RESEARCH ARTICLE

Morphometric, Physico-chemical and Micronutrient Characterization of Rice (*Oryza sativa* L.) Landraces of Sikkim Himalayas

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(Received: 13 September 2017; Revised: 14 May 2019; Accepted: 6 July 2019)

Rice landraces diversity of Sikkim consisting of 51 accessions were studied for morphological, physico-chemical and micronutrient diversity. Analysis of characterization data of 50 DUS characters and 16 physico-chemical traits showed considerable variation among the germplasm. While the landraces Redzomu and Taichung were earliest to flower, Doodhkalam, Taichung, Takmaru (LL), Baelbuty and Anandhi have short stem length and 17 other landraces are strongly aromatic. With regards to important quantitative traits maximum test weight was recorded in Anandhi (33.0 g), head rice recovery in Dudheyjuari and Tulasi (70.6%), kernel length in Kalsati (6.70 mm), grain iron (Fe) in Ramsaree (14.6 ppm) and maximum grain zinc (Zn) content in Chinidhan (30.2 ppm). The Pearson Correlation Coefficient for variables revealed associations among morphological and grain quality parameters. Multivariate Analysis indicated seven principal components (PCs) accounting for 80% of the variability in the rice germplasm and the UPGMA Cluster Analysis grouped the germplasm in to five distinct clusters.

Key Words: DUS characters, Multivariate analysis, Northeastern India, Rice, Sikkim

Introduction

Northeastern region (NE) of India is a biodiversity hotspot rich in crop diversity (Myers et al., 2000). Globally, the region is considered as one of the hot pockets for rice genetic resources and a potential region with extremely diverse conditions for rice cultivation. Traditional cultivars are a storehouse for a number of desirable alleles which are the source material for tailoring genotypes for improved agronomic performance, resilience to stresses and enhancement of quality traits. For large scale adoption of new improved varieties, the best strategy is to breed for improvement of limiting traits without compromising the farmers' preferred traits. For that knowledge regarding the extent of genetic variation and relationships between genotypes is warranted. In this regard, morphological characterization helps in assessment of existing genetic diversity based on which new varieties were developed from germplasm (Sanni et al., 2012; Chakravorty et al., 2013). Rice grain quality is a major factor for varietal adoption by farmers which varies from region to region and is not universal. Although, this crop is a major source of

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nutrients to poor and vulnerable society, yet it doesn't contain sufficient levels of iron and zinc to meet the dietary requirements.

Sikkim is one of the Northeastern states nestled in the western part of East Himalayas and has been recently declared as the first organic state of India. Rice is the principal food crop of the region cultivated over varied altitudinal ranges and approximately covered an area of 11.16 (000' ha) with production and productivity of 20.60 (000' t) and 1815.74 (kg/ha) respectively during 2013-14 (Anonymous). The state is located on the old caravan route connecting India and Tibet and the local rice cultivars were introduced long time back and have established in the region (Subba, 2011). Moreover, the region has been reported to be the centre of differentiation of Indica and Japonica rices (Kihara and Katayama, 1963). Rice landraces of the Himalavan region have been studied and reported by various workers (Agnihotri and Palni, 2007; Rana et al., 2009 and Roy et al., 2016) however, few reports are available for Sikkim (Kihara and Katayama, 1963 and Kapoor et al., 2014). Due to small area of cultivation of landraces in remote and

far-flung inhabitations, collection of all the local rice germplasm is rather difficult. Rice cultivars being grown in the state are adapted to sub-optimal conditions like low temperature, water stress and low manure/fertilizer inputs and knowledge with respect to their superior traits and genetic potential of these landraces remain unexplored. Grouping of cultivars on the basis of their respective traits unravels their strengths and weaknesses and improvement of these local cultivars for limiting traits through different breeding methodologies can be used effectively after proper characterization. As there are no studies carried out on morphological characterization, quality analysis and micronutrient content of rice landraces of Sikkim, hence, the present study has been undertaken to elucidate morphological variablity and grain quality parameters including micronutrient analysis for proper documentation and utilization of these genetic resources in rice improvement.

Material and Methods

Experimental Material

A total of two explorations were conducted during 2013 to collect germplasm samples of local rice landraces from different rice growing areas in all the four districts i.e East, West, North and South Sikkim from where a total of 51 rice accessions were assembled along with passport data containing place of collection, latitude, longitude, altitude, source of collection and special characters of the cultivar (Table 1). Seedlings of each of the 51 entries were planted on a row length of 3m at spacing of 20×20 cm in Augmented Randomized Complete Block Design with four checks namely PD-10, RCPL 123, RCPL 469 and RCPL 473 and raised under organic package of practices under irrigated environment from which five plants/entry were selected for data recording on quantitative and DUS descriptors. The experiment was conducted during kharif season of 2013 and 2014 at the experimental farm of ICAR-National Organic Farming Research Institute (NOFRI), Gangtok situated at 27°19'0''N and 88°36'0''E at an elevation of 1320 msl.

Data Recording

Data on quantitative traits for plant height (PH), days to heading (FL), number of tillers/plant (NOT), panicle length (PL), days to maturity (MAT), 1000 grain weight (SW) and yield/plant (YP) and on descriptors and states consisting of 51 DUS (Distinctiveness, Uniformity and Stability) characters of rice (PPV&FRA, Govt. of India) were recorded on randomly selected five plants. Also,

Indian J. Plant Genet. Resour. 32(3): 327-339 (2019)

recorded were grain quality parameters for 16 traits i.e hulling percentage (HL), milling percentage (ML), HRR (head rice recovery,) KL (Kernel length), KB (Kernel breadth), L/B (length breadth ratio), grain type, grain chalkiness, VER (volume expansion ratio), WU (water uptake), KLAC (kernel length after cooking), ER (elongation ratio), ASV (alkali spreading value), AC (amylose content), GC (gel consistency) and aroma as per standard procedures (IRRI, 1976). While the ASV was estimated following the method of Little *et al.*, 1958 the GC test by the procedure using Cagampang *et al.*, 1973 and the micronutrient analysis (Fe and Zn) by energy dispersive X–ray fluorescence spectrometry (XRF) as per the procedure of Rao *et al.*, 2014.

