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Original Research Article

Agromorphological And Molecular Characterization of Upland Indigenous Rice (*Oryza sativa* L.) Landraces from Kohima District of Nagaland, India

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Abstract: Rice (*Oryza sativa* L.) is an important food crop for more than three billion people around the world. Indigenous rice landraces possess a huge genetic variability. The present study characterized 12 upland indigenous rice landraces from three Angami villages, namely Rusoma, Mima and Dihoma, in Kohima district of Nagaland, India based on agromorphological traits and used 89 Simple Sequence Repeat (SSR) markers for molecular characterization. The genotypes were raised in April, 2019 with three replications in a randomized block design. Five competitive plants were randomly selected in each replication to record the data. Ward's Minimum Variance dendrogram clustering and descriptive statistics of 15 important agromorphological traits were performed, and 12 rice landraces were grouped into five clusters. Considerable variation was observed among the landraces for all traits studied – for example, panicle number per plant (%CV=19.08), 1000-grain weight (%CV=14), panicle main axis length (%CV=13.54), stem thickness (%CV=13.08) and leaf blade width (%CV=11.48). A total of 287 alleles were detected, and the number of alleles per locus ranged from 2 to 7 with an average of 3.21 alleles per locus. The expected heterozygosity ranged from 0.16 (RM19304) to 0.83 (HVSSR04_18) with an average of 0.57. The average PIC value of 0.53 in this study revealed high genetic diversity in 12 rice landraces studied indicating the potential application of these landraces in rice breeding. The present 12 accessions of indigenous rice landraces from three Angami villages would broaden the existing gene pool and provide an opportunity to perform a strategized upland indigenous rice breeding programme in the region.

Key words: Agronomic traits, genetic diversity, germplasm, jhum, polymorphism, SSR

Introduction

Rice (*Oryza sativa* L.) is an important food crop for more than three billion people around the world (Kumbhar *et al.* 2015). It is one of the most diversified crop species because of its adaptability to a wider range of environment conditions (Chang 1976). Indigenous rice landraces provide a valuable source of novel genes for the genetic improvement of rice (Roy *et al.* 2016; Ashraf and Lokanadan 2017). Limited evaluations of genetic divergence and relationship among these landraces narrow the genetic base in improved varieties and advance breeding lines (Kumbhar *et al.* 2015). Collection, characterization and conservation of landraces to broaden the existing gene pool of rice varieties are necessary for crop improvement programmes.

Agromorphological characterization of rice accessions is important to perform crop improvement programmes (Lin 1991; Patra *et al.* 2016). Agromorphological traits, both

qualitative and quantitative are often used to estimate relationships between genotypes (Goodman 1972; Li *et al*. 2000), and several researchers have employed this technique to assess genetic divergence in rice accessions (Patra and Dhua 2003; Medhabati *et al*. 2013; Joshi *et al*. 2015; Roy *et al*. 2016). Specifically, Bajracharya *et al*. (2006) examined the genetic relationships among 147 upland rice landraces from Nepal using 42 agromorphological characters. However, assessment based on agromorphological traits may not be a reliable measure of genetic diversity due to environmental influence. DNA-based markers technology provides highly efficient and reliable tools for measuring genetic diversity among the genotypes at molecular level (O'Neill *et al*. 2003). Simple sequence repeats (SSR) markers are widely used PCR based markers in rice (Temnykh *et al*. 2001; McCouch *et al*. 2002). They are mostly single locus, co-dominant, easily analysed, cost effective, and can detect high level of allelic diversity (McCouch *et al*. 2002; Garcia *et al*. 2004). SSR markers are class of simple, tandemly repeated nucleotide sequence motifs usually 2.6 bp flanked by highly conserved regions (Chambers and Avoy 2000). SSR markers have been used as the most important tools for the assessment of genetic diversity and unambiguous characterization of plant genotypes (Saini *et al*. 2004; Lapitan *et al*. 2007; Das *et al*. 2013).

The North-eastern region of India is one of the hotspots of rice genetic resources and also the secondary centre of origin of rice (Roy *et al*. 2014). This region harbours at least 10000 indigenous rice landraces cultivated under upland, lowland and deep water conditions (Hore 2005). Nagaland is a hilly state in the North-eastern region of India. Recognised tribes of the state include Garo, Kachari, Kuki, Mikir and Naga (Basic facts Nagaland 2016). Different tribes of this state grow various indigenous rice landraces according to their preferences with respect to colour, taste, aroma and cooking quality. These landraces could be broadly classified into three distinct classes – glutinous rice, brown rice and aromatic rice (Roy *et al*. 2014). An initiative of the Government of Nagaland documented rice landraces cultivated by various tribes in jhum (shifting cultivation) and wet cultivation (Roy *et al*. 2014). Combining ability analysis for yield and yield components in some important upland rice germplasm of Nagaland was carried out through line x tester analysis of 45 intervarietal crosses (Kolom *et al*. 2014). Toshimenla *et al*. (2016) studied genetic divergence in 74 genotypes of upland rice from 11 districts of Nagaland.

