

Research Article

Multivariate Analysis of Phenotypic Diversity of Rice (*Oryza sativa* L.) Landraces from Lamjung and Tanahun Districts, Nepal

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The magnitude and nature of genetic divergence play a vital role in the selection of the desirable landraces for its utilization in the breeding program. A study was carried out with 30 rice landraces at the Institute of Agriculture and Animal Science, Lamjung Campus, during June–November 2018 to determine relation among individuals, estimate the relative contribution of various traits of rice using principal component analysis, and identify the potential parents for hybridization using Mahalanobis distance (D^2). The principal component analysis revealed that five among the thirteen principal components were significant (eigenvalue >1) and contributed to 29.96%, 20.26%, 13.56%, 11.68%, and 9.22% of the total variance, respectively. PC1 included the traits that were related mostly to the yield, yield attributing, and grain characteristics. Landraces from Anadi group, Jetho Budo, Jarneli, and Rato Masino performed well in PC1 while landraces such as Mansara, Pakhe Sali, and Aanga performed well in PC2. The landraces were grouped into six clusters where 12 landraces were grouped into cluster I. Cluster analysis showed maximum and minimum intracluster distance in cluster VI ($D^2 = 35.77$) and cluster I ($D^2 = 18.59$), respectively. The maximum intercluster distance was obtained between clusters V and VI ($D^2 = 40.18$) followed by clusters III and VI ($D^2 = 36.17$) and clusters IV and VI ($D^2 = 35.74$). Cluster III showed the highest mean value for grain width, flag leaf breadth, yield, and minimum mean value for plant height while mean values of total grain per panicle, filled grain percentage, and thousand-grain weight were maximum in cluster IV. Mean values of effective tiller and kernel width were found maximum in clusters V and VI, respectively. Landraces from clusters V and VI or clusters III and VI or clusters IV and VI can be used in the hybridization program to develop the superior hybrids by exploiting heterosis in segregating generation.

1. Introduction

Rice (*Oryza sativa* L.) is one of the major and staple foods for more than half of the world's population. Almost 90% of the world's rice is grown and consumed in Asia, where 50% of the population depends on rice for food [1]. In Nepal, rice is the most important cereal crop and ranks 1st in terms of cultivated area with a total area of 1,491,744 ha and production of 5,610,011 metric ton in 2019 [2]. About 2500 rice landraces and 8389 rice accessions from Nepal are recorded [3]. Although Nepal is so rich in rice accessions, Nepal has been using foreign germplasm or landraces as parents in rice breeding programs [4]. Out of all released rice varieties, only 5 landraces have been improved to variety by mass selection and pure line selection [5]; other 10 varieties have been

developed by landrace/exotic genotype crossing and selection where landraces such as Jarneli, Pokhreli Masino, Chhomrong, and Jumli Marsi were used as parents [6].

With the increase in population and increase in the food demand, breeding activities these days are being directed towards high yielding varieties development. Although the traditional varieties or landraces have a low yield, they are believed to be more locally adapted and have both symbolic and social values together with economic benefits [7]. Rice landraces are the reservoir of genetic potential and several resistant genes for biotic and abiotic stress, whereas the modern varieties are devoid of such quality [8]. Landraces are more adapted to local conditions, have a higher chance of survival and reproduction, and pass on their characteristics to the next generation [9].

TABLE 1: List of landraces and site of their collection.

Source	Landraces
Marsyangdi Chepe Community Seed Bank, Lamjung (28°05' N, 84°31' E)	Anadi Tude, Jetho Budo, Gokule Mansuli, Eakle, Kalo Masino, Biramful, Kalo Jhinuwa, Jhinuwa Local, Lekali Marsi, Anadi Local, Pakhe Sali, Pahelo Anadi
Purkot Community Seed Bank, Tanahun (28°04' N, 84°27' E)	Pahela, Chiniya, Anadi Seto, Bihari, Juhari, Kathe, Mansara
Local farmer, Lamjung (28°07' N, 84°33' E)	Rato Anadi, Rato Masino, Thakali Lahare Marsi, Pudke Dhan, Indrabeli, Kalo Namdunge, Aanga, Boryang Masino, Sobhara, Marsi Local, Jarneli

In order to utilize the landraces in breeding activities, we need to understand the genetic variability. The study of the level and patterns of genetic divergence in the rice genotype provides knowledge and idea of the genetic variability [10]. Information on genetic diversity facilitates parental selection from a huge number of landraces or germplasm. The information on the genetic divergence and distance among the parent lines will be useful to assess the potential heterotic combination before attempting the crosses and hence saves time and resources by designing a suitable breeding strategy [11].

