

CHARACTERIZATION AND DIVERSITY ASSESSMENT OF NEPALESE GARLIC (*Allium sativum* L.) LANDRACES

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ABSTRACT

A research was carried out at the field of National Agriculture Genetic Resources Centre (NAGRC), Khumaltar, Lalitpur, Nepal in 2019 with the objective of phenotypic characterization and genetic diversity assessment of thirty-seven local garlic landraces. The phenotypic diversity was assessed based on fifteen qualitative and nine quantitative characters by sing non replicated rod row design following the descriptors developed by IPGRI, 2001. Descriptive statistics were calculated by using MS 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 0.54-0.96 and 16.89-87.85, respectively. Four clusters identified and CO 10307, CO 10482 and CO 10615 of fourth clusters were superior in terms of quantitative characters. Five principal components contributed 95.2% of the cumulative variance. This result will be helpful for breeder and researchers to comprehensively understand the agro-morphological characters as well as diversity of the Nepalese garlic collection.

Key words: Agro-morphological characterization, Bulb crop, Diversity, Landrace, PCA, Variation

INTRODUCTION

Nepal is an ecologically diverse country where 65% populations are involved in agriculture. Due to the diversified geography and climate, large number of species of agricultural crops exist in the country. A total of 1,506 species of agricultural crop and forage genetic resources have been reported in Nepal (Upadhyay and Joshi 2003, Joshi *et al.*, 2020). Till 2020, there are 13,069 landraces of 330 species of different crops conserved in national gene bank (Genebank, 2020). Those plant species which do not produce seeds and need

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vegetative parts to regenerate i.e. recalcitrant and vegetatively propagated plant species are needed to be maintained in the field and regenerated every year for conservation (Mainali *et al.*, 2020). Taro park, field genebank and community field genebank were established in 2012 to conserve non-orthodox crop species in Pokhara, Khumaltar and Kailali, respectively (Joshi, 2019). A total of 241 landraces of 34 various recalcitrant species including 49 landraces of garlic have been collected from different sites of the country are maintained and regenerated every year in Khumal field genebank (Genebank, 2020). The main contributions of landraces to plant breeding are useful traits for more efficient nutrient uptake and utilization and genes associated with adoption to different stress (Newton *et al.*, 2011). The relatively high level of genetic variation of landraces is one of the advantages over improved varieties. Although yields may not be as high, the stability of landraces in face of adverse condition is typically high. But very few numbers of landraces i.e. only 37 local landraces of 19 crops have been utilized in breeding to develop 41 crop varieties in Nepal (Joshi *et al.*, 2020). Lack of knowledge about specific trait(s) of landrace is one of the main reasons behind low utilization of landraces. Besides this, characterization and landrace enhancement are required to increase the utilization of landraces.

Garlic (*Allium sativum* L.) is second most widely cultivated bulb crop after onion in the world, which belongs to family amaryllidaceae. Cultivated garlic is known for at least 5000 years and is believed to be originated in Central Asia where garlic grows wild (Fritsch and Friesen, 2002). There is high diversity of *Allium* species from Mediterranean basin to Central Asia and Pakistan (Fritsch and Friesen, 2002). Garlic is grown globally but China is leading country in area and production followed by India, Republic of Korea, Egypt and Russian Federation (FAO, 2012). Garlic is grown in 8116 ha in Nepal with the production of 56,668 ton (MOALD, 2018/19). Garlic is being cultivated for bulb and fresh vegetables (green bulb), however bulb production is popular in the country.

