.	Tai te	2	III
27.	Zawngin buh	2	III
28.	San	2	III
29.	Kawnglawng	3	III
30.	Biruchuk	3	III
31.	Fare	3	III
32.	Kawnglawng tial	3	III
33.	Buhban Langakthou	3	III
34.	Laithangnu	3	III
35.	Tai sanghar	3	III
36.	Baimasa	3	III
37.	Idaw	3	III
38.	Mangbuh	3	III
39.	Buhpui	3	III
40.	Fazu	3	III

Table 7 Analysis of molecular variance

Source	df	SS	MS	%
Among populations	39	2075.527	53.222	67
Within individuals	400	520.000	1.300	33

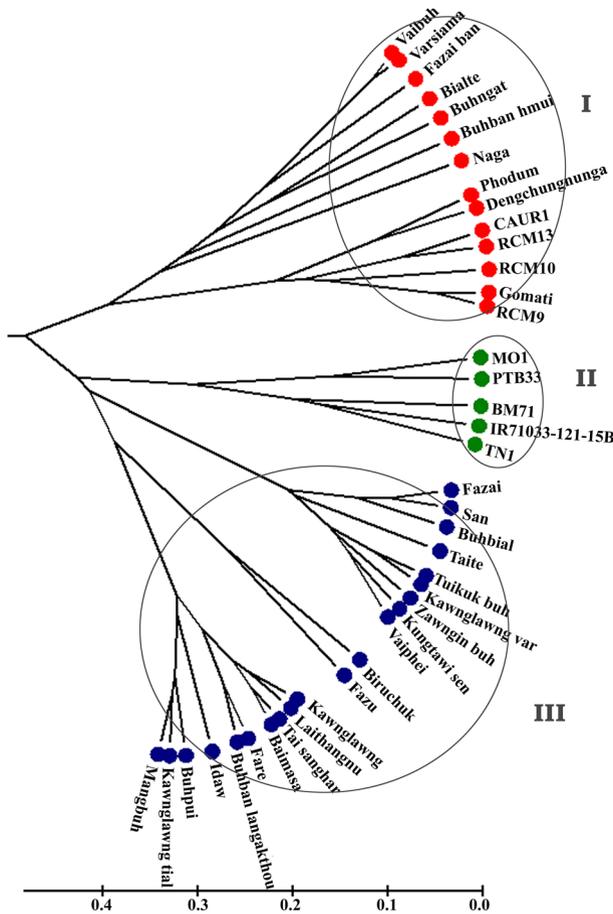*df* degree of freedom, *SS* sum of square, *MS* means of square, *%* percentage variation

Fig. 8 A UPGMA tree of rice cultivars of Mizoram

mean PIC value in this study was higher than previously reported in NE rice cultivars, indicated that the SSR markers used in the current study were good enough for studying genetic diversity. Considering the parameters (Babu et al. 2014) of PIC value (≥ 0.70), expected heterozygosity (≥ 0.71), polymorphic alleles (≥ 6), RM1, RM154, RM131, RM135, and RM72 were found to be the most polymorphic loci among the studied markers.
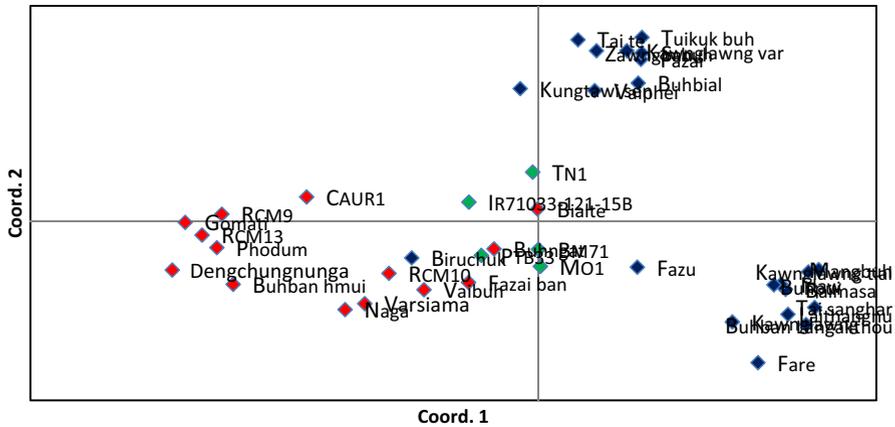
Fig. 9 Principal coordinate analysis (PCoA) of 40 rice cultivars based on Nei's genetic diversity. Group 1, 2 and 3 were indicated by red, green and blue colors, respectively