Multivariate statistical tools are extensively used in summarizing and describing the inherent variations among the landraces. Principal component analysis (PCA) analyses data consisting of several intercorrelated quantitative dependent variables as observations [12]. PCA extracts the information from a table and represents it as a set of new orthogonal variables called principal components. PCA reveals the patterns and eliminates the redundancy in datasets, as variation occurs commonly in plants for yield and yield-related traits [13]. The primary benefit of PCA is to quantify the significance of each dimension for relating the variability of a dataset [14]. Mahalanobis D^2 statistics is an effective tool in quantifying the degree of genetic divergence at the genotypic level based on generalized distance [15]. Multivariate analysis, utilizing Mahalanobis D^2 statistics, has been found to be a potent biometrical tool in quantifying the degree of divergence in a germplasm collection of various crop plants [16]. Mahalanobis D^2 statistics is very useful in selection of the parents in hybridization.

The present study aims to determine the relation among individuals, to estimate the relative contribution of various traits, and to guide the selection of the parents to develop transgressive segregation.

2. Materials and Methods

Thirty landraces were collected from Rainas Municipality, Lamjung and Bhanu Municipality, Tanahun District, and then grown at the Agronomy Farm of Institute of Agriculture and Animal Science (IAAS), Lamjung Campus (28.13°N, 84.40°E, 633 masl) during June–November, 2018. The experiment was laid in an alpha lattice design with two replications. Single seedling was transplanted in 20 × 20 cm distance. All the standard recommended cultural practices were followed. The fertilizer was applied according to the national recommended dose of 100:30:30 kg/ha NPK for irrigated rice [17]. Half dose of nitrogen and a full dose of potassium and phosphorus were applied as a basal dose and

remaining nitrogen was applied in an equal split in tillering and panicle initiation stage.

2.1. Collection of Landraces. The landraces from the similar altitude and agro-climate zone of the research site were collected from one municipality each of two districts. The landraces along with the site of collection of landraces is listed in Table 1.

2.2. Data Recording. Five tillers from each replication were selected randomly to measure 13 quantitative characteristics. The detailed description and evaluation stage of the quantitative characters were according to the descriptor of Biodiversity International, IRRI, and WARDA [18] and are presented in Table 2.

2.3. Data Analysis. Multivariate Analysis was performed in R (4.0.2) using various packages. Principal components analysis, eigenvalues, eigenvectors, and 2D biplots were obtained using FactoMineR and factoextra packages. Similarly, for cluster analysis, the Mahalanobis distance was obtained and clustering was performed using Tocher's method as proposed by Vasconcelos et al. [20], called modified Tocher from biotools packages.

3. Results and Discussion

3.1. Principal Component Analysis. PCA clearly revealed that five of the thirteen principal components were significant (eigenvalue >1) and contributed to 84.67% of the variance. PC1 accounted for the highest variance (29.95%) followed by PC2 which accounted for 20.26% while PC3, PC4, and PC5 accounted for 13.56%, 11.68, and 9.22% variance, respectively. Only these five components which had eigenvalue more than 1 were considered for further analysis. Principal components with eigenvalues >1 explained more total variation in the data than individual attributes [21]. Similar results were obtained by [22] where the first five principal components were found to be significant and contributed 82.9% of the total variation in 31 rice germplasm. In a study of cooking and physicochemical characters using the same landraces, the first four principal components retained 73.8% of the total variance [23]. 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 [24]. Higher the coefficient (regardless of the sign), more will be the effectiveness of those corresponding parameters in

TABLE 2: Quantitative characters along with the procedure and evaluation phase.

