AGRO-MORPHOLOGICAL CHARACTERIZATION AND DIVERSITY ASSESSMENT OF RICE LANDRACES IN NEPAL

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ABSTRACT

Plant genetic resources are raw materials and their use in breeding is one of the most sustainable ways to conserve agro-biodiversity. Field research was conducted at National Agriculture Genetic Resources Centre (NAGRC) during 2018 & 2019 with the objective of agro-morphological characterization and diversity assessment of rice landraces. Forty-two landraces collected from 21 districts of Nepal were characterized and evaluated by using non replicated row design. The phenotypic diversity was assessed based on fourteen qualitative and thirteen quantitative characters following the descriptors developed by Bioversity International, IRRI and WARDA. Basic statistics were calculated by using Excel 2016 and UPGMA clustering and PCA was done with MINITAB-17. The diversity index (H') and coefficient of variation for different traits ranged from 0.16-0.96 and 8.00-47.77 respectively. Clustering grouped the landraces into four clusters with minimum similarity level of 46.33%. Landraces of cluster three i.e. NGRC01917, NGRC03034, NGRC03395, NGRC03057, NGRC03163 are found to be superior in terms of maturity days and yield. PCA partitioned the total variation into three principal components contributing 75.5% of the cumulative variance. Thus, the present study is the preliminary picture for characterization and diversity analysis in Nepalese rice landraces that can be used by the breeder in rice improvement program.

Keywords: Agro-biodiversity, Characterization, PCA, Phenotypic, Trait

INTRODUCTION

Rice (*Oryza sativa* L.) belonging to the family Poaceae is one of the most important food crops grown worldwide. The Sativa rice species found in Asia, America, and Europe are commonly grouped into three sub-species named Indica, Japonica, and Javanica. Rice is believed to originated and been cultivated in tropical Asia, the oldest record dating 5000 years BC (Kandel & Shrestha, 2018). Nepal is also considered as one of the centers of rice genetic resources which include several wild species, their relatives, and thousands of landraces (Joshi et al., 2016). National Agriculture Genetic Resources Centre (NAGRC) has a collection and conservation of 2237 rice germplasms from different parts of the country (Genebank, 2021). Despite this large collection and conservation of rice germplasms, there has been very limited use of these in breeding by rice breeding program in Nepal. They are more dependent on foreign germplasm for varietal development. There are more than 1000 genotypes of rice introduced from IRRI for evaluation (Joshi, 2017). Until now, only 10 rice landraces have been used in the rice breeding program (Paudel et al., 2017). Among the rice landraces, only three (Chhomrong, Pokhreli

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Jethobudho, and Lalka Basmati) out of 76 released rice varieties are the landraces improved and released (Ghimire et al., 2014; Ghimire et al., 2020).

Landraces have been nurtured and cultivated by the farmers through the traditional method of selection over the years. With the increase in population and increase in the food demand, breeding activities these days are being directed towards high yielding varieties development (Dhakal et al., 2020). Though a wide range of genetic resources is available nationally and internationally, the breeders tend to concentrate only on adapted and improved materials avoiding wild and weedy relatives, and landraces in their crossing program (Upadhyaya et al., 2014). The use of landrace diversity in breeding programs is low either because of a lack of knowledge about the genetic worth or the linkage drag associated with the transfer of beneficial traits from such germplasm (Upadhyaya et al., 2014). Non-availability of quality seeds of the local varieties and the introduction of high yielding varieties are also contributing to their deliberate replacement. Although the traditional varieties or landraces have a low yield as compared to improved and hybrid varieties, they have a high capacity to tolerate biotic and abiotic stresses, with high yield stability and an intermediate yield level under a low input agricultural system (Manohara et al., 2018). Landraces are the reservoir of genetic potential and several resistant genes for biotic and abiotic stress, whereas modern varieties are devoid of such quality. They are more adapted to local conditions, have a higher chance of survival and reproduction, and pass on their characteristics to the next generation (Dhakal et al., 2020). Direct use of genebank materials re-introducing with preliminary characterization and evaluation benefits the farming community because, despite lower yield in comparison to improved and hybrid varieties, these landraces acquire several comparative advantages such as premium quality and taste, longer straw suitable for livestock feed, and traditional mat (Gundri) making and resilience to various biotic and abiotic stresses (Ghimire et al., 2020).

