



Multivariate Studies of Bulb Yield and Morphological Characters in Garlic (*Allium sativum* L.) Genotypes at Kulumsa, Southeastern Part of Ethiopia


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ABSTRACT

The experiment was carried out for two consecutive years (2020–2021) from June to October at Kulumsa Agricultural Research Center, Assella, Ethiopia under rain fed conditions using an Alpha lattice design with two replications. One hundred twenty Garlic (*Allium sativum* L.) genotypes were collected from south eastern parts of Ethiopia, were evaluated for genetic diversity of some morphological and bulb yield characters. The investigated traits included vigor, plant height, neck thickness, shaft length/pseudostem, leaf number plant, leaf width, leaf length, cloves number bulb⁻¹, weight of cloves, clove height, clove diameter and total yield. The cluster mean values showed a wide range of variation for all the traits studied. The chi-square (X^2) tests for the six clusters indicate that there are statistically significant differences between all the clusters. The highest inter-cluster distance was recorded between cluster III and VIII ($D^2=10.90$) followed by between cluster III and VII ($D^2=10.55$). One hundred twenty genotypes of garlic were grouped in eleven distinct non- overlapping clusters. The highest number of genotypes were found in cluster I which contained twenty six (26) genotypes, followed by cluster III having twenty one (21) genotypes. From the 12 principal components extracted, the first four principal components (PCs) within an eigenvalue value >1 explained 65.8% of the total variation among the examined genotypes. Selecting materials should be produced among clusters rather than within clusters for future hybridization breeding efforts.

KEYWORDS: Cluster analysis, genetic diversity, principal component analysis

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1. INTRODUCTION

Garlic (*Allium sativum* L.) is a monocotyledonous plant belonging to the family *Amaryllidaceae*, in the order Asparagales. It is native to Central Asia and is cultivated in temperate climates worldwide, with an annual production of 28 million tons on approximately 1.6 million hectares (Anonymous, 2023). China and India are the largest producers of garlic, accounting for 80% of the global production (Anonymous, 2023). Garlic (*Allium sativum* L.) is the most essential crop of the genus *Allium* after onion (*Allium cepa* L.) (Benke et al., 2020; Khandagale et al., 2020). It is widely produced globally and used as a spice, additive, and medicinal plant, as stated (Alare et al., 2020; Avgeri et al., 2020). Garlic has an antimicrobial effect and compounds in garlic have important effects on the immune system (Batiha et al., 2020). It is stated that garlic may contribute to the prevention and treatment of diseases such as obesity, cardiovascular disorders, gastric ulcer and cancer (Sánchez-Sánchez et al., 2020).

In Ethiopia, garlic is a widely cultivated bulb crop with a wide range of climatic and soil adaptation (Zelege and Derso, 2015). Garlic is cultivated on 40,877.55 hectares of land, and 28652.99 thousand tons of yields were harvested in the rainy and belg, with Arsi and East Shewa zones of Oromia region being the major producers (Anonymous, 2022). However, the quality and yield of garlic in Ethiopia are generally low due to biotic and abiotic stresses and management practices under field and storage conditions (Shiferaw, 2016). Although yields are poor, it is widely grown in Ethiopia's central and highlands, both under irrigation and rain-fed conditions (Martha and Marie, 2019). As a result, low production is primarily due to a lack of suitable plant material (Dejen et al., 2021; Tesfaye et al., 2021).

Garlic has a wide range of genetic diversity; depending on soil type, humidity, latitude, altitude, and cultural practices of its cultivation, even a single garlic accession would have a lot of phenotypic variability (Tesfaye et al., 2021). Natural variations in plant parts, for example, have economic significance and suggest the possibility of garlic improvement (Hoogerheide et al., 2017). In addition, a great number of cultivars have resulted through natural and human selection for adaptation in growing areas (Viana et al., 2015). The genetic diversity of desired traits is extremely important to breeding success. However, evidence is scarce on the genetic divergence and cluster distance between garlic accessions in the area. Indeed, the degree of genetic variability in a population (Dejen et al., 2021), which is a universal feature of all species in nature, is a key factor in genetic improvement. When selecting genotypes/ accessions for yield and related traits, the variability of the genotypes

is the most essential component of breeding (Tesfaye et al., 2021). Multivariate analysis is used to determine the degree of divergence and the proportional contributions of certain features to the total divergence (Golakia and Makne, 1992). The D^2 analysis of Mahalanobis (Mahalanobis, 1936) has been used to measure diversity in a variety of crops with great success. Cluster analysis is a useful tool for grouping genotypes into clusters based on weighted averages and gives breeders a way to choose parents (Dangi et al., 2018). Numerous studies have shown the power of a significant ability of principal component analysis (PCA) to determine genetic variation for several polygenic traits leading to the efficient detection of phenotypic variability in crops (Farooq et al., 2015). As a result, this study was designed to estimate the magnitude of genetic distance/divergence among garlic genotypes.

2. MATERIALS AND METHODS

2.1. Description of the study area

The experiment was carried out for two consecutive years (2020–2021) from June to October at Kulumsa Agricultural Research Center, Kulumsa Agricultural Research Center is found in Arsi, Zone Oromia Regional State, Ethiopia, is located 175 km South East of Addis Ababa on the road from Adama to Asella under rain fed conditions. The geographical location of Kulumsa is 8°01' 10"N latitude and 39°09'13"E longitude and at an altitude of 2200 meter above sea level (m.a.s.l). The agro-ecology of the area is characterized by an average annual rain-fall of 850 mm, with short rain between March and April and long rain between June and September, and with annual mean minimum and maximum temperatures of 23.1°C and 7.9°C respectively. The soil type of the area is clay and silt loam with pH of 5.6 (Abayneh et al., 2003).