Statistical Analysis

Analysis of Variance for augmented randomized complete block design was computed (Federer, 1976), Pearson product-moment correlation for measuring relationships among 19 traits, principal component analysis for data reduction and for linear transformation of original variables in to new uncorrelated variables following standard procedures (Wiley, 1981) and cluster analysis for grouping rice germplasm using agglomerative UPGMA hierarchical method (Sneath and Sokal, 1973; Panchen, 1992). The software SAS 9.3 was used for statistical analysis of the data recorded (SAS, 2011).

Results and Discussion

Local rice landraces of Sikkim exhibited considerable variation with respect to plant characters, grain quality parameters and iron and zinc content. Analysis of Variance showed significant differences among the cultivars for plant height, panicle length, number of tillers/ plant, days to 50% heading, days to maturity, 1000 grain weight and yield/plant. Plant height recorded tallest in Tulasi (214.20 cm) while shortest in Doodhkalam (66.0 cm). Bhangeri was the highest tiller bearing cultivar (19.0) followed by Attey (18.0). Krishnabhog and Dhutraj bear long panicles of 29.20 and 28.0 cm respectively while Sanoattey, Taichung and Ramzeera were short panicle bearing cultivars. Heading started at 88th day in Red zomu while it took 131 days for Kalonunia. Earliest maturing cultivars were Redzomu (121 days), Taichung (123 days) and Kalodhan (125 days) while Kalonunia, Krishnabhog, Brihmphool, Khimti and Sanokhamti were last to mature (> 160 days). Highest grain yield per plant recorded in Japani (42.54 g/plant), however, Taichung, Kaleybungey, Dudheyjauri, Pahelodalle and Attey were relatively higher yielding (>30g/plant).

S.No	Landrace	Place of collection	Latitude	Longitude	Altitude
1	Red Zomu	Passingdang, North Sikkim	N 27°33.186'	E 088°28.586'	4882 ft
2	Nepal Dhan	Lower Lingthem, North Sikkim	N 27º31.660'	E 088°30.787'	3519 ft
3	Zomu	Pentong, North Sikkim	N 27°29.258'	E 088°28.913'	5770 ft
4	Takmaru	Pentong, North Sikkim	N 27°29.258'	E 088°28.913'	5770 ft
5	Takmaru LL	Sahgyong, North Sikkim	N 27°24.529'	E 088°33.316'	5341 ft
6	Zokub	Lower Lingthem, North Sikkim	N 27º31.603'	E 088°30.383'	4139 ft
7	Chini Dhan	Upper Lingthem, North Sikkim	N 27°31.381'	E 088°29.970'	4944 ft
8	Taichung	Ramthang, North Sikkim	N 27°24.648'	E 088°33.407'	5469 ft
9	Dharmali	Ramthang, North Sikkim	N 27°24.529'	E 088°33.316'	5341 ft
10	Kalo Dhan	Sahgyong, North Sikkim	N 27°29.258'	E 088°28.913'	5770ft
11	Pahelo Dalle	Yangthang, West Sikkim	N 27º17.366'	E 088º14.015'	4924 ft
12	Tauli	Burmoik (Sumbok), West Sikkim	N 27º15.784'	E 088º15.087'	3675 ft
13	Brihmphool	Toyang, West Sikkim	N 27º16.637'	E 088º14.088'	3438 ft
14	Dudhey Juari	Hee Yangthang, West Sikkim	N 27º16.837'	E 088°14.529'	4769 ft
15	Sijali	Rato Matey, Thamsing, West Sikkim	N 27º14.923'	E 088º14.460'	4283 ft
16	Bhangeri	Sumbok, West Sikkim	N 27º15.559'	E 088º14.435'	3741 ft
17	Ram Saree	Sasbote, West Sikkim	N 27º08.889'	E 088º 12.650'	1559 ft
18	Dhutraj	5 th Mile, Budang, West Sikkim	N 27º07.988'	E 088°13.109'	1814 ft
19	Ram Bhog	East Sikkim	_	_	_
20	Japani	Malbasey, West Sikkim	N 27º08.863'	E 088º12.657'	2387 ft
21	Kalsati	Toyang(GPU, Yangthang), West Sikkim	N 27º16.838'	E 088°14.529'	3195 ft
22	Lal Bacchi	5 th Mile (Budang), West Sikkim	N 27º07.988'	E 088°13.109'	1814 ft
23	Musuli	South Sikkim	_	_	_
24	Katti	8 th Mile (Budang), West Sikkim	N 27º08.889'	E 088°12.650'	2390 ft
25	Marsee	East Sikkim	-	-	_
26	Kataka	Upper Tharpu, West Sikkim	N 27º08.848'	E 088º11.433'	3153 ft
27	Champey	Toyang(GPU, Yangthang), West Sikkim	N 27º16.838'	E 088°14.529'	3195 ft
28	Doodh Kalam	Gerethang, West Sikkim	N 27º18.185'	E 088º13.802'	4450 ft
39	Kaley Bungay	13 th Mile, Sumbog, West Sikkim	N 27º15.361'	E 088°14.557'	4555 ft
30	Khimti	Rinchinpong (Burfok), West Sikkim	N 27º15.873'	E 088º15.732'	2836 ft
31	Timburay	Rungdhu, West Sikkim	N 27º15.781'	E 088º15.086'	2868 ft
32	Phouryal	Lower Martam, West Sikkim	N 27º15.909'	E 088º13.922'	3350 ft
33	Ram Zeera	West Sikkim	-	-	_
34	Sano Khamti	North Sikkim	-	-	_
35	Bael Butty	Barthang, South Sikkim	N 27º15.348'	E 088º15.013'	3014 ft
36	Tulasi	Rungdhu, West Sikkim	N 27º15.827'	E 088°15.149'	2692 ft
37	Chari Masini	Rabitar, South Sikkim	N 27º07.963'	E 088º16.775'	3910 ft
38	Anandhi	South Sikkim	-	-	_
39	Chari Nangrey	South Sikkim	-	-	_
40	Yeidehi	West Sikkim	-	-	_
41	Khamti	Lower Tharpu, West Sikkim	N 27º08.562'	E 088º11.423'	2889 ft
42	Tabrey	Ramthang, North Sikkim	N 27º24.529'	E 088°33.316'	5341 ft
43	Jhapaka	8 th Mile Budang, West Sikkim	N 27°08.889'	E 088º12.650'	2390 ft
44	Attey	Namprik, North Sikkim	N 27°33.186'	E 088°28.586'	4882 ft
45	Zornali	East Sikkim	-	-	_
46	Chirakey	East Sikkim	-	-	_
47	Thulo Attey	Kaluk Rasi, West Sikkim	N 27º13.344'	E 088º14.333'	4527 ft
48	Sano Attey	13 th Mile, Sumbog, West Sikkim	N 27º15.361'	E 088°14.557'	4555 ft
49	Krishna Bhog	5 th Mile Budang, West Sikkim	N 27º08.188'	E 088º13.076'	2151 ft
50	Phool Patta	East Sikkim	-	-	-
51	Kalo Nunia	5 th Mile Budang, West Sikkim	N 27º08.188'	E 088º13.076'	2151 ft