In Nepal, there is subsistence cultivation of few land races of garlic identified by farmers themselves and few other exotic germplasms introduced from adjoining country at different dates. Till now, none of the garlic genotypes has been registered or released from NARC for cultivation in Nepal. The basic pre-requisite for variety improvement is the presence of genetic variability in genetic stocks. In Nepal, Introduction, clonal selection and heterosis breeding are practiced for improvement of vegetatively propagated crops (Joshi, 2017). Clonal selection is a major breeding method for garlic which showed high degree of variation in bulb size, color, growth habits, plant height, number and size of the cloves, days to harvesting, resistance to storage capacity, dormancy and adaptation to agro-climatic situations (Singh *et al.*, 2012). Variability observed among different clones of garlic is due to mutation

providing opportunities through natural and human selection for adaptation to various growing environments (Singh *et al.*, 2018). Evaluation and characterization of preserve traits among garlic landraces is fundamental for their efficient utilization in plant breeding schemes and effective conservation programs (Lampasona *et al.*, 2003). Its evaluation will identify landraces that could be useful to obtain cultivars using clonal selection to be used in breeding programs (Kumar *et al.*, 2015). Wider adoption of short duration and high yielding variety to different agro-ecological zones is necessary to accelerate the garlic production in the country (KC *et al.*, 2004). To increase the production of garlic crop, there is an urgent need to screen the landraces to get more divergent cultivars for qualitative and quantitative traits. This study was therefore conducted with the objective of phenotypic characterization and assessment of genetic diversity of local garlic landraces using morpho-agronomic characters among thirty-seven landraces collected from different parts of the country.

METHODOLOGY

EXPERIMENTAL MATERIALS AND DESIGN

The experiment was carried out at NAGRC Khumaltar, Lalitpur during 2019. Geographically it is located at an altitude of 1368 m, latitude of 27° 40'N and longitude of 085° 20'E. The characterization blocks have black loamy soil. Thirty-seven garlic landraces collected from different parts of the country as depicted in Table 1 and Figure 1 are grown in field gene bank of NAGRC. Experiment was conducted in non-replicated rod row design for agromorphological characterization. Cloves were separated from the bulb, and only the healthy cloves were selected for planting. Each landrace was planted on 6th Nov 2019, in 1.08 m² (120cm × 90cm) plot with the spacing of 20cm × 15cm. Fertilizers were applied @ 100:50:60 kg N₂:P₂O₅: K₂O/ha. Total phosphorus and potassium and half of the nitrogen was applied before planting and rest of the nitrogen was top dressed in two equal splits (30 and 45 days after planting) during weeding. Weeding and pulverizing of soil were done regularly whenever necessary to keep the plots free from weeds and to ensure good aeration in soil. The garlic plants when the tops turn yellowish or light brownish and show signs of drying up indicate the symptom of maturity. The bulbs were lifted, freed from earth and the leaves were tied at the top. The bulbs were cured for 2 to 3 days in the shade before storing them in an ordinary room. Thoroughly cured garlic bulbs stored well in ordinary well-ventilated room.

Table 1: Garlic accessions with their collection districts

S.N.	Accessions	Collected district	Altitude (m)	Latitude (N)	Longitude (E)
1	ARM-02	Kaski	850	28.225	83.975
2	ARM-05	Parbat	952	28.281	83.601
3	ARM-07	Baglung	1993	28.257	83.502
4	ARM-11	Lalitpur	1681	27.475	85.260
5	ARM-14	Sindupalchok	3158	27.965	85.685
6	ARM-20	Lalitpur	1311	27.664	85.368
7	ARM-24	Makwanpur	1828	27.642	85.179
8	ARM-25	Kaski	1170	28.260	83.070
9	ARM-28	Baglung	1183	28.217	83.651
10	ARM-27	Kaski	1392	28.287	83.956
11	ARM-08	Kaski	878	27.937	83.651
12	CO 4812	Rasuwa	1509	28.092	85.250
13	CO 6060	Dolakha	1417	27.596	86.061
14	CO 6077	Dolakha	1200	27.611	86.063
15	CO 10306	Argakhanchi	958	27.985	83.114
16	CO 10307	Argakhanchi	960	27.986	83.115
17	CO 10399	Doti	1529	29.360	80.987
18	CO 10448	Sindhuli	1750	27.152	85.901
19	CO 10482	Sindhuli	1650	27.370	85.750
20	CO 10615	Ramechhap	1150	27.560	86.221
21	ARM-18	Lalitpur	1330	27.679	85.393
22	ARM-09	Kaski	1000	28.250	83.072
23	ARM-03	Parbat	875	28.163	83.651
24	ARM-16	Jumla	3075	29.287	82.118
25	CO 4816	Rasuwa	768	27.200	85.430
26	ARM-26	Kaski	1392	28.287	83.956
27	ARM-19	Lalitpur	1338	27.642	85.343
28	ARM-06	Kaski	1485	28.314	83.969
29	ARM-13	Lalitpur	1388	27.627	85.331
30	Humla Collection-442	Humla	2250	29.873	81.876
31	Mahottari Collection	Mahottari	250	26.958	85.955
32	Surkhet-72	Surkhet	334	28.716	81.335
33	Surkhet-100	Kailali	399	28.733	81.226
34	Surkhet-105	Surkhet	381	28.716	81.335
35	Surkhet-116	Kailali	378	28.732	80.861
36	Surkhet-245	Surkhet	525	28.811	81.553
37	Surkhet-289	Surkhet	526	28.711	81.335

