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Research Article

Phenotypic characterization and diversity of Nepalese garlic (*Allium sativum* L.) landraces

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ABSTRACT

A total 37 garlic landraces collection from Nepal Genebank were characterized using phenotypic trait maps or agro morphological markers at National Agriculture Genetic Resources Centre (Genebank), Khumaltar, Lalitpur, Nepal during 2017 using quantitative and qualitative traits. Shannon and Weaver diversity index (H') analysis revealed significant intra landrace diversity for both quantitative and qualitative traits. Nine principal components contributed 100 % to the cumulative variance and the first three principle components with Eigen value >1 accounted for 69.9 % of the entire variability.The cluster analysis grouped the 37 accessions into five distinct group. The Accession CO4816 from cluster 5 were superior in terms of yield attributing characters such as number of cloves/bulb, weight of cloves and yield but found to be shorter is plant height. Pearson correlation analysis among eight quantitative traits showed highly significant positive correlation between leaf length and leaf width, leaf length and shaft length, leaf length and yield, weight of cloves and yield. The high yield is associated with leaf length, leaf width, weight of cloves and number of cloves. The frequency distribution for disease (*Cercospora leafspot and Alternaria porii*) occurrence were very low in 78 % of the accessions.

Keywords: Diversity index, garlic, quantitative trait, qualitative trait

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INTRODUCTION

Garlic (*Allium sativum* L.) from *Alliaceae* family is vegetatively propagated bulb crop. It is monocotyledonous diploid (2n = 16) plant with rigid asexual life cycle. The fresh leaves and cloves of garlic are widely used raw and cooked in culinary globally. It is also consumed as powder form after processing. Garlic has multiple uses, it is used for its aroma, as well as a traditional medicine (Arreola *et al.*, 2015). In historical periods, garlic has been used as spice to enhance the flavor of dishes in various cultures.

Central Asia is considered its center of origin for Garlic (Vavilov, 1926). It is estimated to have originated from its wild ancestor *Allium longicuspis* Regel present around central Asia (Zohary & Hopf, 2012). Garlic is exclusively propagated by cloves which restricts conventional breeding resulting in narrow genetic base for few traits (*Manjunathagowda et al*., 2017; Benke *et al.*, 2020). The phenotypic variation has been reported in garlic from different locations. Despite its exclusive clonal propagation behavior, occurrence of these variation can be assumed to have originated due to the sexual reproduction of wild genotypes, accumulation of mutations, human selection and history of dissemination of the array of clones from the center of origin. However, the diversity of garlic genotypes have been reported at DNA level by research (Al-Zahim *et al*., 1997; Ipek *et al.*, 2003).

Garlic is very important spice crop in many countries however, due to lack of possibility of conventional breeding and genetic and environmental factors affecting its yield and yield related traits, yield is low in many parts of the world. This problem can be overcome through the selection and breeding of high yielding cultivars. The development of cultural technique which starts with the characterization of the garlic germplasm for identifying elite lines under pre-breeding. Garlic is one of the most important spice crop of Nepalese culinary. In Nepal, garlic was being grown using local landraces since time immoral. Recently, new varieties from China has been dominantly introduced replacing the local germplasms. This study, therefore focuses to study the diversity of the local garlic germplasms available in Nepal to identify the elite lines.

MATERIALS AND METHODS

A total of 37 garlic accessions were collected and maintained in the field genebank in Nepal Genebank. These accessions were phenotyped at Genebank, Khumaltar (latitude of 27°40'N, longitude of 085°20'E and altitude of 1360 m a.s.l.) during winter 2017. Experiment was conducted in non-replicated design for agro-morphological characterization. Single clove was separated and planted in recommended spacing of 20 cm × 15 cm in 1.08 (120 cm × 90 cm) meter square plot. Recommended dose of fertilizers were applied @ 100:50:60 kg N_2:P_2O_5: K_2O/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. The phenotypic traits were recorded using the a local descriptor developed with the reference of different scientific descriptors like IPGRI, NIAS Genebank descriptor and NBPGR descriptor for horticulture crops (IPGRI, 2001; Mahajan *et al*., 2000). The observations were obtained from 10 bulbs per accession.