Table 1. Passport data with source and location of rice landraces collected from Sikkim Himalayas

Cultivars like *Tulasi*, *Dhutraj*, *Krishnabhog*, *Rambhog*, *Kataka*, *Marsee*, *Tauli*and *Attey* are preferred by local farmers due to their tall stature which yield high biomass. Roy and Sharma (2014) studied 84 rice landraces and reported a cultivar with maximum 1000 seed weight with 32.04 g and also recorded the earliest maturing cultivar with 97 days.

Characterization for DUS descriptors and states

Frequencies for DUS descriptors and states were worked out in rice germplasm and the same have been shown in Table 2. Various basal leaf sheath color appeared such as green, uniform purple, light purple and with purple lines. Leaf anthocyanin coloration was present in Kalonunia, Pahelo Dalle, Yeidehi and Ramzeera. Anthocyanin coloration was present on tips in most of the genotypes except in Rambhog and Kalonunia in which coloration was on margins. Leaf auricles were absent in Krishnabhog, Rambhog, Phoolpatta, Ramzeera and Musuli. Anthocyanin colour of collar appeared in Timburey and Yeidehi. Flower stigma was yellow in Rambhog, purple in Redzomu, Chinidhan, Timburey and Yeidehi while white coloured in rest of the cultivars. Panicle number per plant was few (<11) in majority of germplasm. Kalodhan, Tabrey, Chinidhan, Takmaru, Kalonunia and Brihmphool bear awned panicles. 1000 grain weight recorded highest in Anandhi (33.0g) however Zomu, Kalsati, Tabrey, Dudheyjuari, Redzomu, Dhutraj, Kataka, Japani, Chirakey and Taichungbear heavy grains (26-30g/1000 grain weight). These can be used as donor parents in hybridization programmes or as recurring parents for improving one or two agronomically inferior traits as most of the landraces lacked characters for high yield. As paddy straw is a limitation in Sikkim, farmers prefer tall cultivars inspite of their low yields as agriculture is basically a subsistence activity and high yield is not a priority for the farmers and indeed breeding local cultivars for high yield without compromising plant height is a challenge for the breeders. Landrace Attey is most widely cultivated in the region having good quality grain with tall plant habit but the long maturity duration needs to be reduced. Likewise, varieties Red zomu and Kalodhan although early maturing are low yielding. Kalonunia, Brihmphool and Krishnabhog are popular as aromatic rice needs improvement in seed yield and reduction in days to maturity. Kalsati having long kernels, medium amylose content and tall habit is a quality rice but needs to be improved for medium maturity duration to best fit in the local cropping system.

Genotypes *Krishnabhog, Kalonunia, Brihmphool, Khimti* and *Charinangrey* which are aromatic and have medium slender grains are best choice for market purpose but need improvement for high grain yield and medium maturity duration. Many rice workers evaluated rice genetic resources using DUS characters and mention may be made of Chakravorty and Ghosh (2012) who used 46 traits following DUS tests to characterize 51 rice landraces. Subba Rao et al., (2013) used 43 traits to characterize 65 rice landraces and Tripathy *et al.*, (2014) who used 23 characters to characterize 91 early duration landraces.

Grain Quality Parameters

There are no universal quality parameters for rice as it varies from region to region. The variability for grain quality parameters in rice landrace germplasm of Sikkim Himalayas has been depicted in Table 4. Head rice recovery is one of the most important traits from milling point of view which is influenced by a number of factors but chalky grains are more prone to breakage during milling. Maximum head rice recovery was recorded in Dudhey Juari and Tulasi (70.6%) and the minimum in Brihmphool (38.3%). Short bold type grains predominate the rice collections. Dhood Kalam and Kalsati bear long slender grains while Krishnabhog, Kalonunia, Brihmphool, Khimti, Charinangrey and Musuli were of medium slender type. Grain chalkiness was absent in Taichung, Ramsaree, Krishnabhog, Kalonunia, Dhood Kalam, Pahelo Dalle and Anandhi. Amylose content strongly influence the cooking and eating characteristics of rice. Rice with high amylose content (25-30%) tends to cook firm and dry, whereas rice with intermediate amylose content (20-25%) is generally soft and sticky whereas with low amylose content (<20%) cooks soft and sticky. Amylose content ranged from 14.9 to 25.87%. Grains of thirty six cultivars contain medium amylose content while thirteen have low amylose content and one contain high amylose. Kernel length after cooking was highest for Kalsati followed by Nepal Dhan and Yeidehi. Strongly scented aroma observed in 17 entries while 10 were medium scented. Rice with gelatinization temperature at the lower end often cooks to a softer texture and retrogrades less than the rice with a gelatinization temperature at the upper end. Gel consistency measures the tendency of the cooked rice to harden on cooling. Red zomu, Kalo Dhan, Taichung, Pahelo Dalle, Phouryal, Timburey, Thulo attey and Anandhi had soft gel consistency