AGRO-MORPHOLOGICAL TRAITS MEASUREMENT

Agro-morphological traits were measured at various growth stages according to descriptors for garlic developed by International Plant Genetic Resources Institute (IPGRI, 2001). Five random plants from each landrace were selected for agro-morphological traits evaluation. Fifteen qualitative traits i.e. plant vigor, external cloves, leaf color, anthocyanin coloration at the base of pseudo stem, bulb skin color, thickness of neck, bulb shape of base, bulb skin thickness, peeling, cracking of bulb skin, clove skin color, anthocyanin stripes on clove skin, clove shape, easiness of dividing bulb into cloves and compactness of cloves were observed at different growth stages. Likewise, nine quantitative traits i.e. days to emergence, plant height, clove length, clove width, clove diameter, numbers of cloves per bulb and weight/bulb, bulb diameter and biomass yield were recorded as mentioned in descriptors. Fresh biomass yield was recorded by accumulating all parts of the plant in the total plot during harvesting time.

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

$$H' = \left[\sum \left(\frac{n}{N} \right) \times \left\{ \text{Log}_2 \left(\frac{n}{N} \right) \times (-1) \right\} \right] / \text{Log}_2 k$$

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 Shannon-Weaver diversity index (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 standard deviation. The diversity index was considered as low ($0.10 \leq H' \leq 0.40$), intermediate ($0.40 \leq H' \leq 0.60$), high ($0.60 \leq H' \leq 0.80$) and very high ($H' \geq 0.80$) (Eticha *et al.*, 2005).

DATA ANALYSIS

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

RESULTS AND DISCUSSION

DIVERSITY BASED ON QUALITATIVE TRAITS

Frequency distribution for fifteen qualitative traits is presented in Table 2. Among qualitative variables, all characters were polymorphic. The diversity index (H') ranged from 0.54 to 0.96, which indicate tremendous diversity present in the garlic landraces for qualitative traits. Very high diversity index (H') was inferred for most of the traits such as thickness of neck, external cloves, peeling quality, cracking of bulb skin, anthocyanin stripes on clove skin, plant vigor, bulb skin thickness, easiness of bulb dividing into cloves, leaf color, anthocyanin coloration at base of pseudo stem, clove shape 0.83 and compactness of cloves. However, this value of diversity index (H') was found high for bulb skin color and intermediate for bulb shape at base.

The existence of high genetic diversity in the Nepalese garlic landraces increases the space for selection for breeders as well as for farmers. This diversity can be utilized in crop improvement and enhancement of genetic potential of garlic accessions. Agro-morphological traits can be considered by farmers to discriminate varieties regarding selection and adoption of a variety. Similar results are reported in garlic by Pooler and Simon (1993) as well as Simon and Jenderek (2003).