Characters	Procedure	Evaluation phase
Total grains per panicle (TG)	All the grains of the sampled plants were counted.	After harvest
Filled grain percentage (FGP)	No. of fertile grains per panicle of the sampled plants were counted and then FGP was calculated as $FGP = \text{Filled grain} / \text{Total grain} \times 100 \%$	After harvest
Panicle length (PL) (cm)	The length of the main axis of the panicle is measured from base to the tip.	After harvest
Grain length (GL) (mm)	The distance from the base of the lowermost glume to the tip (apiculus) of the fertile lemma or palea, whichever was longer was measured.	After harvest
Grain width (GW) (mm)	The distance across the fertile lemma and palea at the widest point was measured.	After harvest
Kernel length (KL) (mm)	Ten milled grains were randomly taken and their length was measured.	After milling
Kernel width (KW) (mm)	Ten milled grains were randomly taken and their width was measured.	After milling
Flag leaf length (FLL) (cm)	The length of the flag leaf, from the ligule to the tip of the blade, was measured.	7 days after anthesis
Flag leaf breadth (FLB) (cm)	The breadth at the widest part of the flag leaf was measured.	7 days after anthesis
Effective tiller (ET)	Tillers with a panicle filled with grains were counted.	At maturity
Plant height (PH) (cm)	Length measured from the soil surface to the top of the tip of the panicle was measured.	At maturity
Thousand-grain weight (TGW) (g)	1000 grains were randomly selected and then the weight was measured.	After harvest
Grain yield (GY) (ton/ha)	The total yield per plot and its moisture percentage were measured and then the yield per hectare at 12% moisture was estimated using the formula (Paudel, 1995, as cited by Amgain and Dhakal [19]): $GY = (\text{grain yield of plot (kg)} \times (100 - \text{actual moisture \%})) / (\text{plot area} \times (100 - \text{safe moisture \%}) \times 10000)$	After harvest

discriminating the landraces. Figures 1 and 2 show the eigenvalue and contribution of each principal component to the total explained variance in the phenotypic diversity of rice, respectively.

Results from the PCA and vector loading in Figure 3 showed that the first principal component was contributed positively by thousand-grain weight (0.81), grain yield (0.78), filled grain percentage (0.72), grain width (0.66), total grain per panicle (0.59), flag leaf breadth (0.57), kernel width (0.52), and grain length (0.5). This clearly showed that the PC1 included parameters that were mainly related to grain yield, yield attributing, and grain characteristics. Landraces from Anadi group, Jetho Budo, Jarneli and Rato Masino showed good performance in these characters. Kernel length (0.76), grain length (0.72), and flag leaf length (0.69) contributed in a positive way in PC2 while panicle length (−0.62)

contributed in a negative direction. Mansara, Pakhe Sali, and Aanga showed higher kernel length, grain length, and flag leaf length but lower panicle length. Kernel width (−0.61) and grain width (−0.52) played a significant negative role in PC3 while total grain per panicle (0.54) played a significant positive role. Plant height (0.83) and panicle length (0.61) contributed positively to the fourth principal component while the effective tiller (0.66) and flag leaf breadth (0.64) contributed significantly positive in PC5 and PC6, respectively. The magnitude and direction of contribution of different traits in the different principal components are shown in Figures 4 and 5.

The characters coming together in different principal components explaining the variability show the tendency to remain together and must be taken into consideration during the exploitation of these characters in the breeding

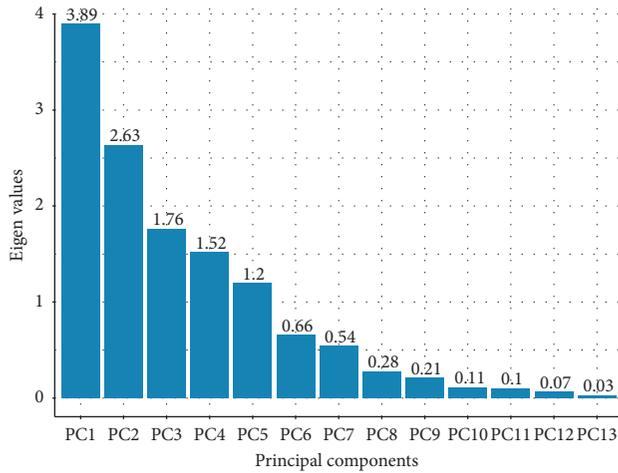


FIGURE 1: Eigenvalues of different principal components as shown by principal component analysis of rice landraces.