Kohima district of Nagaland state is considered as the homeland of Angami Naga, one of 14 Naga tribes of the state (Basic facts Nagaland 2016). Agriculture is the main occupation though significant number of wild biodiversity is foraged from forest and other habitats as source of food. Wet terrace and jhum are the major forms of agricultural systems practiced by Angami Nagas. Traditionally jhum cycle consists of two years of cultivation followed by fallow period of 10-15 years. Farmers hardly use chemical fertilizers and pesticides. They cultivate their heirloom rice landraces chiefly in jhum fields. These landraces are tall, photosensitive, have medium to long duration, are poor yielding, and are susceptible to lodging (Roy *et al*. 2014). However, they hoard a huge genetic variability and resource of biotic and abiotic stress tolerance and/or resistance. They are seeded directly and are grown in rainfed areas. Rice is the staple food of Angami Nagas, supplemented with diverse major and minor dishes collected from farm and wild ecosystems (Singh and Teron 2017). Roy *et al*. (2014) characterized 22 rice accessions from Kohima, while Imsong *et al*. (2015) collected ten rice cultivars known as Kohima special from the Sochunuma area of Dimapur. Toshimenla *et al*. (2016) characterized six upland rice landraces from Kohima based on 12 quantitative traits. Vanlalsanga *et al*. (2019) characterized ten rice landraces from four districts of Nagaland using 22 SSR markers. There is one report of molecular characterization of one rice landrace (suko) from Kohima district of Nagaland (Vanlalsanga *et al*. 2019). But reports of earlier studies revealed scant information on the genetic diversity of upland rice landraces from Kohima district of Nagaland based on molecular characterization. In recent times, local varieties of rice have gradually been replaced with high yielding varieties (HYV) since they are less productive and cannot meet the needs of the supply-chain demand of

the increasing population. Therefore, valuable genetic resources have been gradually eroding from this region. It is imperative to undertake an extensive exploration of the rice landraces of Nagaland state before they are lost. The objective of this study was to characterize 12 upland jhum rice landraces from three Angami villages in Kohima district of Nagaland state based on agromorphological traits. In addition, molecular characterization of the 12 rice accessions was performed by using SSR markers. The generated information will increase the agromorphological diversity and existing gene pool of indigenous rice landraces and contribute to the broadening of the narrow genetic diversity available for rice breeding programmes of the region.

Materials and methods

Collection of germplasm and experimental design

Twelve rice landraces were collected from three Angami villages, namely Rusoma, Dihoma and Mima, during October to December, 2018 (Table 1). Seed samples of each landrace were collected from freshly harvested seed stocks reserved in the farmers' granaries. The experiment was carried out at the research field located at Lerei colony of Kohima Municipality Council, Nagaland. The plot size of 3×3 square feet comprising of 4 rows was maintained with the row to row and plant to plant spacing of 20 cm and 15 cm, respectively. The genotypes were grown in April, 2019 in a randomized block design with three replications consisting of 24 plants per replicated plot. All the agronomic practices were followed to raise good crops as practiced by jhumias (shifting cultivators) in order to achieve the natural performances of genotypes. No synthetic fertilizers were used, although farmyard manure was applied before raising the plants at the rate of 10 tonnes/ha. Five competitive plants were randomly selected in each replication to record the data. The landraces were direct-seeded, grown as rainfed and thus, no artificial irrigation was provided. Pest control measures were not employed but regular manual weeding was carried out. The consent of providing landrace materials and depositing them in gene bank for future utilization in rice breeding programme was obtained from farmers. Germplasm of the 12 indigenous landraces has been deposited in the National Bureau of Plant Genetic Resources, New Delhi, India for long term conservation.

Agromorphological characteristics

Plant morphological traits have been recognized as universal descriptors for Distinctiveness, Uniformity and Stability (DUS) testing and varietal characterization of crop varieties (Joshi *et al.* 2015). Agromorphological characterization of these 12 indigenous rice landraces was conducted following DUS Test Guidelines of the Protection of Plant Varieties and Farmers' Rights Authority, Government of India (2003). Fifty-one agromorphological traits were recorded at different stages of plant growth. The Royal Horticultural Society Colour Chart was used for recording colour and intensity of green and anthocyanin colouration of different parts of the plants. Coleoptile colour (colourless, green, purple) of 20 days old seedlings was recorded. At booting stage, both qualitative and quantitative characteristics were recorded. Qualitative traits included basal leaf sheath colour (green, light purple, purple lines, uniform purple); leaf intensity of green colour (light, medium, dark); presence or absence of anthocyanin colour of leaf and leaf sheath; pubescence of blade surface (absent, weak, medium, strong, very strong); presence or absence of auricle, collar and ligule; anthocyanin colouration of auricles and collar; shape (truncate, acute and split) and colour (white, light purple, purple) of ligule; attitude of culm (erect, semi-erect, open, spreading), whereas quantitative traits considered were length and width of leaf blade. Number of days from sowing to 50% flowering of the plants was also recorded. At the beginning of anthesis, attitude of leaf flag blade and density of pubescence of lemma (absent, weak, medium, strong, very strong) were recorded. Anthocyanin colouration of keel, area below apex of lemma and apex of lemma along with colour of stigma were studied at the halfway of anthesis. During milking stage, thickness of stem, length of stem (from ground level to the first node of panicle) and anthocyanin colouration of nodes and internodes were recorded. Panicle characters (main axis length, curvature, panicle number per plant, exertion, presence or absence of secondary branching, attitude of branches); awn characters (colour, length and distribution); and colour of tip of lemma

and palea were compiled after the terminal spikelets ripened. Colour of sterile lemma was noted when the caryopsis could no longer be dented by thumbnail. The grain characters (weight of 1000 fully developed grains, grain length, width, shape and colour) were collected after harvest.

Diversity analysis

A total of 51 agromorphological traits, *viz*. 14 quantitative and 37 qualitative, were considered for diversity analysis of upland indigenous rice landraces. Normalization of 14 quantitative (continuous) traits was carried out in Microsoft Excel 2016. Twenty-seven out of 37 qualitative/categorical traits were found to be polymorphic. They were dummy-coded with the values of 0 and 1 to avoid any ambiguity and the remaining 10 qualitative traits showing monomorphism were not included in the analysis. The dummy-coded and normalized data were used to access the variability of these rice landraces using Ward's Minimum Variance Cluster analysis which helps in the assessment of the pattern and extent of variation in germplasm (Pachauri *et al*. 2013; Joshi *et al*. 2015). Ward's Minimum Variance dendrogram clustering was performed, and descriptive statistics of important agromorphological traits and Pearson's correlation coefficients for agronomic traits were calculated in Statistical Package for Social Sciences (IBM SPSS version 25).