Table 2: Morphological character-based diversity index of garlic accessions

S.N.	Qualitative characters	Shannon-Weaver index	Descriptor's states	Frequency	Proportion %
1.	External cloves	0.95	0- Absent	18	49
			9- Present	19	51
2.	Plant vigor	0.89	2-Very weak	3	8
			3- Weak	8	22
			4- Slightly weak	5	14
			5- Intermediate	11	30
			6-Slightly vigorous	4	10
			7-Vigorous	3	8
			8-Very vigorous	1	3
3.	Leaf color	0.84	9Extremelyvigorous	2	5
			2-Very light	3	8
			3- Light	4	10
			4- Slightly light	15	41
			5- Intermediate	15	41

4.	Anthocyanin coloration at base of pseudo stem	0.84	2-Very Weak	2	5
			3- Weak	7	19
			4- Slightly Weak	13	36
			5- Intermediate	12	32
			6- Slightly strong	3	8
5.	Bulb skin color	0.63	1- White	26	70
			2- Light pink	1	3
			4- Light brown	10	27
6.	Thickness of neck	0.96	1- Extremely thin	3	8
			2-Very thin	2	5
			3- Thin	5	14
			4- Slightly thin	8	21
			5- Intermediate	5	14
			6- Slightly thick	5	14
			7-Thick	5	14
			8-Very Thick	4	10
7.	Bulb shape of base	0.54	1- Recessed	1	3
			2- Flat	29	78
			3- Round	7	19
8.	Compactness of cloves	0.81	3-Loose	4	11
			5-Intermediate	10	27
			7-Compact	20	54
			9- Very compact	3	8
9.	Bulb skin thickness	0.88	2- Very thin	1	3
			3- Thin	14	37
			4- Slightly thin	8	22
			5- Intermediate	8	22
			6- Slightly thick	6	16
10.	Peeling	0.94	1- Extremely easy	2	5
			2- Very easy	5	14
			3-Easy	7	19
			4- Slightly easy	9	24
			5-Intermediate	4	11
			6- Slightly hard	3	8
			7-Hard	7	19
11.	Cracking of bulb skin	0.93	2-Very frequent	3	8
			3-Frequent	10	27
			4- Slightly frequent	9	24
			5-Intermediate	11	30
			6- Slightly rare	4	11
12.	Clove skin color	0.93	1-White	24	65
			2-Light pink	13	35

13.	Anthocyanin stripes on clove skin	0.90	0-Absent	12	32
			9-Present	25	68
14.	Clove shape	0.83	3- Slim round	18	49
			5- Flat	3	8
			7- Thick round	16	43
15.	Easiness of dividing bulb into cloves	0.86	2- Very easy	1	3
			3-Easy	4	11
			4- Slightly easy	4	11
			5-Intermediate	12	32
			6- Slightly hard	6	16
			7-Hard	7	19
			8- Very hard	2	5
9-Extremely hard	1	3			

DIVERSITY BASED ON QUANTITATIVE TRAITS

Nine quantitative traits were measured for evaluating variation among garlic landraces (Table 3). The result showed existence of high variation with quantitative traits among the landraces. The coefficient of variation ranges from 16.89 (Clove length) to 87.85 (Biomass yield). Out of nine quantitative characters, eight have CV value more than 20% indicating greater variability among the landraces. The result indicated that there was high level of variation in characters of interest i.e. Biomass yield, weight/bulb and number of cloves/bulbs in garlic. Shannon Weaver index ranges from 0.54-0.78 which showed moderate to high level of diversity among the landraces for quantitative traits. High diversity was found in clove width ($H'=0.78$) and intermediate diversity was found in days to emergence and biomass yield ($H'= 0.54$).

The estimate of coefficient of variation depicted a clear picture of extent of variability present in the available landraces (Table 3). High variation in quantitative characters may be due to the collection of garlic germplasm from different ecological regions of the country. Pathak (1994) reported that utilization of existing variability and selection of the best genotypes to produce superior clone might be the best approach of breeding and crop improvement in garlic. The number of cloves per bulb and weight of average 10 cloves attributed to the yield potential of the accessions which varied from each other might be due to their differences in genetic configuration. This result was supported by Andres *et al.* (1996). There was moderate to high variation found in biomass yield, weight/bulb, days to emergence, plant height, length and width of cloves, and bulb diameter among the garlic landraces. This variation can be used by breeder for selection and development of high yielding varieties. High variation in plant height indicated the potential of accessions for the development of garlic varieties

for fresh consumption which have greater number of fresh leaves. Kohli and Prabal (2000), Khae *et al.* (2005), Kumar *et al.* (2006), Panthee *et al.* (2006), Gupta *et al.* (2007), Singh *et al.* (2012) and Singh *et al.* (2015) have also reported the similar results. Recent genetic studies revealed the presence of considerable genetic diversity among the garlic clones (Buso *et al.*, 2008). Garlic shows wide morphological diversity in bulb size and color, leaf length, growth habits, and agronomic traits such as stress and drought tolerance (Panthee *et al.*, 2006).