Shannon–Weaver diversity indices (Shannon & Weaver, 1949) were calculated in order to estimate the phenotypic diversity for each qualitative trait with Microsoft Excel using the formula: Descriptive statistics, Shannon-Weaver diversity index (H') and frequency distribution were employed to estimate and analyze the diversity via MS Excel. The coefficient of variation (CV) was calculated based on the formula

CV (%) = (standard deviation/mean values) × 100

Mean values and standard deviation were calculated on the basis of the 10 individual plants or of the 10 randomly harvested cloves.

The Standardized Shannon-Weaver diversity index (H') (Shannon & Weaver, 1949) was calculated in Excel using the formula:

$$H' = \sum [(pi) \times \log (pi)]$$

Where,
H' = Shannon diversity index;
∑ = Sum
pi = n/N
n = Individuals of a given type/species
N =Total number of individuals in a community

For the quantitative traits, accessions were divided into 10 phenotypic classes, < x-2.5sd, x-2.0sd, x-1.5sd, x-1.0sd, x-0.5s, x, x+0.5sd, x+0.5sd, x+1.0sd, x+1.5sd, x+2.0sd, >x+2.5sd, where x is average and sd is standard deviation. For qualitative traits, the frequency of the descriptive trait map was used for the calculation of Shannon and Weaver diversity indices. The diversity index was considered as low ($0.10 \leq H \leq 0.40$), intermediate ($0.40 \leq H \leq 0.60$) or high ($H \geq 0.60$) (Eticha *et al.*, 2005).

The classifications of landraces on the basis of both quantitative traits were performed using multivariate principal component analysis (PCA) in MINITAB version 17 (Minitab, 2010). For systematic analysis, hierarchical clustering was performed using Euclidean distance and Average method. Distance between clusters were analyzed and reported as a dendrogram of Euclidean distances via MINITAB version 17.

RESULTS AND DISCUSSION

Phenotypic characterization of crops based on morphological traits is pre requisite for the management, and conservation of genetic diversity (Manzano *et al.*, 2001) and is important for the selection of elite lines for pre breeding for development of improved varieties (Fraleigh, 1987; Smith *et al.*, 1991). The genetic diversity needs to be accessed for the vegetatively propagated crop as Garlic to maintain the existing diversity to identify the elite lines as mother plants for further dissemination and to maintain the existing germplasms for future use. Characterization of many garlic cultivars have been carried out in genebanks and research programs (Panthee, 2006). Thirteen seven garlic collections of Nepal Genebank presented in Table 1 was phenotyped.

Table 1. Origin and sources of garlic collections

SN	Code	Accession	District
1	Sur-72	Surkhet-72	Surkhet
2	ARM-24	ARM-24	Lalitpur
3	ARM-28	ARM-28	Baglung
4	Col-21	CN-21	Lalitpur
5	Col-13	C N -13	Lalitpur
6	ARM-19	ARM-19	Lalitpur
7	Col-60	C N -60	Bhaktapur
8	ARM- 13	ARM- 13	Lalitpur
9	ARM-07	ARM-07	Baglung
10	Sur-116	Surkhet -116	Surkhet
11	Col-62	C N -62	Bhaktapur
12	ARM-06	ARM-06	Churunga
13	Col-93	C N -93	-
14	C04812	Co 4812	-
15	ARM-09	ARM-09	Kaski
16	ARM-11	ARM-11	Kathmandu
17	ARM-25	ARM-25	Kaski