DNA extraction

For isolation of DNA, individual landraces were planted on polypots at ICAR Research Complex for NEH Region, Manipur Centre, Lamphelpat, Manipur, India. Young leaves of 15-20 days old seedlings from 12 rice genotypes were clipped and stored in -20°C till DNA extraction. Genomic DNA was then extracted using cetyl trimethylammonium bromide (CTAB) method (Doyle and Doyle 1987). The quality of DNA was checked by DNA quantification using Genova Nano Analyzer UV/VIS (Cole Parmer, India).

SSR markers and PCR amplification

Eighty nine polymorphic SSR markers covering all 12 chromosomes were utilized to characterize and assess genetic diversity among 12 rice landraces. The PCR amplification was carried out in 10µl of reaction mixture containing 2µl genomic DNA, 5µl PCR master mix (RR350A-SapphireAmp® Fast PCR Master Mix160 rxns, DSS Takara), 1µl primer (for forward and reverse 0.5µl each) and 2µl nuclease free water. Thermal profiling was set up with initial denaturation temperature of 95°C for 5 min followed by 35 cycles of denaturation (90°C for 30 s), annealing (55°C - 58°C for 30 s) and extension (72°C for 30 s), and a final extension (72°C for 10 min) and 4°C indefinitely for cooling and storage. The amplified PCR products along with a 100 bp DNA marker ladder were electrophoresed in 2% agarose gel prepared in tris-acetate-EDTA (TAE) buffer stained with ethidium bromide (2µl/ 100ml). The gel was run at a constant voltage of 120 V for 2-3 hours and observed on Gel Doc™ XR + imaging system.

Data scoring and analysis

Clear, unambiguous and reproducible bands obtained from all polymorphic markers were considered for data analysis. Each band of a given primer was considered as a unit character. Data were scored with "1" for the presence and "0" for the absence of band. The binary data of SSR fingerprints were used for genetic analyses. As the number and/or position of band(s) in a lane determine the allelic pattern produced by a primer, all bands in a lane were compared across all other lanes (genotypes) (Kumbhar *et al*. 2015). The unweighted pair group method with arithmetic average (UPGMA) dendrogram was constructed using DARwin software (version 6.0.021) based on Jaccard's dissimilarity index (Perrier and Jacquemould-Collet 2006). The polymorphism information content (PIC) for each marker was calculated as described by Botstein *et al*. (1980) with the following formula:

$$PIC = 1 - \sum_{j=1}^{n} p_{ij}^2$$

Where *Pij* is the frequency of *jth* allele for the *ith* marker, and summed over *n* alleles. Total number of alleles, number of effective alleles, number of polymorphic loci, expected heterozygosity, Shannon's information index and Nei's (1978) unbiased genetic identity and genetic distance were calculated using genetic analysis package POPGENE version 1.32 (Yeh *et al*. 1999).

Results

Agromorphological characterization

Out of the 37 visually assessable DUS descriptors studied, 10 were found to be monomorphic, 17 characteristics were dimorphic and 10 characteristics were polymorphic (Table S1). For qualitative traits, the majority of the rice landraces possessed green basal leaf sheath (91.67%), weak pubescence of leaf blade (75%), erect flag leaf (91.67%), medium density of pubescence of lemma (75%), deflexed curvature of main axis of panicle (91.67%), long bold decorticated grain shape (75%) and white decorticated grain (58.34%), whereas, for quantitative traits, most of them possessed broad leaf blade width (83.33%), medium stem length (58.33%), long and very long panicle main axis (41.67% each), high grain weight (91.67%), short grain length (75%), very broad grain width (83.33%) and basmati type decorticated grain length (50%). All the genotypes possessed long leaf blade length, late heading time, thick stem, few panicles per plant, late maturity and broad decorticated grain width.

Descriptive statistics of 15 important agromorphological characteristics are given in Table 2. There was significant variation in the length of awn (%CV=92.30) ranging from 0.60 mm (nhalenya) to 34.93 mm (rüluoo) with a mean value of 11.17 mm. The shortest landrace was thekelha (111.35 cm), and ketei (154.19 cm) was found to be the tallest. Considerable variation was observed among the collected landraces for all traits studied—for example, panicle number per plant (%CV=19.08), 1000-grain weight (%CV=14), panicle main axis length (%CV=13.54), stem thickness (%CV=13.08) and leaf blade width (%CV=11.48). Time of heading (50% of plants with panicles) varied from 144 days (thevomake, ketsielha and ketei) to 176 days (thekelha and pezonya). Grain

Table 1. Rice landraces collected from three Angami villages in Kohima district of Nagaland, India

Name of village	Number of collections	Altitude (m)	Average annual rainfall in mm	Average annual temperature	Name of landraces
Rusoma	9	1200-1500	1706-1915	2°C-32°C	Khathuo, Thevomake, Ketsielha, Rüluoo, Tenhyo, Pezonya, Pelulha1, Pelulha2, Thekelha
Dihoma	1	1450			Nhalenya
Mima	2	700-1450			Ketei, Kekra