Landraces are raw materials and their use in breeding is one of the most sustainable ways to conserve biodiversity. Agro-morphological characterization of germplasm is fundamental in order to provide information for plant breeding programs (Lin, 1991). Characterization and landrace enhancement are required to increase the utilization of landraces (Thapa et al., 2021). Systematic study and characterization of germplasm are not only important for utilizing the appropriate attribute but also essential in the present era for protecting the unique germplasm. Well-characterized and evaluated germplasm collections would have greater chances of contributing to the development of new varieties and consequently greater realization of benefits for the resource-poor farmer (Manohara et al., 2018). Nepalese rice landraces have a high level of genetic diversity that will be a very important input for future improvement and sustainability of the rice-based production system (Gauchan, 1999). Rice diversity and diversity traits found in Nepalese rice can be commercially utilized in rice breeding program to increase yields and develop biotic and abiotic stress tolerant rice varieties. There is, therefore, a need to discover new sources of variation and assess the pattern of diversity to identify genetically diverse germplasm with beneficial traits to promote utilization of such germplasm in the rice improvement program.

METHODOLOGY

EXPERIMENTAL MATERIALS AND DESIGN

The experiment was carried out at NAGRC Khumaltar, Lalitpur in 2018 and 2019. Geographically it is located at an altitude of 1368 m, latitude of 27°40'N, and longitude of 85°20'E. The characterization blocks have black loamy soil. Forty-two rice landraces collected from twenty-one districts of the country as depicted in Table 1 are grown in the field of NAGRC. The experiment was conducted in a non-replicated row design with a direct seeding method for agro-morphological characterization and diversity assessment. The seeding dates were 31st May and 7th June in 2018 and 2019 respectively. Each accession was seeded in four rows of 3-meter length with 20 cm × 20 cm spacing with 2 to 3 seeds per hill. The chemical fertilizer was applied at the rate of 80, 60, 40 kg ha-1 N, P2O5, K2O. The entire dose of phosphorus and potassium and half dose of nitrogen was applied as a basal dose. The remaining dose of nitrogen was applied in two equal splits, one at the time of tillering and the remaining another at the panicle initiation stage. Weeding and pulverizing of soil were done regularly whenever necessary to keep the plots free from weeds and to ensure good aeration in the soil.

Table 1. Rice landraces with their local name and collection sites

S.N.	Landraces	Local Name	Collected site	Altitude (m)	Latitude	Longitude
1	NGRC01676	Masino dhan	Dudhpokhari, Lamjung	1920	28.28	84.40
2	NGRC01685	Seto dhan	Gaunsahar, Lamjung	1981	28.28	84.40
3	NGRC01780	Kalo marse dhan	Dharabari, Humla	2350	30.00	81.90
4	NGRC01837	Rambilash Dhan	Kuntadevi, Okhaldhunga	1384	27.91	85.25
5	NGRC01917	Ghaiya dhan	Orang, Dolakha	2251	27.80	86.24
6	NGRC02819	Ekle dhan	Nauthar, Lamjung	1402	28.28	84.40
7	NGRC02829	Lahare dhan	Bichaur, Lamjung	1996	28.28	84.40
8	NGRC02833	Anadi dhan	Nauthar, Lamjung	1402	28.28	84.40
9	NGRC02851	Madhise dhan	Dhorphirdi, Tanahun	1311	27.95	84.21
10	NGRC03034	Rate ghaiya	Latamandau, Doti	605	29.16	80.88
11	NGRC03057	Churi dhan	Patan, Baitadi	1266	29.49	80.60
12	NGRC03089	Dudhraj dhan	Dahathum, Syangja	1304	28.07	83.76
13	NGRC03093	Kalo dhan	Deurali, Doti	508	28.30	84.77
14	NGRC03097	Mansara Dhan	Hespur, Gorkha	3296	28.30	84.77
15	NGRC03141	Jado Dhan	Bagaicha, Myagdi	1124	28.57	83.50
16	NGRC03158	Jumli Dhan	Chandannath, Jumla	2290	29.22	82.25
17	NGRC03161	Kalo marsi	Depal, Jumla	2340	29.22	82.25
18	NGRC03163	Jumli Dhan	Mahat, Jumla	2815	29.22	82.25
19	NGRC03195	Darmali Dhan	Yarsha, Dolakha	1855	27.80	86.24
20	NGRC03322	Aaun dhan	Bhojpur bazar,	1528	27.17	87.05