18	ARM-18	ARM-18	Bhaktapur
19	Col-154	C N -154	
20	ARM-27	ARM-27	Kaski
21	ARM-23	ARM-23	Kathmandu
22	Sur-100	Surkhet-100	Surkhet
23	Sur-105	Surkhet-105	Surkhet
24	Col-66	C.N-66	
25	C04816	Reg. No. 4816	
26	ARM-08	ARM-08	Syanja
27	ARM-14	ARM-14	Sindhupalchowk
28	Sur-289	Surkhet-289	Surkhet
29	ARM-05	ARM-05	Parbat
30	ARM-16	ARM-16	Jumla
31	Col-79	C.N 79	
32	ARM-02	ARM-02	Kaski
33	Sur-245	Surkhet-245	Surkhet
34	ARM-20	ARM-20	Lalitpur
35	ARM-03	ARM-03	Parbat
36	Col-48	C N - 48	
37	ARM-26	ARM-26	Kaski

Descriptive statistics and Shannon Weaver Diversity analysis

Range and average of observations as well as Shannon–Weaver diversity indices for different quantitative traits has been presented in Table 2.

Coefficient of variance of the nine quantitative traits ranged from 11.21 -35.55 %. Shaft length (21.21 %), number of cloves/bulb (21.64 %), weight of cloves (25.02 %), yield/plot (35.42 %) and Plant height (35.55 %) possessed CV higher than 20 % revealing the wider range of variability within the accessions for these phenotypic traits (Table 2). Leaf length (12.08%), leaf width (18.92 %), leaf thickness (1.21 %) and shaft thickness (11.21 %) have low CV (<20 %) indicating minimal intra-variability in these traits. The higher coefficient of Variance (CV>20 %) for qualitative traits like shaft length, number of cloves/bulb, weight of cloves , yield/plot and plant height revealed the wider range of variability within the accessions for these phenotypic traits. Likewise, low CV (<20 %) for Leaf length, leaf width, leaf thickness and shaft thickness have indicates minimal intra-variability for these traits. The higher variability for yield contributing characters demonstrates the selection of elite lines from the existing collections.

Leaf thickness with the range (1.07-1.72 mm) has the highest diversity index of 0.85 and plant height with range (49.15-175.75 cm) has the low diversity index of 0.36. The yield attributing characters such as number of cloves/bulb (0.83), weight of cloves (0.83) and total yield/plot (0.84) showed high diversity indices. Further, other traits; leaf length (0.75), leaf width (0.72), shaft length (0.76) and shaft thickness (0.79) are with diversity index above high diversity indices indicating the existence of high inter genetic diversity (Table 2) within the garlic landraces.

Table 2. Descriptive statistics and Shannon–Weaver diversity index of nine quantitative traits

Traits	Minimum	Maximum	Mean±SE	Std dev	CV (%)	(H’)
Leaf length (cm)	21.96	42.89	32.98±0.65	3.98	12.08	0.75
Leaf width (cm)	1.28	3.01	20.3±0.06	0.38	18.92	0.72
Plant height (cm)	49.15	175.75	70.02±4.09	24.89	35.55	0.36
Shaft length (cm)	3.32	10.32	6.07±0.21	1.28	21.12	0.76
No. of cloves/ bulb	13	35	24.93±0.89	5.39	21.64	0.83
Weight. of cloves (g)	0.79	2.47	1.48±0.06	0.37	25.02	0.83
Yield (g/plot)	105.00	515.00	262.84±15.31	93.11	35.42	0.84
Leaf thickness (mm)	1.07	1.72	1.39±0.03	0.16	11.21	0.85
Shaft thickness (mm)	10.14	18.78	12.63±0.32	1.96	15.54	0.79

Table 3. Shannon–Weaver diversity index, descriptor states and frequency of six qualitative traits