Table 2. Agromorphological characteristics of rice landraces

Characteristics	Minimum	Maximum	Mean	Std. Deviation	Coefficient of Variation (%CV)
Length of leaf blade (cm)	70.76	92.36	79.55	6.27	7.88
Width of leaf blade (cm)	1.64	2.46	2.09	0.24	11.48
Time of heading (days)	144.00	176.00	154.92	13.38	8.64
Stem thickness (cm)	0.75	1.28	1.07	0.14	13.08
Stem length (cm)	111.35	154.19	129.54	14.07	10.86
Panicle main axis length (cm)	21.74	36.57	29.47	3.99	13.54
Panicle number per plant	4.07	7.33	5.45	1.04	19.08
Length of longest awn (mm)	0.60	34.93	11.17	10.31	92.30
Time of maturity (days)	157.00	210.00	183.67	15.44	8.41
1000-grain weight (g)	21.78	40.60	34.72	4.86	14
Grain length (mm)	7.28	9.31	8.34	0.58	6.95
Grain width (mm)	2.80	4.29	3.69	0.36	9.76
Decorticated grain length (mm)	5.44	7.18	6.41	0.58	9.05
Decorticated grain width (mm)	2.63	3.64	3.29	0.25	7.60
Decorticated grain: length/breadth	1.66	2.28	1.95	0.19	9.74

length recorded the lowest variation (%CV=6.95) among the traits studied. The variation of grain and kernel morphology of the collected landraces is depicted in Fig. 1.

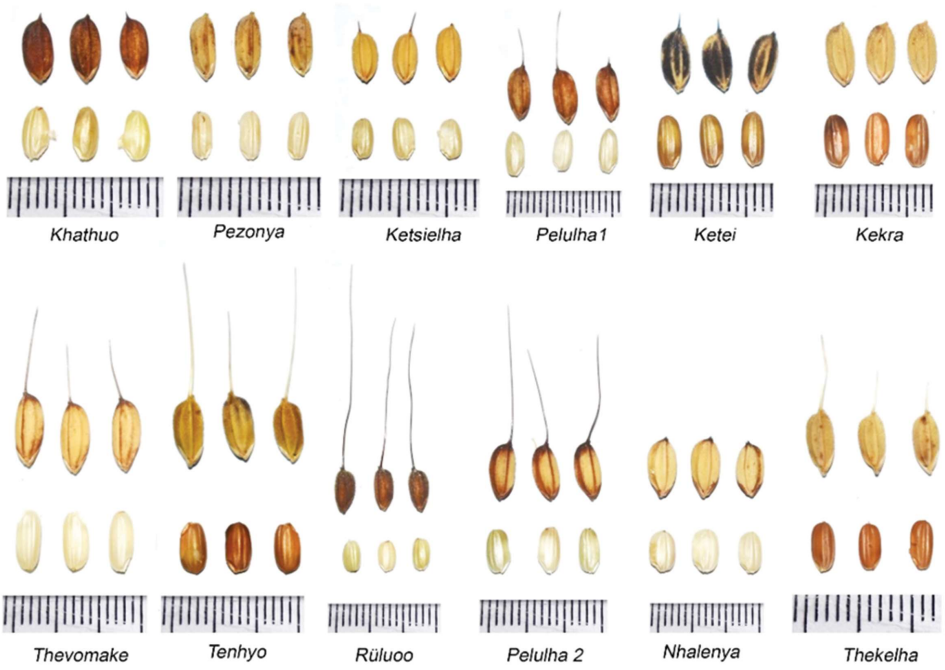

Fig. 1. The variation of grain and kernel morphology of the 12 rice landraces from Kohima district of Nagaland, India.

Pearson's correlations among the agromophological traits are given in Table 3. Positive associations were found between leaf width and grain width; between stem thickness and 1000-grain weight; between panicle number and time of maturity; and between grain length and 1000-grain weight. Negative associations were found between leaf length and grain width; between leaf width and time of heading; and between panicle length and time of maturity. This study revealed significant positive correlations between leaf width and 1000-grain weight; between grain width and 1000-grain weight; and between time of heading and time of maturity.

Ward's Minimum Variance Clustering

Analysis of variance revealed substantial variation/deviation in all the characters studied. The 12 landraces could be grouped into five clusters (Fig. 2). Three clusters, *viz.* I, III and IV consisted of three landraces each. Cluster II had two landraces, while cluster V had only one landrace (ketei). Ketei was the only landrace with black lemma and palea and awn for which it was not grouped with any other landraces. Tenhyo, rüluoo and thekelha had short bold decorticated grain shape, while the remaining landraces had long bold decorticated grain shape. However, rüluoo was grouped in cluster IV in which nhalenya and pelulha2 were included. This can be attributed to decorticated grain colour (white) and purple lemma and palea of rüluoo. The cluster mean values for agromorphological traits are given in Table 4. Cluster V represented the tallest (154.19 cm) landrace with the longest panicle (36.57 cm). Cluster II was characterized by shorter (118.10 cm) and very late maturing (199 days) landraces. Cluster I included landraces which had the longest grain (8.76 mm). Landraces of the thickest stem (1.13 cm), widest grains (3.92 mm), highest panicle number per plant (6.29) and highest 1000-grain weight (38.76 g) were included in cluster III. Landraces with the longest awns (18.38 mm) were integrated into cluster IV. Results on cluster means revealed that the minimum and maximum cluster mean values were distributed in relatively distant clusters.

SSR polymorphism and molecular characterization

Assessment of genetic diversity forms a vital component in germplasm characterization for crop improvement programmes. In this study, 150 SSR markers were screened,

Table 3. Pearson's correlation coefficient among 11 agromorphological traits

Characters	LBL	LBW	TH	ST	SL	PL	PN	TM	GW	GL	GWD
LBL	1	-.159	.203	-.430	.404	.496	-.089	.103	-.482	-.274	-.577*
LBW		1	-.690*	.348	.263	.468	.468	-.602*	.748**	.239	.697*
TH			1	-.402	-.266	-.561	.427	.755**	-.513	-.127	-.319
ST				1	.506	-.071	.371	-.012	.620*	.481	.489
SL					1	.370	.219	.137	.313	.105	.073
PL						1	-.269	-.592*	.229	.249	-.128
PN							1	.601*	.304	.459	.341
TM								1	-.231	.015	-.212
GW									1	.676*	.790**
GL										1	.319
GWD											1

LBL, leaf blade length; LBW, leaf blade wide; TH, time of heading; ST, stem thickness; SL, stem length; PL, panicle main axis length; PN, panicle number per plant; TM, time of maturity; GW, 1000-grain weight; GL, grain length; GWD, grain wide; * Correlation is significant at the 0.05 level; ** Correlation is significant at the 0.01 level.