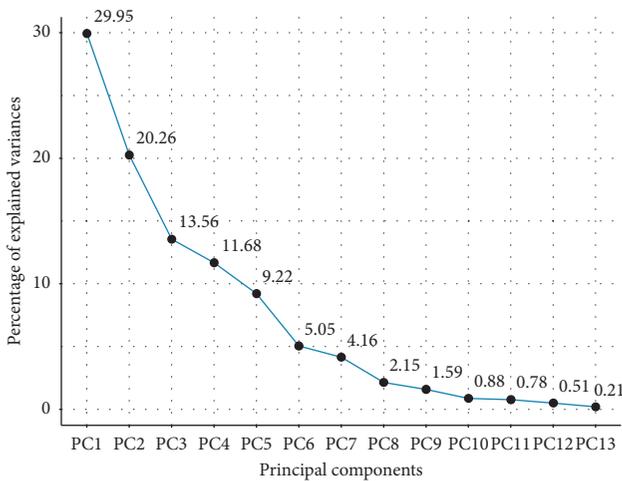


FIGURE 2: Contribution of each principal component to total explained variance in the phenotypic diversity of rice landraces.

program [25]. Eight characters (thousand-grain weight, grain yield, total grains per panicle, filled grain percentage, flag leaf breadth, kernel width, grain width, and grain length) contributed highly to PC1 which are yield, yield attributing, and grain characters (reproductive characters). The dominating characters in PC2 and PC4 were weighted average of both vegetative and reproductive characters. PC3 was dominated by reproductive characters. PC5 and PC6 were dominated by vegetative characters. An inference can be made based on thousand-grain weight, kernel length, grain width, kernel width, panicle length, and plant height which contributed the most in the observed variation and can be effectively used in the selection of the parental line for breeding purposes.

3.2. Mahalanobis Distance. Mahalanobis generalized distance (D^2) was used to measure the genetic divergence among the landraces and their grouping was done by Tocher's method given by Vasconcelos et al. [20]. The

	PC1	PC2	PC3	PC4	PC5	PC6
TG	0.59	-0.27	0.54	-0.1	0.24	-0.2
FGP	0.72	-0.27	0.34	-0.08	0.24	-0.22
PL	-0.07	-0.62	0.34	0.61	-0.24	-0.11
GL	0.5	0.72	-0.05	0.28	-0.27	-0.02
GW	0.66	-0.41	-0.52	0.13	0.12	0.18
KL	0.39	0.76	-0.04	0.13	-0.4	-0.19
KW	0.52	-0.41	-0.61	0.36	0.17	0.06
FLL	0.15	0.69	0.22	0.19	0.44	0.19
FLB	0.57	-0.08	0.34	-0.17	-0.26	0.64
ET	-0.36	0.38	-0.13	0.31	0.66	0.06
PH	-0.4	-0.07	0.25	0.83	-0.14	0.1
TGW	0.81	0.12	-0.42	0.09	-0.1	-0.17
GY	0.78	0.12	0.42	0.17	0.17	0

FIGURE 3: Principal component analysis and its component value for 13 qualitative traits of 30 landraces. The blue colour indicates the positive contribution, and red indicates the negative contribution (TG: total grain, FGP: filled grain percentage, PL: panicle length, GL: grain length, GW: grain width, KL: kernel length, KW: kernel width, FLL: flag leaf length, FLB: flag leaf breadth, ET: effective tiller, PH: plant height, TGW: thousand-grain weight, and GY: grain yield).

landraces were grouped into six clusters as listed in Table 3. Cluster I was the largest cluster which included 12 landraces followed by cluster II which included 6 landraces. Cluster III included 4 landraces, clusters IV and V included 3 landraces each while cluster VI comprised two landraces. The intra-cluster and intercluster distance (D^2) presented in Table 4 indicated that the intracuster distance was maximum in cluster VI ($D^2 = 35.77$) followed by cluster V ($D^2 = 32.35$) and cluster III ($D^2 = 27.32$). The cluster with maximum no. of landraces (cluster I) showed intracuster distance of 18.59.