Traits	H’	Phenotypic class	Shape	Frequency	Proportion (%)
Waxiness	0.78	1	Absent	20	54.05
		2	Intermediate	15	40.54
		3	High	2	5.41
Mature dry bulb shape	0.81	1	Flat	1	2.56
		2	Flat globe	6	15.38
		3	Rhomboid	4	10.26
		4	Broad oval	19	48.72
		5	Globe	7	17.95
Bulb skin colour	0.71	1	White	8	22.22
		2	Cream	22	61.11
		4	White stripes	4	11.11
		99	Creamish white	2	5.56
Bulb structure type	0.64	1	Loose	18	50
		2	Semi loose	12	33.33
		3	Compact	7	17.95
Vigour	1.13	1	Extremely weak	8	22.22
		2	Very weak	2	5.56
		3	Weak	10	27.02
		4	Slightly weak	4	11.11
		5	Intermediate	6	16.67
		6	Slightly vigourous	4	11.11
		7	Vigorous	3	8.10
Disease	0.64	1	Low	29	78.37
		2	Intermediate	5	13.51
		3	High	3	8.10

Shannon –Weaver diversity analysis for qualitative traits are presented in Table 3. The diversity index ranged from 0.64 (Bulb structure type, and Disease) up to 1.13 (Vigour). All six qualitative traits have high diversity index ($H \geq 0.60$) (Eticha *et al.*, 2005) confirming high genetic diversity among the garlic landraces in terms of qualitative phenotypic traits (Simon & Jenderek, 2003; Pooler & Simon, 1993; Thapa *et al.*, 2021).

Higher average Shannon-Weaver diversity indices (H') for quantitative characters (0.75) and qualitative characters (0.79) indicates the existence high diversity and suggests the possibility of selection of elite lines based on requirement and breeding objective. The estimate of H' considers both richness and evenness of the phenotypic classes of the traits (Yadav *et al.*, 2018). Study of Shannon diversity index has been widely used in the estimation of existing diversity in germplasm collection of different crops like barley (Tolbert *et al.*, 1979).

of phenotypic diversity in germplasm collection of wheat (Jardat, 1992; Yang *et al*., 1991) and used for improvement of the germplasms.

Principal Component Analysis

For the quantitative traits, the first three principle components with Eigen value >1 accounted for 69.9 % of the entire variability (Table 4).

Table 4. Principal component analysis and Eigen analysis of the correlation matrix

Variable	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8	PC9
Eigenvalue	3.4559	1.4905	1.3415	0.9067	0.6171	0.5269	0.3196	0.2205	0.1213
Proportion	0.384	0.166	0.149	0.101	0.069	0.059	0.036	0.024	0.013
Cumulative variance (%)	0.384	0.550	0.699	0.799	0.868	0.927	0.962	0.987	1.000
Leaf length (cm)	0.423	-0.179	0.069	0.001	0.314	-0.502	0.630	0.191	-0.007
Leaf width (cm)	0.397	0.026	-0.299	0.046	-0.459	-0.500	-0.254	-0.472	-0.045
Plant height (cm)	0.008	0.387	0.457	-0.651	-0.371	-0.180	0.036	0.199	0.081
Shaft length (cm)	0.278	0.148	-0.403	-0.514	0.565	0.038	-0.360	0.037	0.149
No. of cloves/ bulb	0.153	-0.626	0.310	-0.339	-0.008	0.281	0.063	-0.483	0.239
Wt. of cloves(g)	0.258	0.518	0.346	0.370	0.212	0.108	0.018	-0.316	0.503
Yield (g/plot)	0.440	0.069	0.389	0.072	0.142	0.202	-0.229	-0.025	-0.728
Leaf thickness (mm)	0.335	0.227	-0.405	-0.104	-0.315	0.563	0.491	-0.008	-0.062
shaft thickness (mm)	0.436	-0.283	0.058	0.205	-0.264	0.122	-0.330	0.605	0.354