S. No.	Descriptor	Descriptor state (Frequency %)
1.	Coleoptile colour	Colourless (100%)
2.	Basal leaf sheath colour	Green (84%): purple lines (6%): uniform purple (2%): light purple (8%)
3.	Leaf intensity of green color	Medium (94%): light (6%)
4.	Leaf anthocyanin coloration	Present (8%): absent (92%)
5.	Leaf: distribution of anthocyanin coloration	On margins (4%): on tips (96%)
6.	Leaf sheath anthocyanin coloration	Present (12%): absent (88%)
7.	Leaf sheath : intensity of anthocyanin coloration	Medium (4%): weak (6%): very weak (90%)
8.	Leaf pubescence of leaf blade	Weak (36%): absent (24%): medium (30%): strong (10%)
9.	Leaf auricles	Absent (10%): present (90%)
10.	Leaf anthocyanin coloration of auricles	Colorless (56%): light purple (44%)
11.	Leaf collar	Present (100%)
12.	Leaf anthocyanin coloration of collar	Present (4%): absent (96%)
13.	Leafligule	Present (100%)
14.	Shape of ligule	Split (100%)
15.	Leaf: colour of ligule	Light purple (6%): white (94%)
16.	Leaf length of blade	Medium (16%): long (84%)
17.	Leaf width of blade	Narrow (26%): medium (74%)
18.	Culm attitude	Semi-erect (30%): open (2 %): erect (68%)
19.	Time of heading (50% of plants with panicles)	Early (8%): late (40%): medium (52%)
20.	Flag leaf attitude (early observation)	Erect (98%): erect (2%)
21.	Spikelet: density of pubescence of lemma	Strong (22%): very strong (2%): weak (10%): medium (66%)
22.	Male sterility	Absent (100%)
23.	Lemma: anthocyanin coloration of keel	Very strong (2%): strong (2%): weak (4%): absent (92%)
24.	Lemma: anthocyanin coloration of area below apex	Very strong (2%): strong (10%): weak (4%): absent (84%)
25.	Lemma: anthocyanin coloration of apex	Very strong (8%): strong (12%): weak (4%): absent (76%)
26.	Spikelet color of stigma	Purple (8%): yellow (2%): white (90%)
27.	Stem thickness	Thick (24%): thin (4%): medium (72%)
28.	Stem length	Very short (6%): short (4%): medium (42%): long (32%): very long (16%)
29.	Stem anthocyanin colouration of nodes	Present (4%): absent (96%)
30.	Stem anthocyanin colouration on internodes	Absent (100%)
31.	Panicle: length of main axis	Long (32%): medium (68%)
32.	Flag leaf attitude of blade(late observation)	Erect (100%)
33.	Panicle: curvature of main axis	Straight (12%): semi-straight (8%): semi-erect (4%): deflexed (76%)
34.	Panicle number per plant	Medium (2%): few (98%)
35.	Spikelet: colour of tip of lemma	Black (12%): purple (10%): vellow (6%): brown (72%)
36.	Lemma and palea colour	Straw (36%): brown furrows on straw (16%): brown tawny (32%): purple black (8%): brown spots on straw (8%)
37.	Panicle awns	Absent (88%): present (12%)
38.	Panicle: presence of secondary branching	Present (100%)
39.	Panicle secondary branching	Strong (64%): weak (36%)
40.	Panicle attitude of branches	Erect to semierect (22%): semierect (70%): erect (2%): semierect to spreading (6%)
41.	Panicle exertion	Mostly exerted (6%): well exerted (94%)
42.	Time maturity	Medium (8%): late (54%): very late (38%)
43	Leaf senescence	Late (50%): medium (50%)
44.	Sterile lemma colour	Red (10%) ; purple (12%) ; straw (78%)
45.	Grain: weight of 1000 fully developed grains	Low (42%) ; high (6%) ; medium (52%)
46	Grain lenoth	Very short (84%): short (16%)
47	Grain width	Narrow (6%): medium (56%): long (38%)
48	Grain shape	Short hold (72%): medium slender (12%): long hold (12%): long slender (4%)
49	Content of amylose	Low (26%): medium (74%)
50	Gelatinization temperature	Low (94%): medium (6%)
51.	Aroma	Strongly scented (34%): medium scented (22%): non scented (44%)

Table 2. Frequencies for DUS descriptors and states recorded on rice landraces from Sikkim Himalayas

	Character	Cultivars
Time of heading	Early (71 -90 days)	Red zomu, Tabrey, Zomu, Taichung
Stem length	Very short (< 91 cm)	Doodh Kalam, Taichung, Takmaru (lowland)
	Short (91-110 cm)	Bael Buty, Anandhi
1000 grain weight	Very high (> 30g)	Anandhi
	High (26-30 g)	Zomu, Kalsati, Tabrey, Dudhey Juari, Red zomu, Dhutraj, Japani, kataka, Chirakey, Taichung
Stem thickness	Thick (> 0.55 cm)	Krishnabhog, Kataka, Rambhog, Japani, Lal bacchi, Sano attey, Kalsati, Khimti, Ramzeera, Zornalli, Sano khamti
Panicle length	Long (26-30 cm)	Krishnabhog, Tulasi, Dhutraj, Rambhog, Zornalli, Sijali, Tauli, Takmaru lowland, Dudhey juari, Attey, Chirakey, Bhangeri, Japani, Phool patta, Ramsaree, Kataka, Chini dhan, Kaley bungey, Champey, Brihmphool
Panicle: curvature of main axis	Straight	Champey, Khimti, Zomu, Jhapaka, Khamti, Katti,
Kernel length	Long (> 6.60 mm)	Kalsati
	Medium (5.50 mm-6.60 mm)	Doodh kalam, Dhutraj, Japani, Charimasini, Phouryal, Champey, Zornalli, Nepal dhan, Khimti, Anandhi, Kataka, Zomu, Lal bacchi
Maturity	Medium (121-140 days)	Red zomu, Taichung, Kalodhan and Tabrey
Aroma	Strongly scented	Kalo dhan, Tabrey, Ramsaree, Krishnabhog, Rambhog, Kalonunia, Lal bacchi, Chari masini, Pahelo dalle, Tauli, Brihmphool, Kalsati, Khimti, Anandhi, Phool patta, Chari nangrey, Ramzeera

Table 3. Promising rice landraces identified against important traits

(61-100), this is a preferred character as the cooked rice has high degree of tenderness. The time required for cooking milled rice is determined by gelatinization temperature (GT). GT of milled rice is determined by evaluating the alkali spreading value. It correlates positively with the time required to cook rice. High alkali spreading value recorded for most of the cultivars which denotes low gelatinization temperature (55° to 69 °C). Consumers prefer length wise elongation of rice kernels and is influenced by factors like type of genotype, water uptake, amylose content, gelatinization temperature, aging and water uptake. KLAC varies from 7.7 mm in Kaleybungey to 15.5 mm in Kalsati. However elongation ratio recorded highest for Takmaru (lowland type) 5.2 mm. Values of volume expansion ratio were high in Sijali and Dhutraj (5.6 each) while low in Tauli, Lal Bacchi and Attey (4.0). It is a positive character for lower income group farmers for whom quantity is important, however more the volume expansion ratio, less will be energy content per unit volume. Rice with low amylose content which cooks sticky are preferred in Manipur, Nagaland and Mizoram and some parts of Arunachal Pradesh while short slender to medium slender with intermediate amylose content are preferred in Assam, Tripura and Sikkim. Short and bold grains with intermediate amylose content which become fluffy on cooking are mostly preferred in Sikkim. Subudhi et al. (2012) reported that HRR (%) in rice germplasm varied from 43.5% to 68.0% while kernel length after