Table 3: Analysis of garlic accessions in terms of quantitative characters

S.N.	Characters	Mean±SE	Std.	CV %	Min.	Max.	SWD (H')
1.	Days to emergence	9±0.32	1.95	20.82	7.00	18.00	0.54
2.	Plant height at 30 days (cm)	15.9±0.62	3.82	24.06	8.82	27.00	0.68
3.	Plant height at 120 days (cm)	35.6±1.50	9.11	25.64	22.40	60.80	0.69
4.	Clove length (cm)	3.7±0.10	0.62	16.89	2.50	4.90	0.68
5.	Clove width (mm)	10.4±0.33	2.01	19.27	6.30	14.10	0.78
6.	Number of cloves/bulbs	19±1.27	7.71	39.90	5.00	39.00	0.71
7.	Weight/bulb (g)	17.16±2.01	12.25	71.39	2.33	62.67	0.63
8.	Bulb diameter (mm)	31.9±1.18	7.19	22.53	18.79	53.51	0.70
9.	Biomass yield (kg/plot)	1.79±0.25	1.57	87.85	0.21	8.03	0.54

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

The cluster analysis grouped the landraces into four clusters for nine quantitative traits (Figure 2). The critical examination of the dendrogram revealed four clusters with minimum of 35.60% similarity level in UPGMA clustering. Cluster-1 has landraces having lowest mean value for plant height, clove length, weight/bulb, number of cloves per bulb, longitudinal diameter of bulb, biomass yield and intermediate mean value for days to emergence and clove width. Landraces in Cluster-2 have intermediate mean value for days to emergence, number of cloves per bulb, plant height, clove length, weight/bulb, longitudinal diameter of bulb and biomass yield. Cluster-3 consists of landraces having longer days to emergence and lower plant height, clove length and width, weight/bulb, number of cloves per bulb, longitudinal diameter of bulb and biomass yield than that of Cluster-4. Similarly, Cluster-4 consists of landraces with shortest days to emergence and highest mean value for plant height, clove length and width, number of cloves/bulbs, weight/bulb, longitudinal diameter of bulb and biomass yield than remaining cluster. Characterization of landraces and clustering of them based on their morphological and genetic similarity helps to identify and select the best parents for hybridization. Hence, 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 crop improvement. Landraces of Cluster-4 i.e. CO 10307, CO 10482 and CO 10615 are superior in terms of plant height, clove length and width, weight/bulb, no of cloves per bulb, longitudinal diameter of bulb and biomass yield. These landraces can be included in garlic improvement program after further selection process.