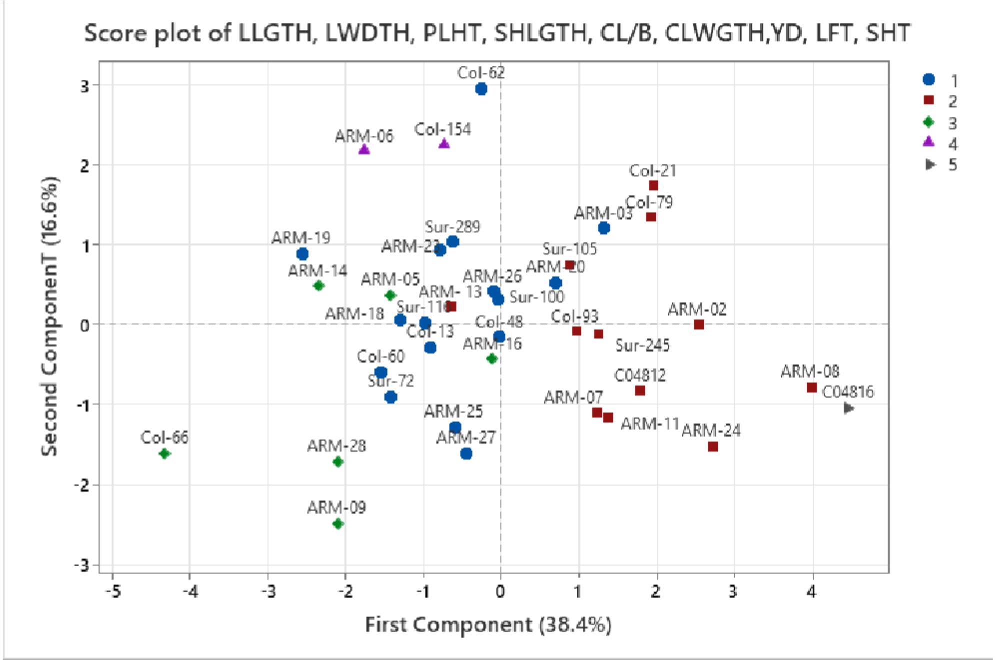

Figure 1. Scatter plots of garlic landraces based on first two principal components

In the first principal component, yield (0.440) leaf length (0.423), and shaft thickness (0.436) with positive loading were most important traits accounting for 38.4 % variation. Similarly, in the second principal component accounting for 16 % of the variation, plant height (0.387), weight of cloves (0.518) and leaf thickness (0.227) with positive loading are important traits. Third component explaining 14.9 % variation includes plant height (0.457), number of cloves/bulb (0.310) showed major contribution. The remaining fourth (10 %), fifth (6 %), sixth (5 %), seventh (3 %), eight (2 %) and ninth (1 %) accounted for quantitative traits (Table 4).

The scatter plot of the first two principal components in Figure 1 accounts for 55 % of cumulative variance. The distribution of accessions based on the first two principal components shows quantitative phenotypic variations among the accessions and the magnitude of spread towards the two axes (Figure 1).

The PCA results show that the most appropriate traits for grouping the garlic landraces comprises of shaft thickness, leaf length, yield, leaf width, leaf thickness, weight of cloves and number of cloves per bulb. As, the first two principal components largely discriminate the garlic landraces it can serve as an important breeding tool for the characterization of these accessions.

Cluster Analysis

The further investigation of phenotypic traits combining quantitative traits were done to group the 37 accessions into clusters with phenotypic similarities using Euclidean distance and average linkage methods. The cluster analysis grouped the 37 accessions into five distinct group (Figure 2). Group I comprised 16 accessions (43.24%), group II of 12 accessions (32.43%), group III of 6 accessions (16.21%), group IV of 2 accession (5.40%) and group V of 1 accession (2.70%) (Table 5).