cooking from 7.9 to 12.5 mm in 41 rice varieties. Bajpai et al. (2012) reported elongation ratio of 1.60-2.33 mm in Kalanamak rice collections. Verma et al. (2015) found variation from 2.0-24.5% in amylose content while grain length after cooking varied from 5.5-12.7 mm. Devi et al. (2008) studied 15 important indigenous rice genotypes from different rice ecosystems of Northeastern India of which majority of rice genotypes were bold and amylose content varied from 2.3-24.5% while Devi et al. (2012) studied rice quality parameters in 18 indigenous rice cultivars of Tripura of which majority of them have low amylose content (< 20%). Aromatic rices have been popular in the state due to their quality traits and premium price in local market. A considerable variation in aromatic rice signifies ample scope of their improvement for reaping more economic benefits. Due to the complex inheritance of rice grain quality traits (Srivastava et al., 2012) initial improvement can be made through pureline breeding through local selection and purification (Singh et al., 2000). However, pedigree selection, backcross and convergent breeding can be employed for isolating superior segregants. Genetic enhancement employing pedigree method along with molecular markers were also employed for improvement of plant stature, aroma and grain quality traits (Patnaik et al., 2015).

Iron and Zinc content

Iron and Zinc content ranged from 6.4 to 14.6 ppm and 13.4 to 30.2 ppm, respectively (Table 5). Highest

Table 4. Variability for grain quality parameters in rice landraces from Sikkim Himalayas

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S No.	andrace	Hulling (%)	Milling %)	HRR (%)	KL mm)	(mm)	J/B (mm)	Grain type	Grain Chalk	VER	ΜŪ	KLAC	ER	ASV	AC	ЭС	Aroma
1	Red Zomu	78.2	61.2	39.2	5 11	2.89	1 76	SB	VOC	41	270	9.8	1 91	5.0	20.26	64	NS
2	Kalo Dhan	82.4	67.5	64.9	5.01	2.54	1.97	SB	OC	4.8	280	9.8	1.95	7.0	22.02	67	SS
3	Zokub	78.7	70.7	70.4	3.73	2.42	1.54	SB	OC	4.2	275	12.0	3.21	7.0	17.77	22	NS
4	Tabrey	80.4	68.4	60.8	4.82	2.69	1.79	SB	VOC	4.6	265	11.0	2.28	7.0	21.13	50	SS
5	ChiniDhan	78.0	67.5	61.7	5.43	2.29	2.37	SB	OC	5.4	260	11.1	2.04	7.0	22.99	45	NS
6	Nepal Dhan	78.0	67.9	62.8	6.01	2.07	2.90	LB	VOC	4 8	370	14.6	2.42	7.0	17.95	43	MS
7	Zomu	76.2	65.0	59.2	5.62	2.63	2.13	SB	VOC	4.2	265	10.7	1.90	4.0	22.88	58	NS
8	Takmaru	76.2	65.0	65.0	4.32	2.65	1.63	SB	OC	4.5	220	8.1	1.87	6.0	21.88	50	NS
9	Takmaru (Lowland type)	79.2	69.3	65.8	2.26	2.50	1.70	SB	OC	4.6	275	11.8	5.22	7.0	24.55	48	NS
10	Taichung	80.8	71.3	68.6	4.56	2.91	1.56	SB	А	4.6	310	8.6	1.88	7.0	17.21	68	NS
11	Jhapaka	78.4	63.7	56.7	5.11	2.36	2.16	SB	OC	5.0	240	10.3	2.01	7.0	23.20	56	NS
12	Khamti	77.6	66.5	60.1	5.19	2.39	2.17	SB	OC	4.8	320	11.8	2.00	7.0	21.23	47	NS
13	Katti	77.2	66.6	66.2	4.12	2.38	1.73	SB	OC	4.1	200	11.6	2.81	7.0	21.53	47	MS
14	Ram Saree	79.7	70.6	70.2	3.82	2.50	1.52	SB	А	4.6	200	8.7	2.27	7.0	20.53	22	SS
15	Dhut Raj	78.0	68.7	65.6	6.51	2.26	2.88	LB	OC	5.6	300	14.2	2.18	7.0	18.94	52	MS
16	Krishnabhog	78.7	67.0	64.5	5.41	2.08	2.60	MS	А	4.6	120	14.2	2.62	6.0	22.46	60	SS
17	Marsee	76.7	67.3	64.3	4.81	2.57	1.87	SB	VOC	4.8	195	10.8	2.24	6.0	18.97	55	NS
18	Kataka	79.6	68.9	66.6	5.69	2.34	2.37	SB	VOC	5.4	290	10.5	1.84	7.0	21.23	22	NS
19	Rambhog	79.2	70.9	70.4	4.39	2.45	1.79	SB	OC	4.2	300	10.6	2.41	7.0	17.68	22	SS
20	Japani	74.8	69.2	66.4	6.33	2.20	2.87	LB	OC	4.8	260	13.4	2.11	7.0	19.27	42	MS
21	Kalonunia	77.7	67.2	63.2	5.46	2.10	2.60	MS	А	5.0	200	12.5	2.28	6.0	21.61	43	SS
22	Lal Bacchi	78.9	67.2	61.2	5.51	2.23	2.47	SB	VOC	4.0	295	12.8	2.32	7.0	20.76	22	SS
23	Chari Masini	79.8	69.2	65.6	6.31	2.22	2.84	LB	VOC	4.4	260	13.8	2.18	7.0	20.97	51	SS
24	Champey	69.8	60.2	53.8	6.02	2.02	2.98	LB	VOC	4.1	305	13.8	2.29	7.0	16.48	45	NS
25	Dhood Kalam	80.0	69.0	62.4	6.57	2.10	3.12	LS	OC	4.8	290	13.9	2.11	6.0	21.26	35	NS
26	Pahelo Dalle	77.8	68.0	66.1	5.17	2.12	2.43	SB	А	4.5	215	14.1	2.72	4.0	14.90	77	SS
27	Tauli	81.3	69.7	63.5	4.87	2.60	1.87	SB	VOC	4.0	305	11.9	2.44	7.0	23.90	58	SS
28	Brihmphool	78.3	60.6	38.3	5.19	1.99	2.60	MS	VOC	4.8	300	11.1	2.13	7.0	23.40	43	SS
29	Dudhey Juari	81.6	71.0	70.6	4.99	2.68	1.86	SB	VOC	5.5	310	11.1	2.22	7.0	22.90	52	NS
30	Kaley Bungey	78.8	68.8	67.9	3.89	2.53	1.53	SB	VOC	4.6	280	7.7	1.97	6.0	20.97	46	MS
31	Bael Butty	80.1	69.0	59.6	5.14	2.34	2.19	SB	OC	4.3	230	11.5	2.23	7.0	21.47	58	NS
32	Sano Attey	80.0	69.4	68.9	3.85	2.33	1.65	SB	VOC	4.6	225	8.8	2.28	7.0	20.70	60	NS
33	Kalsati	76.2	67.8	64.8	6.70	2.17	3.08	LS	VOC	4.6	295	15.5	2.31	6.0	17.68	51	SS
34	Phouryal	79.7	71.3	68.7	6.19	2.26	2.73	LB	VOC	5.4	335	13.8	2.22	7.0	18.24	61	MS
35	Timburey	79.4	65.4	64.7	4.09	2.73	1.49	SB	VOC	4.6	335	8.6	2.10	7.0	23.11	63	MS
36	Thulo Attey	79.5	69.3	65.7	4.75	2.55	1.86	SB	VOC	4.1	315	10.6	2.23	6.0	22.70	61	MS
37	Tulasi	80.9	71.3	70.6	4.21	2.65	1.58	SB	VOC	4.2	300	9.6	2.28	7.0	22.79	50	MS
38	Sijali	78.8	72.9	65.7	4.99	2.73	1.82	SB	VOC	5.6	305	11.2	2.24	7.0	25.87	55	NS
39	Khimti	77.6	66.5	60.1	5.90	2.14	2.75	MS	OC	4.8	320	11.8	2.00	6.0	17.62	53	SS
40	Bhangeri	76.3	65.4	64.8	4.60	2.57	1.78	SB	VOC	4.3	310	10.6	2.30	6.0	20.73	44	NS
41	Anandhi	79.4	65.5	57.8	5.87	2.82	2.08	SB	F/A	4.1	260	12.6	2.14	7.0	18.06	77	SS
42	Phool Patta	77.3	67.3	64.6	4.67	2.51	1.86	SB	VOC	4.2	395	11.7	2.50	7.0	22.82	54	SS
43	Chari Nangrey	78.9	68.8	64.5	5.45	1.93	2.82	MS	VOC	4.6	340	13.8	2.53	7.0	22.29	49	SS
44	Yeidhei	79.0	69.7	66.8	5.39	2.44	2.20	SB	OC	4.6	285	14.3	2.65	7.0	23.46	53	NS
45	Ramzeera	78.0	70.8	70.3	3.91	2.42	1.61	SB	VOC	4.6	305	8.0	2.04	7.0	23.49	54	SS
46	Musuli	78.7	67.3	61.1	5.36	1.94	2.76	MS	VOC	5.5	320	12.5	2.33	7.0	22.61	56	MS
47	Zornalli	79.4	68.8	61.6	6.02	2.27	2.65	LB	OC	4.6	320	13.5	2.24	6.0	21.64	55	NS
48	Chirakey	79.8	69.4	68.8	4.48	2.90	1.54	SB	VOC	4.5	230	9.1	2.03	5.0	20.76	52	NS
49	Sano Khamti	77.0	66.5	60.1	5.10	2.36	2.16	SB	OC	4.5	310	10.7	2.00	7.0	23.05	57	MS
50	Attey	81.8	71.4	68.0	4.65	2.56	1.81	SB	VOC	4.0	275	9.5	2.04	7.0	24.64	58	NS
51	Dharmali	82.0	69.6	63.2	5.88	2.55	2.30	SB	VOC	4.6	300	11.6	1.97	7.0	23.14	50	NS