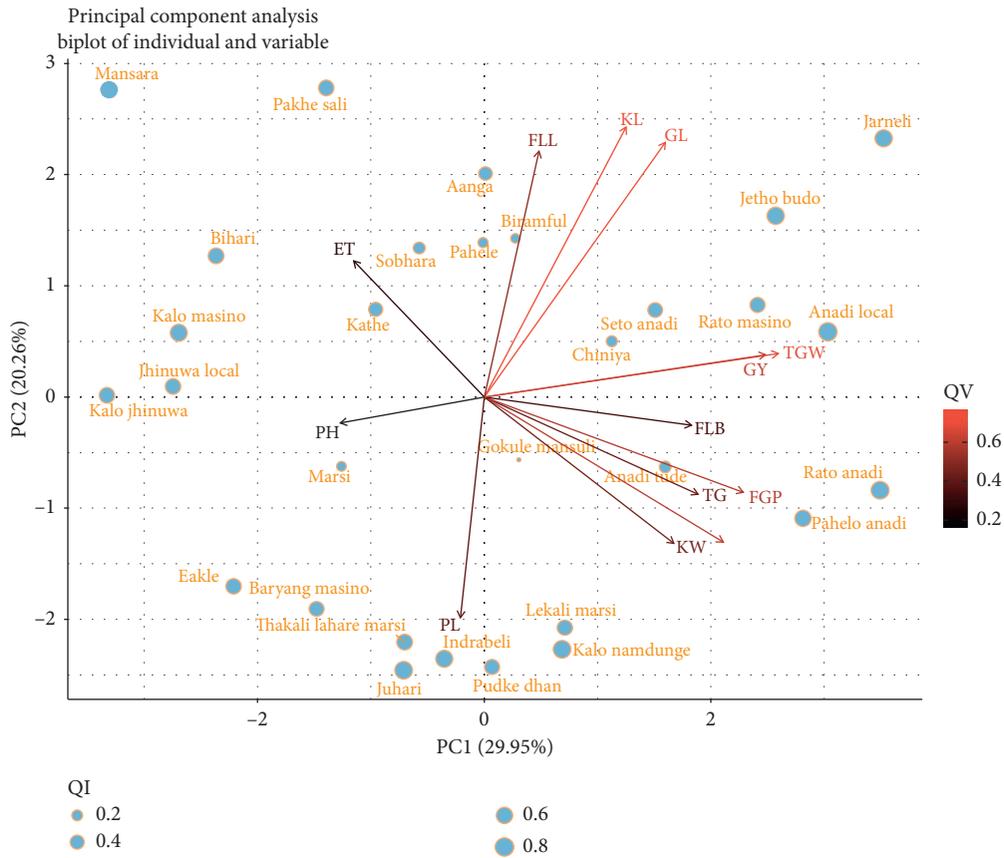


FIGURE 4: The biplot of 30 rice landraces for PC1 and PC2. The arrows show the contribution (magnitude and direction) of the trait in PC1 and PC2. QI is the quality of representation of individuals, and QV is the quality of representation of variables. (TG: total grain, FGP: filled grain percentage, PL: panicle length, GL: grain length, GW: grain width, KL: kernel length, KW: kernel width, FLL: flag leaf length, FLB: flag leaf breadth, ET: effective tiller, PH: plant height, TGW: thousand-grain weight, GY: grain yield).

The intracluster distance is due to the heterogeneous nature of the landrace within a cluster. The low intracluster distance indicated that the landraces in the clusters were closely related [26]. From Table 4, the intercluster distance was found to be maximum between clusters V and VI ($D^2 = 40.18$) followed by clusters III and VI ($D^2 = 36.17$). Minimum cluster distance was obtained between cluster I and cluster II ($D^2 = 22.86$). The distances to other clusters are presented in Table 4.

The cluster mean values for 13 characters are presented in Table 5. The data indicated a wide range of mean values among the characters. The mean value of length of the panicle (28.51 cm) was found to be maximum in cluster I. Mean values of grain length (9.40 mm), kernel length (6.91 mm), and flag leaf length (42.93 cm) were found to be maximum in cluster II. The mean values of characters such as grain width (3.41 mm), flag leaf breadth (1.71 cm), and grain yield (3.26 t/ha) were found to be maximum while mean value of plant height (145.93 cm) was found to be minimum in cluster III. Mean values of total grain (186.50), filled grain percentage (88.06%), and thousand-grain weight (30.37 g) were found to be maximum in cluster IV. Character effective tiller (7.67) and kernel width (2.89 mm) were found to be maximum means in clusters V and VI, respectively.