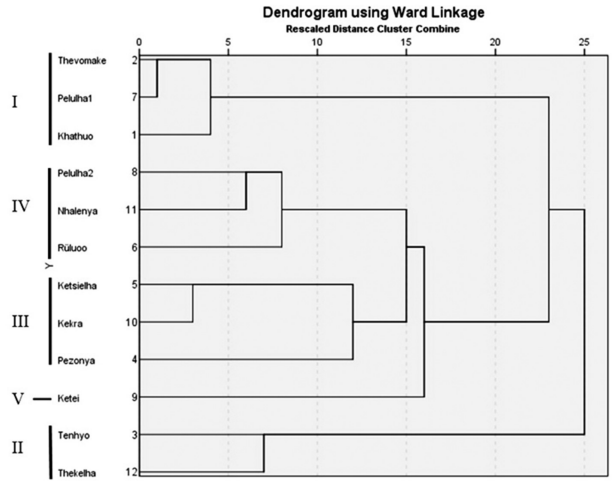

Fig. 2. Grouping of 12 rice landraces using Ward's hierarchical clustering method.

of which 89 SSR markers which displayed clear, unambiguous and reproducible polymorphic bands were selected for analysis of genetic diversity among 12 rice landraces (Table S2). These 89 polymorphic markers detected a total of 287 alleles. The number of alleles per locus ranged from 2 to 7 with an average of 3.21 alleles per locus (Table 5). The SSR marker HVSSR04_18 (Chromosome-4) detected the highest number of alleles (7) which showed its high polymorphic nature, while 25 markers detected two alleles each. The expected heterozygosity ranged from 0.16 (RM19304) to 0.83 (HVSSR04_18) with an average of 0.57, and the average heterozygosity was found to be 0.04. The effective number of alleles varied from 1.18 (RM19304) to 4.76 (HVSSR04_18) with an average of 2.38. The PIC value of SSR markers used in this study ranged from 0.15 (RM19304) to 0.79

Table 4. Cluster mean values for agromorphological traits.

Characteristics	Cluster I	Cluster II	Cluster III	Cluster IV	Cluster V
Leaf: Length of blade (cm)	75	82.09	78.11	79.6	92.36
Leaf: Width of blade (cm)	2.09	1.66	2.15	2.23	2.06
Timing of heading (days)	146.67	175.5	160.67	147.33	144
Stem: Thickness (cm)	1.10	0.89	1.13	1.08	1.10
Stem: Length (cm)	120.40	118.10	136.28	131.34	154.19
Panicle: Length of main axis (cm)	29.96	25.18	29.40	29.56	36.57
Panicle: Number per plant	5.09	5.20	6.29	5.38	4.73
Panicle: Length of longest awn (mm)	6.29	13.97	4.90	18.38	4.86
Time of maturity (days)	174.67	199.50	190.67	178	175
1000 Grain: Weight (g)	36.30	25.99	38.76	35.40	33.22
Grain: Length (mm)	8.76	7.65	8.74	7.94	8.46
Grain: Width (mm)	3.66	3.25	3.92	3.89	3.45
Decorticated grain: Length (mm)	6.90	5.57	6.70	6.13	6.65
Decorticated grain: Width (mm)	3.28	2.92	3.41	3.45	3.24

Table 5. Polymorphism in 12 rice landraces as detected by 89 polymorphic SSR markers.

	Sample size	Na	Ne	Exp Het	Ave Het	I
Mean	21	3.21	2.38	0.57	0.04	0.93
Std. Deviation		1.02	0.77	0.16	0.09	0.32

Na, observed number of alleles; Ne, effective number of alleles; Ext Het, expected heterozygosity; Ave Het, average heterozygosity; I, Shannon's Information index.

(HVSSR04_18) with mean value of 0.53 (Table S2). Shannon's information indices (I) varied between 0.29 (RM19304) and 1.70 (HVSSR04_18) with an average of 0.93 (Table 5). Gel image showing SSR banding profile obtained by four primers (RM3482, RM1387, RM282, RM122) is presented in Fig. 3.

Cluster analysis and genetic divergence

A dendrogram (Fig. 4) based on Jaccard's dissimilarity index was constructed using UPGMA (Umakanth *et al.* 2017). The 12 rice landraces were grouped into two main clusters. Cluster I consisted of one landrace (thekelha), while cluster II had eleven landraces. Moreover, cluster II was sub-divided into two sub-groups, *viz* cluster IIA and cluster IIB. Cluster IIA consisted of four landraces (pezonya, tenhyo, khathuo and thevomake), while cluster IIB had seven landraces (kekra, ketei, nhalenya, pelulha2, pelulha1, ketsielha and rüluoo). The unbiased genetic identity and genetic distances (Nei 1978) among 12 landraces are presented in Table 6.