* SB: Short bold; LB: long bold; MS: medium slender; LB: long bold; LS: long slender; VOC: very occasional; OC: occasional; A: absent; NS: nonscented; SS: strongly scented; MS: medium scented

Table 5. Variability for Iron and Zinc content (ppm) in rice landraces from Sikkim Himalayas

S.No	Landrace	Fe	Zn	S.No	Landrace	Fe	Zn
1	Red Zomu	12.7	25.3	27	Tauli	10.5	21.9
2	Kalo Dhan	9.7	18.0	28	Brihmphool	9.5	21.7
3	Zokub	9.6	20.5	29	DudheyJuari	10.1	19.8
4	Tabrey	8.7	21.5	30	KaleyBungey	10.4	21.0
5	ChiniDhan	10.1	30.2	31	BaelButty	8.8	23.5
6	Nepal Dhan	9.6	20	32	Sano Attey	10.8	22.3
7	Zomu	11.1	25.6	33	Kalsati	6.4	18.9
8	Takmaru	11.4	23.9	34	Phouryal	9.5	19.9
9	Takmaru	9.1	20.4	35	Timburey	10.6	21.3
10	(Lowland type)	12.1	260	26	Thule Atter	10.1	21.0
10	Taichung	12.1	20.8	27	Thulo Alley	10.1	21.9
11	Ларака	10.5	20.7	3/	Tulasi	11.3	23.1
12	Khamti	8.0	19.1	38	Sıjalı	10.8	25.1
13	Katti	12.5	25.5	39	Khimti	10.5	20.6
14	Ram Saree	14.6	21.3	40	Bhangeri	10.8	16.2
15	Dhut Raj	9.9	16.1	41	Anandhi	11.1	19.8
16	Krishnabhog	10.2	13.6	42	Phool Patta	12.5	16.4
17	Marsee	12.7	20.5	43	Chari	13.3	15.3
					Nangrey		
18	Kataka	8.7	22.6	44	Yeidhei	9.2	22.5
19	Rambhog	8.0	14.7	45	Ramzeera	11.6	16.9
20	Japani	9.6	18.1	46	Musuli	12.5	18.4
21	Kalonunia	8.2	13.4	47	Zornalli	9.8	20.2
22	Lal Bacchi	8.4	18.8	48	Chirakey	12.2	26.9
23	Chari Masini	8.6	21.9	49	Sano Khamti	10.4	27.3
24	Champey	7.9	18.0	50	Attey	11.2	23.4
25	Dhood Kalam	8.2	16.8	51	Dharmali	12.3	20.8
26	Pahelo Dalle	9.4	15.4				