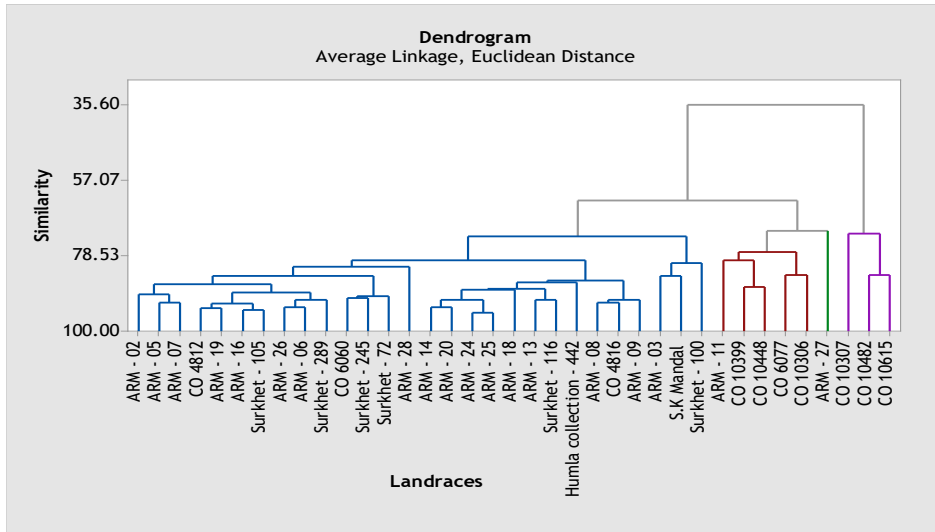


Figure 1: Dendrogram of 37 garlic landraces derived by UPGMA from nine quantitative traits

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

Variables	Cluster-1	Cluster-2	Cluster-3	Cluster-4
Number of accessions	28	5	1	3
Days to emergence	9.60	8.6	11	7.66
Plant height (30 days)	14.32	18.5	16.2	25.80
Plant height (120 days)	31.34	45.00	39.20	57.80
Clove length (cm)	3.48	4.12	3.60	4.80
Clove width (mm)	10.48	9.84	9.50	11.21
Weight/bulb (g)	12.12	28.44	14.60	50.56
Number of cloves/bulbs	16.27	22.84	29.29	34.26
Bulb diameter(mm)	29.17	36.72	31.91	49.51
Biomass yield (kg/plot)	1.41	2.71	1.51	6.33

PRINCIPAL COMPONENT ANALYSIS

Table 5 shows the principal component analysis with eigen values, eigen vectors and variances which partitioned the total variation into five principal components contributing 95.2% of the cumulative variance. The first two principal components with eigen value greater than 1 accounted for 78.7% of the entire variability. The first PC explained 63.5% of the total variance, the traits such as Biomass yield, bulb diameter, plant height and weight/bulblargely contributed in the positive direction while days to emergence loaded in the negative direction. The second PC accounting for 15% of the total variance which was mainly influenced negatively by clove width and positively by number of cloves/bulbs. The traits contributed negatively on the third PC (9%) were days to emergence and clove width. Principal component analysis partitions the total variance into components and measures how each component contributes to total phenotypic variation. Biomass yield, number of cloves per bulb, Bulb diameter and plant height contributed positively for phenotypic variation in our experiment. These traits are directly correlated with yield. These traits can be used for the development of high yielding garlic varieties.

Table 5: Principal Component Analysis of nine quantitative characters

Variables	PC-1	PC-2	PC-3	PC-4	PC-5
Eigen value	5.71	1.37	0.82	0.40	0.25
Proportion	0.63	0.15	0.09	0.04	0.02
Cumulative Variance (%)	63.5	78.7	87.9	92.3	95.2
Days to emergence	-0.181	0.279	-0.922	0.058	-0.119
Plant height at 30 days (cm)	0.382	0.164	0.093	0.213	0.010
Plant height at 120 days (cm)	0.377	0.196	0.045	0.170	-0.583
Clove length (cm)	0.322	-0.298	-0.162	-0.745	0.079
Clove width (cm)	0.113	-0.744	-0.246	0.507	0.168
Number of cloves/Bulb	0.323	0.437	-0.066	0.073	0.677
Weight/bulb (g)	0.377	-0.121	-0.183	-0.232	-0.238
Bulb diameter (mm)	0.398	-0.071	-0.122	0.062	0.245
Biomass yield (kg/plot)	0.397	0.065	-0.000	0.219	-0.193

CONCLUSION

There was high diversity found in both qualitative as well as quantitative traits among Nepalese garlic landraces. This result will obviously be helpful for breeder and researchers to comprehensively understand the agromorphological characters as well as diversity of the Nepalese garlic collection and more easily select the target landraces. Garlic landraces named as CO 10307 from Arghakhanchi, CO 10482 from Sindhuli and CO 10615 from Ramechhap were found superior based on economically important traits such as biomass yield plant height, clove length and width, no of cloves per bulb and longitudinal diameter of bulb. These landraces could be evaluated further in multiple environments and used to develop new garlic varieties.

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