			Bhojpur			
21	NGRC03332	Sokan Dhan	Nijgadh, Bara	193	27.20	85.23
22	NGRC03341	Kataura dhan	Nijgadh, Bara	192	27.20	85.23
23	NGRC03352	Jumli Dhan	Depal, Jumla	2290	29.22	82.25
24	NGRC03371	Ruwani dhan	Bansagadhi, Banke	150	27.05	81.80
25	NGRC03291	Rato basmati Dhan	Bharatganj, Bara	194	27.20	85.23
26	NGRC03314	Naiharwa Dhan	Bharatganj, Bara	195	27.20	85.23
27	NGRC03333	Langhi Dhan	Nijgadh, Bara	192	27.20	85.23
28	NGRC03342	Sankharika dhan	Nijgadh, Bara	193	27.20	85.23
29	NGRC03348	Palsa moto dhan	Kadmaha, Morang	73	26.45	87.37
30	NGRC03349	Bhatte dhan	Laxmipur, Dang	606	28.00	82.49
31	NGRC03361	Kariya kamod dhan	Chuhadwa, Siraha	81	26.29	86.10
32	NGRC03362	Panchali Dhan	Rayapur, Saptari	116	26.36	86.45
33	NGRC03363	Makhul sayar dhan	Deliya, Saptari	120	26.60	86.72
34	NGRC03364	Basmati Dhan	Chinnamasta, Saptari	90	26.60	86.72
35	NGRC03366	Nanhiya Dhan	Chinnamasta, Saptari	90	26.60	86.72
36	NGRC03367	Matiya dhan	Haripur, Sunsari	85	26.31	87.01
37	NGRC03384	Baruwa Dhan	Bankatwa, Banke	106	27.05	81.80
38	NGRC03393	Jiri Dhan	Murarwa, Rautahat	250	26.48	85.40
39	NGRC03395	Jiri Dhan	Murarwa, Rautahat	250	26.48	85.40
40	NGRC03272	Local Dhan	Lakhanpur, Parsa	90	27.10	84.79
41	NGRC03403	Local Dhan	Madar, Siraha	80	26.63	86.18
42	NGRC03418	Local Dhan	Madar, Siraha	85	26.63	86.18

DATA RECORDING AND ANALYSIS

Agro-morphological traits were measured at various growth stages according to descriptors for rice (IPGRI, 2007). Five random plants from each landrace were selected for agro-morphological traits evaluation. Fourteen qualitative traits like leaf blade pubescence, leaf blade color, basal leaf sheath color, flag leaf attitude, ligule color, collar color, etc. were observed at different growth stages. Likewise, thirteen quantitative traits like days to emergence, days to maturity, plant height, leaf length and width, seed length and width, test wt. and yield etc. were recorded as mentioned in descriptors.

Shannon–Weaver diversity indices (Shannon & Weaver,1949) were calculated in order to estimate the phenotypic diversity for each qualitative and quantitative trait with Microsoft Excel using the formula:

$$H' = \left[\Sigma\left(\frac{n}{N}\right) \times \left\{Log2\left(\frac{n}{N}\right) \times (-1)\right\}\right] / Log2\kappa$$

Where, H′ is the standardized Shannon–Weaver diversity index, *k* is the number of phenotypic classes for a character, n is the frequency of a phenotypic class of that character and N is the total number of observations for that character. For the H′ of quantitative traits, accessions were divided into 10 phenotypic classes as <x-2sd, x-2sd, x-1.5sd, x-sd, x-0.5sd, x, x+0.5sd, x+sd, x+1.5sd,

x+2sd, and >x+2sd are as the margins of the classes, where x is average and sd is the standard deviation. The diversity index was considered as low (0.10≤H′≤0.40), intermediate (0.40≤H′≤0.60), high (0.60≤H′≤0.80), and very high (H′≥0.80) (Eticha et al., 2005).

DATA ANALYSIS

Basic statistics including mean, maximum, minimum, coefficient of variation (CV), and diversity index (H') were calculated by using Excel 2016 and UPGMA clustering, and Principal Component Analysis was done with MINITAB 17 for quantitative characters. Estimates of similarities among the landraces were calculated using Euclidean distance and average linkage and PCA was conducted to know the contribution of traits in total variation among the landraces.