iron concentration in grains recorded for Ramsaree (14.6 ppm) followed by Chari Nangrey (13.3 ppm) while highest zinc content in Chini Dhan (30.2 ppm) followed by Sano Khamti (27.3 ppm). Grain iron and zinc content of a genotype depends on various factors such as the nature of grain, soil properties, time of harvest, phloem sap loading and unloading rates and genotype \times environment interaction. Anuradha *et al.*, (2012) analysed brown rice samples of 126 accessions and reported grain iron and zinc concentration ranging from 6.2 ppm -71.7 ppm and 2.6-67.3 ppm, respectively while iron content of 6.6-16.7 ug/g and zinc of 7.1 to 32.4 µg/g in 192 brown rice accessions (Nachimuthu et al., 2014). Biofortification programmes like Harvest plus and All India Coordinated Rice Improvement Project (AICRIP) have fixed 24 ppm as target for zinc in well polished rice grain and varieties having ≥ 24 ppm zinc content are promoted without compromising yield as well as grain quality. Mostly consumers prefer polished rice and percentage losses of iron and zinc during polishing were observed to be as high as 60 to 70% and 20 to

40%, respectively. Considering the above, nine landraces appeared to be promising for zinc content in polished rice, however, their performance in multiple locations within this region needs to be verified through a separate study. Iron content is low in brown rice which might be due to the soil properties as farming in Sikkim is low input and hardly any soil amendment is done.

Correlation Coefficients and Association

Pearson Correlation Coefficient revealed associations among 19 variables (Table 6). Plant height showed positive significant correlation with panicle length (r=0.504). Days to maturity correlated positively with days to heading (r=0.740) along with low degree of association with kernel length, VER and KLAC. Yield/ plant, seed weight and kernel breadth were negatively correlated with days to maturity. Kernel length (r=0.531), kernel breadth (r=0.278), KLAC (r=0.361) and gel consistency were positively correlated with 1000 seed weight. Kernel length showed positive correlation with KLAC while negative association with kernel breadth, elongation ratio and amylose content. Kernel breadth and KLAC were strongly negatively associated (r=-0.644). Head rice recovery showed high significant positive correlation (r=0.80) with milling percentage while negative correlation with kernel length and L/B ratio. Amylose content did not showed high correlation with any of the traits, however moderately positively correlated with hulling percent (r=0.26) and negatively correlated with kernel length (r=-0.29), L/B (r=-0.27) and KLAC (r=-0.32). ASV showed moderate positive correlation (r=0.26) with water uptake and negative correlation with gel consistency. Sinha and Mishra (2013) reported high correlation of 50% heading with maturity time and stem length and also of grain length and grain weight with kernel length and kernel weight respectively. Sharma et al. (2012) reported highest correlation between days to flowering and days to maturity, positive and significant correlation among grain weight, width and length.

PCA and Cluster Analysis

Multivariate analytical techniques are important tools for classifying the germplasm and analyzing genetic relationships among breeding material (Mohammadi and Prasanna, 2003). These statistical algorithms are useful in delineating variability in germplasm and reducing the complex data of number of variables for meaningful inferences. Eigen values of the correlation matrix showed

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Hd	FL	NOT	ΡL	MAT	ΥP	SW	KL	KB	VER	ΜŪ	KLAC	ER	ASV	AC	GC	HL	ML	HRR
Hd	0.236	0.136	0.504*	0.165	-0.059	-0.173	-0.089	0.115	0.066	-0.024	-0.193	-0.215	0.142	0.210	-0.106	0.143	0.227	0.226
FL		0.071	0.045	0.740*	-0.075	-0.393*	0.021	-0.378*	0.122	-0.107	0.071	-0.018	0.371^{*}	-0.003	-0.306*	-0.264	-0.070	0.122
NOT			0.187	0.057	0.277	-0.153	-0.009	-0.056	0.008	0.085	-0.032	-0.046	0.024	0.310*	0.033	-0.104	0.018	0.008
PL				0.062	0.107	-0.015	-0.056	0.039	0.097	-0.013	0.056	0.162	-0.011	0.209	-0.291*	0.045	0.138	0.102
MAT					-0.336*	-0.303*	0.293*	-0.562*	0.288*	-0.129	0.334^{*}	-0.049	0.134	0.049	-0.220	-0.350*	-0.113	-0.019
ΥP						060.0	-0.118	0.141	-0.050	0.262	-0.033	0.146	0.072	-0.117	0.130	0.084	0.293	0.290
SW							0.531^{*}	0.278*	0.096	0.183	0.361^{*}	-0.148	-0.164	-0.177	0.329*	0.018	-0.110	-0.196
KL								-0.474*	0.275	0.162	0.695*	-0.460*	-0.107	-0.318*	0.067	-0.241	-0.196	-0.284*
KB									-0.245	-0.029	-0.644*	-0.112	-0.107	0.184	0.266	0.332*	0.178	0.147
VER										0.094	0.133	-0.134	0.194	0.113	-0.026	0.065	0.172	0.104
WU											0.111	-0.069	0.312^{*}	0.045	-0.034	-0.003	0.048	-0.025
KLAC												0.261	0.011	-0.304*	-0.022	-0.269	-0.059	-0.114
ER													0.118	0.081	-0.122	0.025	0.173	0.155
ASV														0.228	-0.268	0.187	0.229	0.182
AC															0.044	0.317*	0.093	-0.019
GC																0.129	-0.119	-0.143
HL																	0.595*	0.264
ML																		0.791*
HRR																		
* values denc	te significe	me at 5% le	vel															

Table 6. Pearson Correlation Coefficient for 19 characters recorded on rice landraces from Sikkim Himalayas