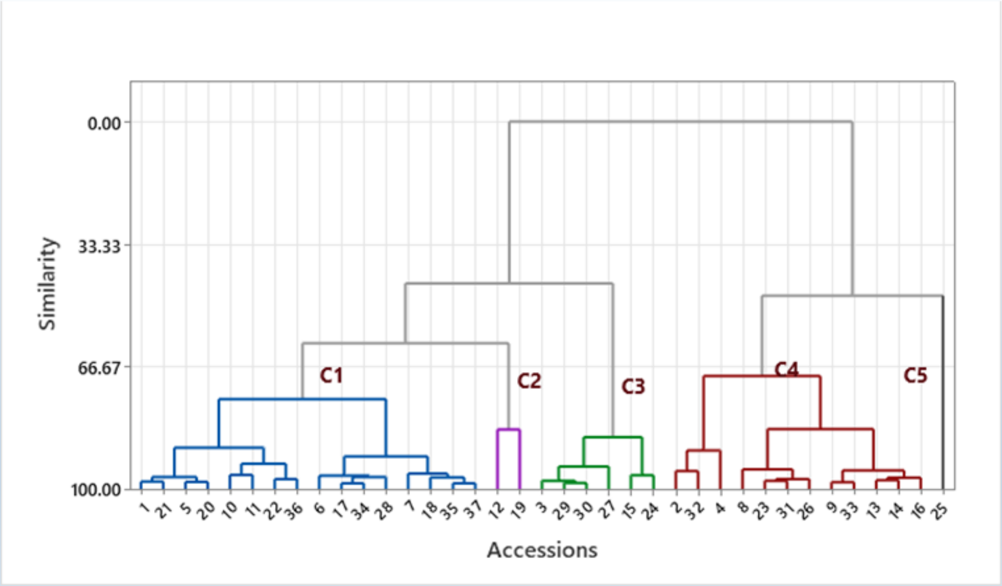

Fig. 2 UPGMA clustering of 37 garlic landraces based on Euclidean distance

Cluster analysis of accessions based on quantitative characters is presented in Table 5 shows that Cluster I has accessions with intermediate yield (85-275 g/plot), height (56-71 cm) and number of cloves (13-27). Cluster II comprises of high yielders (310-425 g/plot), higher clove weight (1.41-1.93 g) and number of cloves (19-35). Cluster III includes accessions with lowest yield (105-160 g/plot), leaf width (1.27-3.0 mm), leaf thickness (1.0-1.48) and shaft thickness (10.41-11.71 mm). Cluster IV has accessions with tall plants (162.3-175.7). Cluster V includes one accession of the highest yielder (515 g/plot), number of cloves (30) and shaft thickness (18.78 mm) and leaf length (40.6 mm). Cluster analysis clearly showed the five cluster with no relation with the geolocations (Panthee *et al*., 2006). Cluster V with garlic accession C04816 found superior in terms of quantitative character as compared to the other clusters.

Table 5. Grouping of 37 garlic andraces by Euclidean UPGMA method

Cluster	**I**	**II**	**III**	**IV**	**V**
Number	**16**	**12**	**6**	**2**	**1**
Accessions	Sur-72 Col-13 ARM-19 Col-60 Sur-116 Col-62 ARM-25 ARM-18 ARM-27 ARM-23 Sur-100 Sur-289 ARM-20 ARM-03 Col-48 ARM-26	ARM-24 Col-21 ARM-13 ARM-07 Col-93 C04812 ARM-11 Sur-105 ARM-08 Col-79 ARM-02 Sur-245	ARM-28 ARM-09 Col-66 ARM-14 ARM-05 ARM-16	ARM-06 Col-154	C04816

The collections from Genebank requires a good phenotyping and evaluation to identify the elite lines for pre breeding. Based on the phenotypic traits, the study demonstrated the existence of a high amount of agro morphological diversity in 37 local germplasm accessions of *A. sativum*. The different collections exhibited good amount of intra varietal variations in yield attributing characters. C04816 under Cluster V were superior in terms of number of cloves/ bulb (13-35), yield (105-515 g/plot) and weight of cloves (0.79-2.47g) (Table 6).