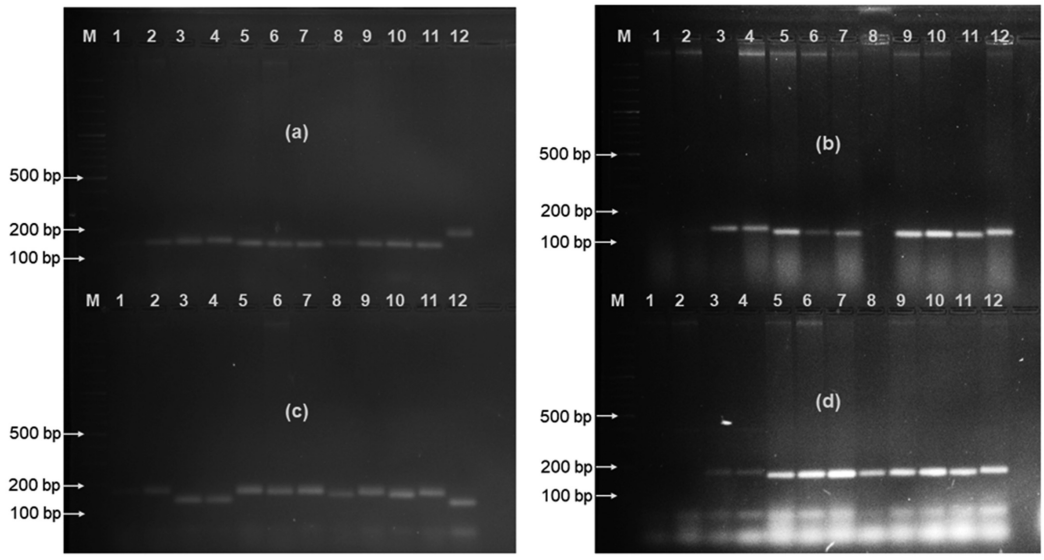

Fig. 3. SSR banding profile obtained by four markers. (a) RM3482, (b) RM1387, (c) RM282, (d) RM122. M represents 100 bp DNA ladder. Lane 1-12 represents rice landraces used in the present study.

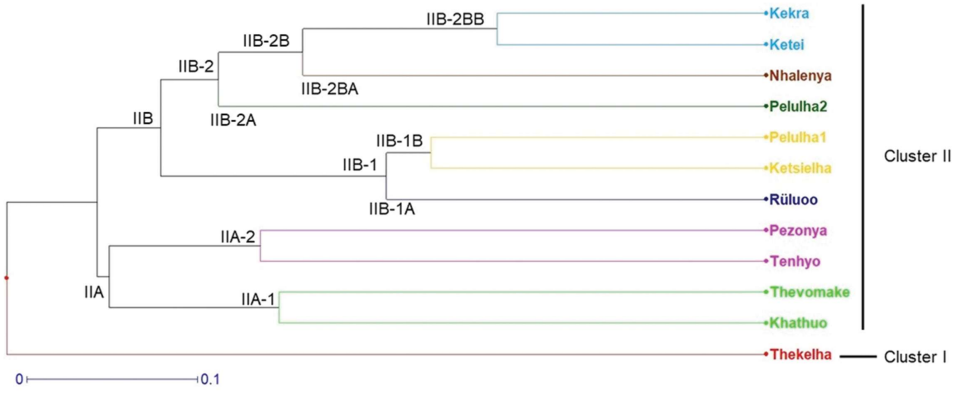

Fig. 4. Dendrogram based on Jaccard's dissimilarity index for grouping 12 rice landraces into two main clusters.

Discussion

The present study revealed 17 dimorphic and 24 polymorphic traits that could establish distinctiveness among the collected rice landraces, while 10 monomorphic traits studied had no role in establishing distinctiveness. Similar attempts for varietal characterization based on agromorphological traits have been earlier reported by various workers (Patra *et al*. 2010; Nascimento *et al*. 2011; Roy *et al*. 2014). Eighteen basmati rice varieties were characterized by using 46 visually assessable agromorphological characters and examined their distinctiveness, uniformity and stability (Patra *et al*. 2010). Characterization of 146 upland rice accessions from Japan based on 28 agromorphological traits recorded higher diversity in panicle number per plant, plant height, leaf length and width, days to heading, panicle main axis length and awn length (Nascimento *et al*. 2011). Assessment of 124 rice landraces from different parts of Nagaland state based on 38 agromorphological traits showed significant variation in panicle number, panicle weight, grains per panicle and grain yield per plant (Roy *et al*. 2014). Characterization of 74 upland rice accessions from Nagaland based on 13 quantitative traits recorded higher diversity in unfilled grains number, yield per plant, panicle weight, filled grains number and 100 seed weight (Toshimenla and Changkija 2013). In the present study, the 12 rice landraces tested exhibited considerable variation in important agronomic traits such as panicle main axis length, panicle number per plant, 1000-grain weight, grain length, grain width, decorticated grain length, decorticated grain width, days to heading and days to maturity. Some studies reported significant positive correlations between leaf width and 1000-grain weight; between grain width and 1000-grain weight; and between time of heading and time of maturity (Roy *et al*. 2014; Chhangte and Devi 2019) which were consistent with the results of this study. Results of the present study suggested that parental lines selected from cluster III (ketsielha, kekra and pezonya) having desirable agronomic traits such as highest panicle number per plant and highest 1000-grain weight and from cluster V (ketei) having the longest panicle main axis length can be selected as potential parents for jhum rice breeding programme in the future. Joshi *et al*. (2015) reported similar results in diversity analysis of 28 rice varieties.