Table 7. Eigen values of the correlation matrix deduced for 16 variables

	Eigen	Values of the Cor	relation Matrix	
	Eigen Value	Difference	Proportion	Cumulative
1	3.06512862	0.36522576	0.1916	0.1916
2	2.69990286	0.95432561	0.1687	0.3603
3	1.74557725	0.13762996	0.1091	0.4694
4	1.60794729	0.28967657	0.1005	0.5699
5	1.31827071	0.16953303	0.0824	0.6523
6	1.14873768	0.08329421	0.0718	0.7241
7	1.06544348	0.25759360	0.0666	0.7907
8	0.80784987	0.06251302	0.0505	0.8412
9	0.74533685	0.18909656	0.0466	0.8878
10	0.55624028	0.09837642	0.0348	0.9225
11	0.45786386	0.12613647	0.0286	0.9511
12	0.33172739	0.10092477	0.0207	0.9719
13	0.23080263	0.09281185	0.0144	0.9863
14	0.13799077	0.07636523	0.0086	0.9949
15	0.06162554	0.04207063	0.0039	0.9988
16	0.01955492		0.0012	1.0000

that seven principal components (PCs) have Eigen value > 1 which account for approximately 80% of the variability which indicate the contribution of many traits with higher level of correlation to explain the genetic variability in the rice collections (Table 7). First five

PCs were employed for cluster analysis using UPGMA clustering which divided rice accessions in to five clusters (Fig. 1) and the mean values of the clusters have been shown in Table 8. Accessions of cluster I have lowest mean days to heading and maturity along with lowest yield per plant while cluster II members have highest days to heading and maturity. Cluster III comprised of entries having shortest plant height, heaviest grains, longest kernel length and kernel length after elongation while cluster IV have tallest plants, highest number of tillers, longest panicle length, highest water uptake and highest grain yield per plant. Entries of cluster V have lowest 1000 grain weight, short panicle length, shortest kernel length and highest elongation ratio. Cultivars in cluster IV like Attey and Tulasi are preferred due to tall height having good straw yield and relatively high grain yield but prone to lodging and take longer duration to mature. Hybridization of entries in cluster IV with elite high yielding medium to early duration lines/varieties may result in isolating superior segregants. Earliness is a preferable trait of cluster I, however improvement in grain yield can be made by crossing with high yielding varieties which perform better under organic conditions

Table 8. Mean values of 1	6 characters for fiv	e clusters grouped ba	ased on UPGMA
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Character	Cluster I	Cluster II	Cluster III	Cluster IV	Cluster V
Plant height (cm)	135.00	133.84	121.33	149.25	125.83
Days to 50% heading	99.25	116.50	111.55	109.75	113.85
Number of tillers	9.30	11.36	9.76	13.90	9.68
Panicle length (cm)	25.04	25.02	24.95	26.50	24.71
Days to maturity	142.13	164.00	155.09	147.25	154.00
Yield per plant (g)	12.61	14.33	20.38	27.03	17.62
1000 grain weight (g)	24.93	22.74	25.88	22.82	20.44
Kernel length (mm)	4.87	5.34	6.19	4.66	4.29
Kernel breadth (mm)	2.68	2.25	2.24	2.57	2.45
Volume expansion ratio	4.45	5.07	4.65	4.43	4.58
Water uptake	255.00	301.50	299.09	307.50	252.69
Kernel length after elongation (mm)	9.99	11.59	13.81	10.80	10.62
Elongation ratio	2.05	2.11	2.23	2.30	2.58
Alkali spreading value	5.75	6.90	6.73	6.75	6.85
Amylose content (%)	21.33	22.35	19.20	22.78	21.15
Gel consistency	57.13	48.30	48.55	51.88	47.62
	Red zomu, Zomu, Kalodhan, Tabrey, Thulo attey, Takmaru, Marsee, Chirakey (8)	Chinidhan, Kataka, Sijali, Jhapaka, Sanokhamti, Khimti, Khamti, Brihmphool, Charinangrey, Musuli (10)	Nepaldhan, Phouryal, Lalbacchi, Charimasini, Champey, Zornalli, Dhoodkalam, Kalsati, Anandhi, Dhutraj, Japani (11)	Tauli, Kaleybungey Bhangeri, Tulasi, Attey Dudheyjuari, Phoolpatta, Yeidehi (8)	Timburey, Ramzeera, Ramshog, Ramsaree, Baelbutty, Krishnabhog, Kalonunia, Sanoattey, Katti, Taichun Pahelodalle, Takmaru (LL) Zokub (13)



Fig. 1. UPGMA based clustering of rice germplasm based on five principal components

likewise entries in cluster III have better plant type for high yield but needs improvement in number of effective tillers and higher number of filled grains per panicle. Characters having maximum value for PC1 are days to maturity, days to heading and KLAC while kernel breadth contributed negatively towards first PC. Variables highly and positively correlated for PC2 were amylose content and plant height while seed weight and kernel length contributed negatively towards PC2. First component differentiated those accessions having long flowering and maturity duration alongwith minimum kernel breadth, hence PC1 identified mainly the phenological variables for differentiating the accessions while PC2 identified plant height and amylose content along with the grain parameters which contributed most negatively. Ahmed et al. (2016) reported first three PCs for 72.24% of the total variation among 10 agro-morphological traits and obtained seven cluster groups using UPGMA. They also found that first five PCs with vector value >1 for 82.90% of total variation and grouped 31 rice germplasm in to four clusters. These above findings are in line with Caldo *et al.* (1996) who reported maturity, heading and plant height among characters contributed to the parental lines of Philippines rice cultivars while Sharma *et al.* (2012) and Guei *et al.* (2005) reported negative contributions of phenological like days to flowering and maturity characters for PC1.

Maintaining landraces *ex-situ* is one of the best conservation strategies for genetic resources as marginal farmers prioritize reaping maximum production instead of maintaining genetic diversity (Lipton and Longhurst, 1989). The information generated during the present study is worthy of attention and could be utilized for conservation, utilization and improvement of local rice cultivars.

Conclusions

Sikkim is one of the smallest states in India and the occurrence of tremendous variability in rice landrace germplasm within the small geographical area is indeed noteworthy with many of the landraces collected having not been reported in the past. The agro- morphological characterization unraveled important traits of immense value to the breeders having ample scope for improvement of popular landraces like Attey, Kalonunia, Bhrimphool and Krishnabhog for the economic benefit of local farmers. Due to their small population size, these landraces are prone to genetic erosion and oblivion without any benefit to local farmers. Therefore, registration of important landraces with Protection of Plant Varieties and Farmers Rights Authority under Farmers' Variety category by the biodiversity management committees may help the farming communities not only in protecting their varieties but also in accruing some royalties through access and benefit sharing mechanism.

Acknowledgement

The authors deeply acknowledge and thank the Director, ICAR Research Complex for NEH Region, Barapani for providing necessary funds, the Director ICAR- Indian Institute of Rice Research for quality analysis of rice germplasm and farmers of Sikkim and officials of State Agriculture Department for their cooperation during survey.

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