RESULT AND DISCUSSION

DIVERSITY IN QUALITATIVE TRAITS

The morphological character-based diversity index of rice landraces is presented in Table 2. Among qualitative variables, all characters are found to be polymorphic. The diversity index *(*H'*)* ranged from 0.16 to 0.96, which indicates low to very high diversity present in the rice landraces for qualitative traits. A very high diversity index (H') was inferred for the panicle exertion trait (0.96). However, this value of diversity index (H') was found high for secondary branching of panicle (0.70) and coloration of apiculus (0.66). Qualitative characters are considered as marker characters in the identification of landraces of rice, which are less independent of the environmental responses (Singh et al., 2014). Flag leaf angle is an important growth character in which maximum photosynthesis occurred. It might be due to the higher light penetration in the crop canopy due to erect leaves (Zafar et al., 2004). In our study, 83% of the landraces had erect, 15% semi erect, and 2% horizontal flag leaf attitudes. Farmers prefer awnless grain because awns are objectionable in threshing and milling (Singh et al., 2014). Among the landraces, 96% were awnless and the remaining 4% were having awn.

Table 2. Morphological character-based diversity index of rice landraces

S.N.	Qualitative characters	Shannon-weaver index	Descriptor's states	Frequency	Percentage
1.	Leaf blade pubescence	0.37	1-Glabrous	39	93
			2-Intermediate	3	7
2.	Leaf blade color	0.34	2-Green	37	88
			3-Dark green	3	8
			5-Purple margin	1	2
			7-Purple	1	2
3.	Basal leaf sheath color	0.20	1- Green	40	96
			2-Green with purple line	1	2
			4-Purple	1	2

4.	Flag leaf: Attitude	0.47	1-Erect	35	83
			3- Semi-erect	6	15
			5-Horizontal	1	2
5.	Ligule color	0.20	1-White	40	96
			2-Purple lines	1	2
			3-Purple	1	2
6.	Collar color	0.27	1-Pale green	40	96
			3- Purple	2	4
7.	Auricle color	0.27	1-Pale green	40	96
			2-Purple	2	4
8.	Panicle exsertion	0.96	3-Moderately well	12	29
			exserted	8	19
			5-Just exserted	7	17
			7-Partly exserted	15	35
			9-Enclosed		
9.	Culm habit	0.16	3-Semi-erect	41	98
			5-Open	1	2
10.	Panicle type	0.27	5- Intermediate	40	96
			9-Open	2	4
11.	Panicle: Secondary branching	0.70	0-Absent	17	40
			1-Light	24	58
			2-Heavy	1	2
12.	Shattering	0.71	5-Moderate	1	2
			7-Moderately high	21	50
			9-High	20	48
13.	Awn distribution	0.27	0-Absent	40	96
			7-Long and partly awned	2	4
14.	Lemma: Coloration of Apiculus	0.66	1-White	2	5
			2-Straw	27	64
			3-Brown	11	26
			6-Purple	2	5

DIVERSITY IN QUANTITATIVE TRAITS

Thirteen quantitative traits were measured for evaluating variation among rice landraces (Table 3). The result showed the existence of high variation with quantitative traits among the landraces. The coefficient of variation ranges from 8.00 (days to emergence) to 47.77 (yield). Out of thirteen quantitative characters, five have a CV value of more than 20% indicating greater variability among the landraces. The result indicated that there is a high level of variation in characters of interest i.e., Plant height, test wt. and yield in rice landraces. This signifies that selection for these traits could be effective. Shannon Weaver index ranges from 0.48-0.89 which showed intermediate to a very high level of diversity among the landraces for quantitative traits. Very high diversity was found in test wt. (0.89), Leaf length (0.84), seed length (0.82), ligule length (0.82), plant height (0.81), and no of grains/panicle (0.80), and remaining characters showed intermediate to a high level of diversity. Knowledge of existing diversity and its distribution in crop species is useful for landrace conservation and selection of parents with the diverse genetic background to make improvement more efficient (Teklu et al., 2006). The existence of high genetic diversity in the Nepalese rice landraces increases the space for selection for breeders as well as for farmers. This diversity can be utilized in crop improvement and enhancement of the genetic potential of rice landraces. Agro-morphological traits can be considered by farmers to discriminate varieties regarding the selection and adoption of a variety.