Table 6. Nei's genetic identity (above diagonal) and genetic distance (below diagonal) between populations of rice landraces analysed

P ID	L1	L2	L3	L4	L5	L6	L7	L8	L9	L10	L11	L12
L1	****	0.6037	0.3197	0.2246	0.4013	0.3107	0.3674	0.2444	0.3035	0.2659	0.2630	0.1536
L2	0.5047	****	0.5241	0.4098	0.5161	0.4230	0.4028	0.2383	0.3133	0.2614	0.2376	0.1803
L3	1.1405	0.6460	****	0.6084	0.4328	0.4007	0.3186	0.1759	0.1983	0.1693	0.1871	0.1423
L4	1.4933	0.8921	0.4969	****	0.5453	0.5201	0.4163	0.2360	0.3001	0.2834	0.2318	0.1750
L5	0.9131	0.6615	0.8376	0.6063	****	0.7832	0.7882	0.4348	0.5362	0.4405	0.3767	0.2198
L6	1.1690	0.8604	0.9145	0.6537	0.2444	****	0.7045	0.4020	0.4646	0.4079	0.3500	0.2134
L7	1.0014	0.9094	1.1437	0.8763	0.2380	0.3503	****	0.5738	0.6219	0.5068	0.3675	0.2000
L8	1.4088	1.4342	1.7378	1.4439	0.8328	0.9114	0.5555	****	0.6354	0.5779	0.4507	0.2659
L9	1.1925	1.1607	1.6178	1.2036	0.6232	0.7667	0.4750	0.4535	****	0.8270	0.6150	0.3524
L10	1.3246	1.3416	1.7762	1.2609	0.8199	0.8968	0.6797	0.5484	0.1899	****	0.6448	0.4139
L11	1.3358	1.4373	1.6761	1.4618	0.9764	1.0498	1.0010	0.7969	0.4861	0.4388	****	0.5116
L12	1.8735	1.7129	1.9497	1.7429	1.5151	1.5448	1.6092	1.3247	1.0431	0.8822	0.6702	****

P ID, population identity; L1, khathuo; L2, thevomake; L3, tenhyo; L4, pezonya; L5, ketsielha; L6, rüluoo; L7, pelulha1; L8, pelulha2; L9, ketei; L10, kekra; L11, nhalenya; L12, thekelha.

This study evaluated the level of genetic relationship among 12 upland indigenous rice landraces collected from three Angami villages in Kohima district of Nagaland state. Several workers had shown high genetic diversity in rice cultivars of Northeast India using random markers (Choudhury *et al*. 2013; Das *et al*. 2013; Roy *et al*. 2016). One study of blast resistance in 232 rice landraces of Northeast India reported an average of 153 landraces which showed resistance to leaf blast and 135 landraces resistance to neck

blast, and 91 landraces resistance to both leaf and neck blast (Umakanth *et al*. 2017). In many regions of Northeast India indigenous rice landraces adapt well to dry jhum ecosystems. These landraces may be good candidates to look for these variable traits (Vanlalsanga *et al*. 2019). Evaluation of genetic variation and relationship among these landraces is important for their utilization in future rice breeding programmes. The average number of alleles per locus (3.21) observed in this study was higher than that reported in the study of 12 genotypes assessed by five markers with an average of 2.2 alleles per locus (Prabakaran *et al*. 2010), and slightly lower than that obtained in the study of 50 aromatic rice accessions using 32 SSR markers with an average of 4.4 alleles per locus (Aljumaili *et al*. 2018). Further, some studies reported number of alleles per locus as much as 6.60 to 14.60 (Thomson *et al*. 2007; Jin *et al*. 2010) which was higher than the numbers of alleles per locus recorded in this study. The allelic data generated from the study of 629 rice germplasm using 39 SSR markers with an average of 3.28 alleles per locus (Anandan *et al*. 2016) and from 232 rice landraces assessed by 120 SSR markers with an average of 3 alleles per locus (Umakanth *et al*. 2017) agreed with the results of this study in terms of the average number of alleles per locus (3.21). These variations on the number of alleles per locus can be attributed to differences in rice genotypes, variability of markers used and methods to detect and score PCR products (Pathaichindachote *et al*. 2019).

Expected heterozygosity is the most widely used parameter to estimate genetic variability within populations (Toro and Caballero 2005). PIC corresponds the ability of a marker to detect polymorphism among individuals of a population, and the higher that capacity, the greater its value (Serrote *et al*. 2020). Co-dominant markers like SSR markers with PIC > 0.50 are more recommended to genetic studies, while PIC < 0.25 are not recommended (Botstein *et al*. 1980). Results from this study with the average expected heterozygosity of 0.57 (range of 0.16 to 0.83) and the average PIC of 0.53 (range of 0.15 to 0.79) agreed well with several previous reports (Babu *et al*. 2014; Salem and Sallam 2016; Pathaichindachote *et al*. 2019). Study of 82 rice accessions from different parts of Asian countries using 39 SSR markers reported the average expected heterozygosity of 0.55 (range of 0.02 to 0.80) and the average PIC of 0.50 (range of 0.02 to 0.77) (Babu *et al*. 2014). Characterization of 22 rice genotypes from India, Philippines and Egypt using 23 SSR markers recorded the average expected heterozygosity of 0.62 (range of 0.38 to 0.79) and the average PIC value of 0.57 (range of 0.34 to 0.76) (Salem and Sallam, 2016). The average expected heterozygosity of 0.59 (range of 0.27 to 0.87) and the average PIC value of 0.56 (range of 0.26 to 0.86) were reported in the study of 114 Thai rice accessions and 23 exotic rice accessions using 13 SSR markers (Pathaichindachote *et al*. 2019). Out of 89 polymorphic SSR markers used in this study, 82 markers were considered informative markers, of which 56 were very informative (PIC > 0.5) and 26 were somewhat informative markers (0.25 < PIC < 0.50) (Botstein *et al*. 1980). The results suggested that these 82 markers were suitable to assess the genetic relationships among 12 rice landraces. Higher average values of I (0.93) and PIC (0.53) in this study revealed the high genetic diversity in the 12 rice landraces studied and thus showing a clear correspondence with the substantial variation in these landraces in terms of agromophological traits.