The clustering clearly indicated that neither of any cluster contained landraces with all desirable characters

which could be selected and utilized directly in the breeding activities. Thus, in order to develop a desirable genotype, hybridization is needed between the genotype of different clusters [27]. No cluster showed the landraces collected from a single location; this indicated that there was no role of ecological distribution on the genetic divergence. The possible reason for this could be a free exchange of landraces among the farmers, unidirectional selection of the landraces by the farmers, [10] and also the sites of collection of the landraces used in this research were very near and of similar geography. In hybridization programs, parental lines must be selected from the clusters that are at highest genetic divergence because divergent parents are expected to produce wide variability and transgressive segregations with high heterotic effects [28, 29]. Chakma et al. [30] pointed out the role of the magnitude of genetic distance, the contribution of different characters towards the total divergence, and the magnitude of cluster mean for different characters performance in the selection of the parents for the hybridization program. Nisar et al. [31] suggested selecting the lines belonging to diverse clusters and showing high mean performance in desirable directions for different traits can be used as parents in the breeding program. The genetic gains in yield or any characters can be exploited by recombining the elite

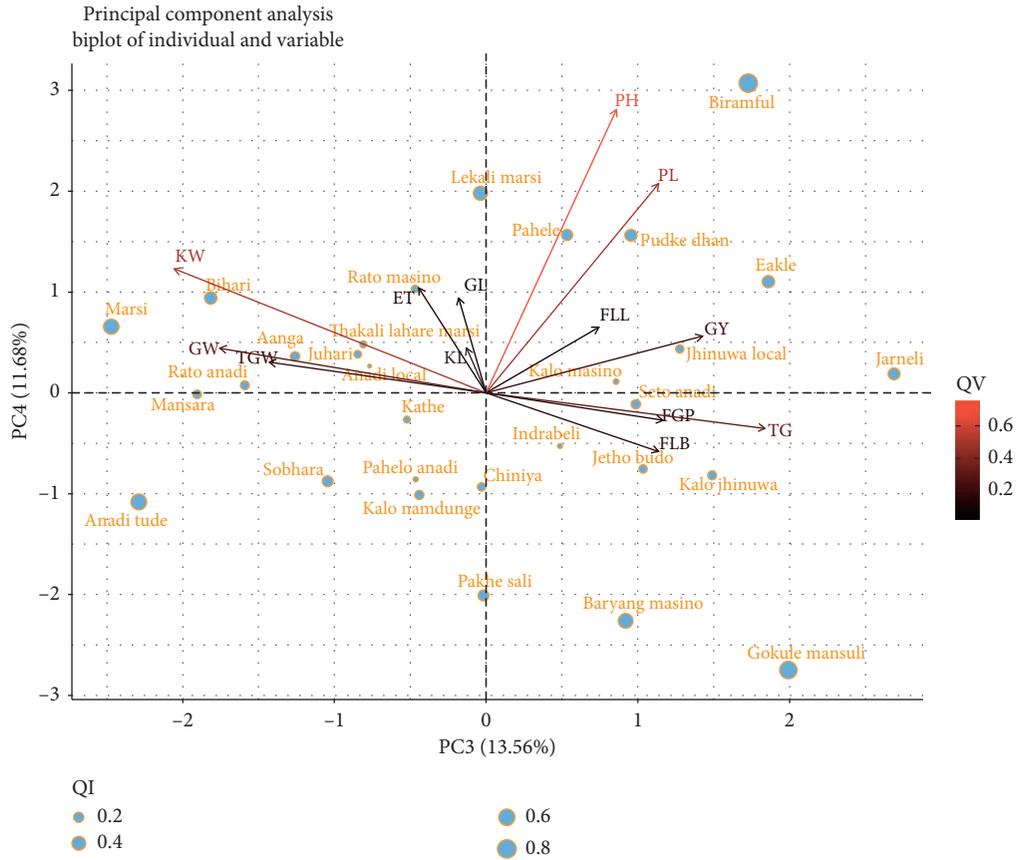


FIGURE 5: The biplot of 30 rice landraces for PC3 and PC4. The arrows show the contribution (magnitude and direction) of the trait in PC3 and PC4. QI is the quality of representation of individuals, and QV is the quality of representation of variables. (TG: total grain, FGP: filled grain percentage, PL: panicle length, GL: grain length, GW: grain width, KL: kernel length, KW: kernel width, FLL: flag leaf length, FLB: flag leaf breadth, ET: effective tiller, PH: plant height, TGW: thousand-grain weight, GY: grain yield).