Table 3: Diversity based on quantitative traits

S.N.	Characters	Mean±SE	Std.	CV(%)	Max.	Min.	SWD(H')
1	Days to emergence	11±0.14	0.88	8.00	13	9	0.48
2	Days to 50% heading	107±2.34	15.19	14.10	125	79	0.75
3	Days to maturity	157±2.67	17.28	10.95	174	123	0.59
4	Leaf length (cm)	34±1.12	7.27	20.96	48	16.2	0.84
5	Leaf width (cm)	0.98±0.03	0.19	19.5	1.4	0.7	0.79
6	Ligule length (cm)	1.66±0.06	0.38	22.8	2.7	1.0	0.82
7	Plant height (cm)	101±4.19	26.14	25.75	157	63	0.81
8	Panicle length (cm)	59±1.16	7.16	12.1	72	42	0.79
9	No of grains panicle^{-1}	54±1.74	10.80	19.9	77	35	0.80
10	Seed length (mm)	35.1±0.70	4.37	12.45	43.7	28.0	0.82
11	Seed width (mm)	29.1±0.66	4.12	14.17	37.9	21.9	0.78
12	Thousand grain weight (g)	23.9±0.76	5.26	22.06	36.1	14.5	0.89
13	Yield (t ha^{-1})	2.08±0.14	0.99	47.77	4.69	1.01	0.51

SE = Standard Error, Std. = Standard Deviation, CV = Coefficient of Variation, Min. = Minimum, Max. = Maximum, SWD = Shannon–Weaver diversity, H' = Notation for Shannon–Weaver diversity index

CLUSTER ANALYSIS

A dendrogram was constructed by using UPGMA clustering method based on average linkage and Euclidean distance across the 42 landraces. The cluster analysis grouped the landraces into four clusters for 13 quantitative traits (Table 4). The accessions were clustered using days to emergence, days to heading, days to maturity, plant height, Ligule length, leaf length, leaf width, panicle length, grains per panicles, seed length, seed width, test wt. and yield as variables. The critical examination of the dendrogram revealed four clusters with a minimum of 46.33% similarity level in UPGMA clustering. Clusters were obtained based on similarity percentage and related characters.

Cluster 1, 2, 3 and 4 consist of 13, 10, 5 and 14 rice accessions respectively. Cluster-1 has accessions having a higher value for plant height, panicle length, no of grains/panicle, seed length and seed width, and intermediate value for yield and days to maturity. Accessions in cluster-2 were early mature and high yielder than that of cluster-1 and 4 and had higher test weight. Accessions in cluster-3 were early mature and high yielder and had intermediate values for plant height, panicle length, and no of grains/panicle than the remaining clusters. Similarly, cluster-4 consists of late mature and low yielder accessions with lower plant height.

Characterization of accessions and clustering of them based on their morphological and genetic similarity helps to identify and select the best parents for hybridization. Hence, a grouping of landraces using multivariate analysis such as UPGMA clustering would be valuable for the breeders in such a way that the most promising landraces in the population may be selected from different clusters for pre-breeding and further evaluation. Accessions of the cluster- 3 i.e. NGRC01917, NGRC03034, NGRC03395, NGRC03057, NGRC03163 are superior in terms of yield and days to maturity. After further selection, these accessions can be included in the rice improvement program to develop early and high yielding varieties of rice. Accessions having higher plant height may be useful as a potential donor for increasing total plant biomass. The accessions with early maturity, higher test wt., higher panicle length, and higher yield might serve as potential donors for increasing grain yield of predominant rice varieties.

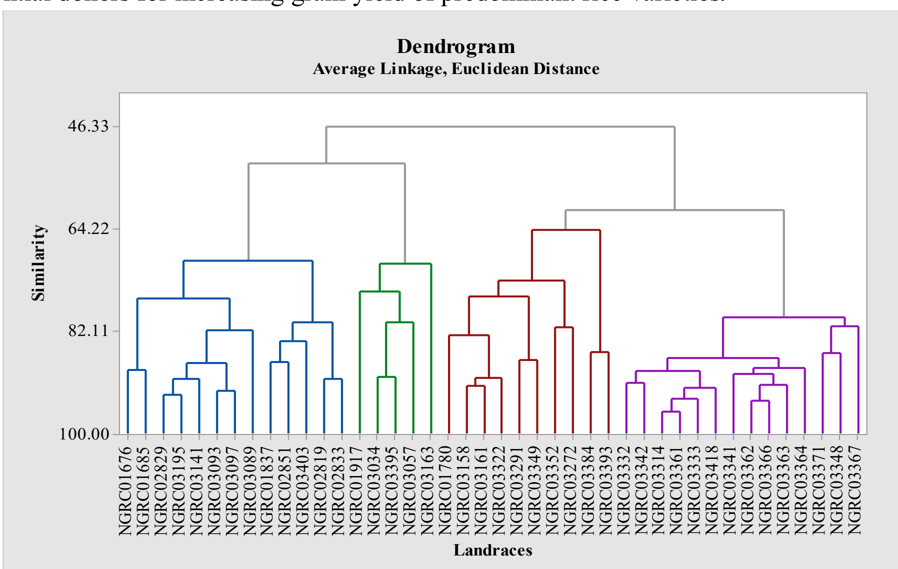

Figure 2: Dendrogram of 42 rice landraces derived by UPGMA from thirteen quantitative traits