Heterozygosity is one of the parameters to determine the proportion of heterozygous individuals at a locus in populations (Liu and Muse 2005). The present study recorded the average heterozygosity of 0.04 which was higher than the average heterozygosity (0.019) of 82 upland rice landraces from Java Island, Indonesia assessed by 16 SSR markers (Sutoro *et al*. 2015), and slightly lower than that (0.07) of 82 rice accessions using 39 SSR markers (Babu *et al*. 2014). This discrepancy of low level of heterozygosity with an average of 0.04 was likely due to inbreeding status within this rice collection.

Separation of thekelha from other landraces in cluster analysis based on Jaccard's dissimilarity index corresponded well with Ward's Minimum Variance clustering with the exception of tenhyo which was grouped with thekelha. Grouping of thekelha with tenhyo in Ward's hierarchical clustering can be attributed to the presence of similar traits such as shorter stem length and very late maturing. However,

thekelha had lesser degree of genetic identity (0.14 to 0.51) and larger genetic distance (0.67 to 1.94) with other landraces. This showed a clear correspondence with the traditional classification of rice landraces into two groups based on the year of growing in jhum cultivation. Pelulha is a group of rice landraces cultivated in the first year, while thekelha is for second year. This study characterized only one type of thekelha and eleven landraces of pelulha. Unfortunately, no other types of thekelha could be obtained, and the present thekelha studied was found to be very rare since many jhumias discontinue growing this landrace in the second year. Ketei and kekra in cluster IIB were found to have larger genetic distance with other landraces of pelulha (range of 0.43 to1.77) which was likely due to their occurrence in different geographical regions located at an altitude <1000 m above sea level. Nhalenya was genetically more identical with ketei (0.61) and kekra (0.64) than with any other landraces of pelulha. This can be attributed to their adaptation to dry jhum ecosystem in Dihoma and Mima villages where amount of annual rainfall is comparatively less. Khathuo, thevomake, ketsielha, rüluoo, tenhyo, pezonya, pelulha1 and pelulha2 landraces which were collected from Rusoma village showed considerable genetic divergence among each other with the genetic distance ranged from 0.23 to 1.73. The high level of genetic diversity shown by the 12 rice landraces indicated the potential application of these landraces for rice breeding.

The jhum rice landraces cultivated in Kohima district of Nagaland state are generally poor yielding compared with improved HYVs because they are tall, more prone to lodging and photosensitive. However, these landraces possess a huge genetic variability and thus can be a great genetic pool for biotic and abiotic stress tolerance—for example, pest resistance and drought stress tolerance (though pest resistance and drought tolerance traits have not been characterized or targeted in the present study). The landraces are not given any irrigation at any stage of development, and hence rely entirely on rain. In addition, they are grown on hillsides where there is no stagnant water, instead, only run-off water and no proper recommended cultural practices are followed at any growth stage. Despite the lack of any extra measure, these landraces grow well, indicating their tolerance to drought. Since there is no improved HYV suitable for the jhum ecosystem, local farmers have been growing these landraces for years with an average yield of 1.5-2 tonnes/ha which is much lesser than the yield of improved HYV, > 5 tonnes/ha. These indigenous rice cultivars constitute a precious gene pool in terms of resistance/tolerance to biotic and abiotic stresses. This can be taken advantage of for improving landraces to produce better strains that can sustain the adverse impacts of climatic change (Patra *et al*. 2016; Mishra *et al*. 2018). Farmers select and maintain landraces in response to varying ecological, social and cultural conditions to meet their needs (Roy *et al*. 2014). However, indigenous rice landraces are gradually being replaced with the so-called HYV since landraces are less productive and cannot meet the increasing demand for food (Chakravorty and Ghosh 2012; Choudhury *et al*. 2013). This has led to a gradual disappearance of indigenous landraces from farmers' field in just a few decades (Roy *et al*. 2014) and is a significant threat to the wealth of genetic resources accumulated over millennia in this area. Farmers save seeds from their harvest or obtain them from their neighbours for the next growing season. These seeds are selected using traditional methods. Though poor yielding, farmers prefer to grow the indigenous landraces for the obvious reasons of palatability, aroma, soft and sticky nature. Indigenous sticky rice is culturally important as it is used for the preparation of traditional rice beer which constitutes an indispensable ingredient of Angami ritual ceremonies. This may contribute to the conservation of these landraces on-farm.

Conclusions

This study generated valuable information on agromorphological traits of indigenous upland jhum rice landraces. There is a need for further exploration of the genetic diversity of rice landraces among indigenous farmers to develop a rich repository of traits that can contribute to crop improvement programmes. Pezonya and ketsielha landraces having desirable agronomic traits such as highest panicle

number per plant and highest 1000-grain weight, respectively, and ketei landrace having the longest panicle main axis length can be selected as potential parents for jhum rice breeding programme in the future. The average PIC value of 0.53 in this study revealed the high level of genetic diversity in 12 rice landraces studied indicating the potential application of these landraces in rice breeding. Improvement of rice with genes from local landraces will improve production of smallholder farmers who are confronting adverse impacts of climate change. Further, collection of 12 jhum rice landraces from three Angami villages will contribute to the existing gene pool of indigenous landraces of North-eastern hilly regions of India. Farmers persist in growing these landraces in their jhum fields, since no HYV suitable for dry jhum ecosystem is available. The agromorphological traits documented will be immensely useful in carrying out a strategized rice improvement programme for jhum farmers in the region. An identified superior landrace from such adverse environment with desirable agromorphological and grain quality traits may be crossed with semi-dwarf high yielding varieties, thus producing grains with superior quality in the succeeding generations. It would provide the rice breeder with better quality starter crops for carrying out selections and thus giving an opportunity to carry out a well strategized jhum rice breeding programme in the future.

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