TABLE 3: Distribution of 30 landraces from Lamjung and Tanahun districts into six clusters.

	No. of landraces	Name of landraces
Cluster I	12	Kalo Masino, Jhinuwa Local, Kalo Jhinuwa, Baryang Masino, Anadi Local, Kathe, Kalo Namdunge, Anadi Tude, Lekali Marsi, Thakali Lahare Marsi, Juhari, Eakle
Cluster II	6	Biramful, Jarneli, Jetho Budo, Sobhara, Bihari, Aanga
Cluster III	4	Pahelo Anadi, Pudke Dhan, Gokule Mansuli, Rato Anadi
Cluster IV	3	Rato Masino, Seto Anadi, Indrabeli
Cluster V	3	Pakhe Sali, Chiniya, Mansara
Cluster VI	2	Marsi Local, Pahele

TABLE 4: Intracluster (diagonal) and intercluster distance among 6 clusters.

	Cluster I	Cluster II	Cluster III	Cluster IV	Cluster V	Cluster VI
Cluster I	18.59	—	—	—	—	—
Cluster II	22.86	21.93	—	—	—	—
Cluster III	24.27	28.26	27.32	—	—	—
Cluster IV	25.69	29.24	32.35	25.70	—	—
Cluster V	28.26	30.72	34.78	33.92	32.35	—
Cluster VI	26.96	30.61	36.17	35.74	40.18	35.77

TABLE 5: Mean performance of different traits in each cluster.

	Cluster I	Cluster II	Cluster III	Cluster IV	Cluster V	Cluster VI
TG	154.46	166.58	174.50	186.50	112.67	129.25
FGP (%)	73.10	74.67	87.63	88.06	69.53	65.66
PL (cm)	28.51	26.08	27.97	27.88	21.33	27.17
GL (mm)	7.74	9.40	7.82	8.71	8.19	9.38
GW (mm)	3.00	2.93	3.41	2.99	2.65	3.06
KL (mm)	5.74	6.91	5.57	6.27	6.68	5.91
KW (mm)	2.64	2.63	2.87	2.71	2.37	2.89
FLL (cm)	32.93	42.93	38.42	35.16	38.73	37.91
FLB (cm)	1.49	1.45	1.71	1.54	1.44	1.47
ET	5.71	7.42	5.25	5.83	7.67	6.75
PH (cm)	160.40	155.59	145.93	158.52	150.57	169.04
TGW (g)	23.92	26.47	26.70	30.37	26.43	25.15
GY (t/ha)	1.91	3.05	3.26	2.44	1.98	1.63

TG: total grain; FGP: filled grain percentage; PL: panicle length; GL: grain length; GW: grain width; KL: kernel length; KW: kernel width; FLL: flag leaf length; FLB: flag leaf breadth; ET: effective tiller; PH: plant height; TGW: thousand-grain weight; GY: grain yield.

germplasm followed by selection [32]. Thus, landraces with high performance from clusters V and VI or clusters III and VI or clusters IV and VI can be used in the hybridization program as a parent line due to greater intercluster distance. Crossing genotypes belonging to the same cluster do not yield superior hybrids or segregates [33]. While selecting a landrace as a parent in a breeding program, other numerous factors need to be considered. Thus, the result from this study along with the result of Pokhrel et al. [5]; Sharma et al. [26], and Dhakal et al. [9] can set a foundation for future rice breeding programs. Further study at the gene level can be carried out to study the variation at the genetic level.

4. Conclusion

Both multivariate statistical analysis tools showed the existence of the wide genetic diversity among the landraces in the study. PCA revealed that five of the thirteen principal components were significant (eigenvalue >1) and contributed to 84.67% of the variance. PC1 was dominated by the yield, yield attributing, and grain characteristics. So, selecting the landrace with a high score in PC1 could result in higher yield and improved grain character. Landraces with high performance from clusters at a greater intercluster distance (clusters V and VI or III and VI or IV and VI) can be used in the breeding program to develop the superior hybrids by exploiting heterosis in segregating generation. Thus, the study will be highly beneficial to breeders for selection of the potential parental lines from the landraces in the study.

Data Availability

The field raw data and code used to support the findings of this study are available from the corresponding author upon request.

Disclosure

The authors did not receive any fund for this research from any funding source.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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