Table 6. Cluster means for nine traits of garlic landraces

Traits	**Cluster**				
	I	**II**	**III**	**IV**	**V**
Leaf length (cm)	32.102	35.786	29.669	29.361	40.600
Leaf width (cm)	1.946	2.187	1.914	1.806	2.680
Plant height (cm)	64.815	67.592	55.031	169.050	74.180
Shaft length (cm)	5.989	6.599	5.425	5.408	6.220
No of cloves/bulb	22.488	27.772	25.306	23.667	30.200
Weight of cloves(g)	1.465	1.686	0.978	1.654	1.824
Yield (g/plot)	228.438	351.250	134.167	267.500	515.000
Leaf thickness (mm)	1.372	1.474	1.317	1.323	1.393
Shaft thickness (mm)	12.150	13.872	11.109	10.486	18.780

Correlation Analysis

Correlation analysis studies the relationship between yield and its components. Pearson correlation analysis among eight quantitative traits presented in Table 7 showed highly significant positive correlation between leaf length and leaf width, leaf length and shaft length, leaf length and yield, weight of cloves and yield (Atinafu, 2021; Sultan & Raina, 2020). Further, number of cloves showed negative correlation with weight of cloves. There is significant but positive correlation between number of cloves and shaft thickness, leaf width and yield, number of cloves/bulb and yield. The association between weight of cloves and yield suggests that weigh of cloves with higher leaf length and shaft thickness should be selected for high yield.

Table 7. Pearson's correlation analysis between eight quantitative traits

Traits	Leaf length (cm)	Leaf width (cm)	Plant height (cm)	Shaft length (cm)	No. of cloves/ bulb	Weight of cloves (g)	Yield (g/plot)	Leaf thickness (mm)
Leaf width (cm)	0.518**							
Plant height (cm)	-0.057	-0.056						
Shaft length (cm)	0.357**	0.383**	0.016					
No of cloves/ bulb	0.336**	0.019	-0.008	-0.005				
Weight of cloves (g)	0.273	0.191	0.232	0.084	-0.252			
Yield (g/plot)	0.588**	0.386	0.186	0.261	0.315	0.636**		
Leaf thickness (mm)	0.280	0.529**	-0.023	0.483	-0.077	0.242	0.317	
Shaft thickness (mm)	0.593**	0.577	-0.161	0.189	0.415*	0.216	0.657**	0.390

The yield of garlic is significantly and positively correlated to the weight of the cloves. The result of this experiment related to morphological trait is very important to characterize the existing accessions, as it helped to estimate variability existing in the landraces which were due to genotype character. The promising landraces included under cluster V and II requires further morphological and molecular evaluation for identification of elite lines. The promising landraces needs to be maintained as core collection of garlic germplasms for Nepal Genebank.

CONCLUSION

The collections from Genebank requires a good phenotyping and evaluation to identify the elite lines for pre breeding. Based on the phenotypic traits, the study demonstrated the existence of a high amount of agro morphological diversity in 37 local germplasm accessions of A. sativum. The different collections exhibited good amount of intra varietal variations in yield attributing characters. C04816 under Cluster V were superior in terms of Number of cloves/ bulb (13-35), yield (105-515 g/plot) and weight of cloves (0.79-2.47g). The yield of garlic is significantly and positively correlated to the weight of the cloves. The result of this study is based on one year was one-year experiments and the interaction between environment and genotype is not considered. However, the preliminary result of the experiment related to morphological trait is very important to characterize the existing accessions, as it helped to estimate variability existing in the landraces which were due to individual genotype character. The promising landraces included under cluster V and II requires further morphological and molecular evaluation for identification of elite lines. The promising landraces needs to be maintained as core collection of garlic germplasms for Nepal Genebank.

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Authors' contribution

D. Singh Shrestha conceptualized the experiment, recorded and analyzed data and wrote the manuscript. K. H. Ghimire supported in data analysis. J. N. Chaudhary and J. Shrestha helped in editing the manuscript. All authors listed have made a substantial, direct and intellectual contribution to the study, and approved it for publication.

Conflict of Interest

The authors declare that there is no conflict of interest regarding the publication of this manuscript.

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