The ΔK peak at $K = 3$ indicated that all the studied rice cultivars could be divided into three clusters. This result was strongly in agreement with principal coordinates analysis obtained and UPGMA tree. Cluster I represents *Japonica* test cultivars and cluster II represents *Indica* group while cluster III cultivars could be admixed or interchanged between *Japonica* and *Indica*. Choudhury et al. (2013) have also found out that Kawnglawng cultivar was interchanged between *Indica* and *Japonica*. Nine cultivars which are present together in cluster III in dendrogram formed a group with *Indica* varieties in bar plot, represented by same color. Another twelve cultivars in cluster III formed one group different from *Indica* and *Japonica* group in bar plot which may be indicated that these cultivars are admixed. Indigenous rice cultivars of Cluster I were from one village which indicated that clustering in the present study is more or less affected by geographic condition. This geographic clustering was also reported in aromatic and quality rice of North East India (Roy et al. 2015). This may be due to the chance of gene flow within intra-species is higher in geographically close cultivars than far distance. Genetic diversity and levels of gene flow were also influenced by human activities, farming practices, cultivar preferences, etc (Roy et al. 2015). According to Evanno et al. (2005), an alpha value close to zero implies most of the individuals are from one population or another, and an alpha value greater than 1 indicated that most individuals are admixed. The mean value of alpha (the degree of admixture) was 0.0341. According to Wright (1978), Fst values from 0 to 0.05 indicate little genetic differentiation, 0.05 to 0.15 indicate moderate, 0.15 to 0.25 indicate great and more than 0.25 indicate very great genetic differentiation. In this study, the average Fst value of three clusters indicated the existence of great genetic differentiation among clusters. Comparing the three phylogenetic trees (Seed tree = T1, Protein tree = T2, SSR tree = T3) in the present study, mention may be made that seed morphology had the least effect on the genetic relationship since the distribution of rice cultivars in T1 was dissimilar to the other T2 and T3. This could be due to less number of morphometric parameters employed in the current study.

However, T2 and T3 were comparable. For instance, cluster II of T2 possessed nine cultivars which were also present together in a cluster in T3 except one cultivar (Bialte). Similarly, Cluster I of T3 possessed nine indigenous cultivars which were also grouped together in Clusters III of T2. It is cleared from these results that the studies of genetic diversity based on seed storage protein and SSR markers had more or less kinship.

Many farmers of Mizoram practice shifting cultivation or jhuming cultivation and prefer indigenous cultivars, not only rice but also other crops, due to their ability to grow in different local conditions, drought to heavy rain, salinity, etc, easily available and lack of lowland for farming practices. Though shifting cultivation causes environmental issues, the practice serves as a conservation field for indigenous crop cultivars. Previous researchers also pointed out that the upland regions of India represent a valuable center for the conservation of the diversity of indigenous rice varieties (Gayacharan et al. 2015). Landraces of rice may contain considerable genetic diversity and genetically variable traits, thus are good sources for future rice improvement because they are thought to be an intermediate stage between wild rice species and cultivated rice (Choudhury et al. 2013; Li et al. 2014). Knowledge on genetic diversity of these landraces of rice is essential for conservation, utilization and management because it is the basis of the plant breeding (Rao and Hodgkin 2002; Sohrabi et al. 2012).

The high genetic diversity observed in the indigenous rice samples such as Biruchuk, Baimasa, Kawnglawng, etc. may be useful as a resource for future rice improvement or breeding program. Some cultivars exhibited a low level of genetic diversity (Table 4) suggesting necessity action with respect to conservation strategies. The present study is a preliminary report on genetic diversity and grain quality traits of landraces of rice of Mizoram. Although it will be helpful for conservation and utilization, further investigations on agronomy, qualitative and quantitative traits are needed to be undergone to select valuable parental lines for successful future breeding programs.

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