Table 4: Number of accessions with an average of major quantitative traits in each cluster

Variables	Cluster-1	Cluster-2	Cluster-3	Cluster-4
No of accessions	13	10	5	14
Days to emergence	11	11	11	12
Days to 50% heading	114	93	83	120
Days to maturity	167	141	132	170
Ligule length (cm)	1.8	1.4	1.8	1.6
Leaf length (cm)	40.8	27.7	36.5	33.3
Leaf width(cm)	1.05	0.90	1.11	0.98
Plant height (cm)	130	84	118	77
Panicle length (cm)	68.3	51.1	57.9	56.6

No of grains panicle^{-1}	67	46	59	45
Seed length (mm)	40.3	32.5	36.4	31.7
Seed width (mm)	33.3	27.8	31.1	25.4
Thousand grain weight (g)	23.8	26.6	25.8	22.8
Yield (t ha^{-1})	1.89	2.32	2.83	1.64

PRINCIPAL COMPONENT ANALYSIS

In this study, the first three principal components are most important in reflecting the variation pattern among accessions, and traits associated with these are more useful in differentiating accessions. The first three components with eigen value greater than 1 accounted for 75.5 % of total variation (Table 5). The first component accounted for 42.7% of the total variance indicating that no of grains/panicle, panicle length, plant height, seed length, and width were the variables that contributed most positively. The second PC accounted for 21% of the total variance which was mainly influenced positively by days to maturity, days to heading, days to emergence, and negatively by yield. The third component accounted for 11 % of the total variance which was positively associated with leaf length, leaf width, and yield and negatively with test wt. trait. The proper value measures the importance and contribution of each component to total variance, whereas each coefficient of the proper vectors indicates the degree of contribution of every original variable with the principal component it is associated with (Dhakal et al., 2020). Higher the coefficient (regardless of the sign), more will be the effectiveness of those corresponding parameters in discriminating the landraces. The results of PCA suggested that traits, viz. Days to maturity, no of grains/panicle, grain yield, plant height,
and panicle length were the principal discriminatory characteristics of the Nepalese rice landraces.

Table 5: Principal Component Analysis based on thirteen quantitative characters

Variables	**PC-1**	**PC-2**	**PC-3**
Eigen value	5.54	2.83	2.43
Proportion	0.42	0.21	0.11
Cumulative Variance (%)	42.7	64.5	75.5
		Coefficient vector	
Days to emergence	-0.135	0.350	-0.082
Days to 50% heading	0.061	0.484	-0.064
Days to maturity	0.090	0.525	-0.124
Leaf length (cm)	0.351	0.185	0.146
Leaf width (cm)	0.184	-0.073	0.538
Ligule Length (cm)	0.233	0.090	0.477
Plant height (cm)	0.393	-0.137	-0.054
Panicle length (cm)	0.396	0.179	-0.060
No of grains panicle^{-1}	0.407	-0.069	-0.051
Thousand grain weight (g)	-0.033	-0.287	-0.432
Seed length (mm)	0.395	-0.089	-0.245
Seed width (mm)	0.352	-0.196	-0.299
Yield (t ha^{-1})	-0.011	-0.368	0.302

CONCLUSION

The rice landraces exhibited sufficient genetic variation for most of the qualitative and quantitative traits. Agro-morphological traits, namely days to maturity, no of grain panicle^{-1}, grain yield, plant height, and panicle length were the principal discriminatory characteristics of the Nepalese rice landraces. Rice landraces, Ghaiya dhan from Dolakha, Rate Ghaiya from Doti, Jiri dhan from Rautahat, Churi dhan from Baitadi, and Jumli dhan from Jumla were found superior based on preliminary evaluation of important traits such as days to maturity and yield. These landraces could be evaluated further in multiple environments and used to develop new rice varieties.

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