2.2. Experimental materials

One hundred twenty garlic genotypes (one hundred genotypes were collected from the Bale, West Arsi and Arsi areas and 4 released varieties from the Kulumsa Agricultural Research Center which were basically released by Debre-Zeit Agricultural Research Center) were used for the experiment.

2.3. Experimental design and field management

The garlic accessions/germplasm and released varieties were planted in an Alpha Lattice Design with two replicates in the 2020 and 2021 cropping/Meher season at Kulumsa highlands of Southern Eastern part of Ethiopia. The spacing between double rows, rows and plants was 60 cm, 40 cm and 20 cm. respectively. All agricultural management practices was carried out at the same time to all treatments such as weeding, watering, and hoeing. Fertilizers were also applied at the rate of 243 kg ha⁻¹ NPS during planting and 130 kg

ha⁻¹ urea in split application once during planting and the second application was done after 45 days

2.4. Data collection

Morphological traits were measured at deferent growth stages according to descriptors for garlic developed by the International Plant Genetic Resources Institute (Anonymous, 2001). The following data were recorded based on plant and plot basis vigor, plant height (cm), neck thickness (cm), shaft length pseudostem⁻¹ (cm), leaf width (cm), leaf length (cm), leaf number plant (number), cloves number bulb⁻¹ (no.), weight of cloves (g), clove height (cm), clove diameter (cm) and bulb yield (t ha⁻¹). The bulb yield was recorded from the middle of each plot and converted to t ha⁻¹.

2.5. Data analysis

The analysis of variance (ANOVA) was performed on the data collected for the twelve morphological parameters using R software (Anonymous, 2024), in accordance with the steps specified in Gomez and Gomez (1984). To determine the characteristics that directly influenced bulb yield, the factors that contributed most to overall variation, and the grouping of garlic genotypes, bivariate and multivariate analyses were carried out. To examine the relationships among the quantitative traits that were correlated with each other, principal component analysis was performed using a correlation matrix. This analysis was used to convert the correlated variables into uncorrelated components using R software (Anonymous, 2024). Based on twelve quantitative variables, the genotypes were sorted into appropriate clusters using the ward linkage method. The number of clusters was calculated by Nb-Clust package (Charrad et al., 2014). To calculate the genetic distance between and within clusters,

Mahalanobis' (1936) generalized distance statistics were used. The values calculated between cluster pairs were considered chi-square values and were tested for significance using "p" degrees of freedom, which represents the number of studied traits.

3. RESULTS AND DISCUSSION

3.1. Cluster mean analysis

The cluster mean values showed a wide range of variation for all the traits studied (Table 1). Cluster I was characterized by low mean values for vigor (2.4) and leaf weight (1.52). Cluster II exhibited a high mean for clove diameter (2.00). Cluster III had a high mean for plant height (77.52), shaft length/pseudostem length (29.14), leaf weight (2.04) and leaf length (51.70). Cluster IV was characterized by medium mean for leaf width (1.54). Cluster V was characterized by high mean for vigor (3.38), leaf length (47.70), weight of clove (38.40) and total yield (10.44). Cluster VI was characterized by medium weight of clove (26.62) and clove length (2.59). Cluster VII was characterized by low mean for clove diameter (0.91). Cluster VIII was characterized by low mean for total yield (4.47). Cluster IX was characterized by medium to low mean for total yield (4.48). Cluster X was characterized by medium to low mean for plant height (67.36), neck thickness (0.97), shaft length (23.43) and leaf width (1.74). Cluster XI was characterized by low mean for number of leaf (10.58) and number of clove (11.74) (Table 1). In general, the mean values of each parameter in each cluster indicated that genotypes could be selected for different breeding purposes. Hence, genotypes grouped under clusters III and V were found to be high-yielders based on their mean values for bulb yield ha⁻¹ and other important characteristics. These

Table 1: Cluster means for 12 traits of 120 garlic genotypes at Kulumsa; during the main cropping season of 2020/2021

Clusters	V	PLH	NTH	SHL	NL	LW	LL	NC	WC	CL	CD	Y
I	2.40	66.53	0.96	24.88	11.03	1.52	44.53	18.70	29.20	2.59	1.21	6.92
II	2.81	73.57	1.05	27.10	11.08	1.68	45.60	20.28	22.35	2.36	2.00	6.17
III	3.38	77.52	1.16	29.14	10.99	2.04	51.70	18.59	35.12	2.80	1.35	10.38
IV	2.83	72.34	1.04	28.83	11.26	1.54	45.82	15.15	19.22	2.31	1.08	5.71
V	3.48	73.74	1.23	27.98	11.43	1.71	47.70	21.48	38.40	2.84	1.22	10.44
VI	2.53	71.06	0.88	28.42	11.00	1.73	45.93	22.25	26.62	2.59	1.14	6.48
VII	2.68	65.90	0.89	24.54	11.61	1.54	43.80	20.82	16.54	2.01	0.91	4.55
VIII	2.82	65.43	0.92	23.22	10.44	1.57	44.07	19.61	17.85	2.22	0.97	4.47
IX	2.77	67.70	0.90	24.26	11.70	1.75	46.38	16.94	19.11	2.29	1.07	4.48
X	3.06	67.36	0.97	23.43	11.29	1.74	45.52	23.06	22.87	2.44	1.09	6.37
XI	2.75	68.37	0.75	23.51	10.58	1.61	45.68	11.74	25.51	2.73	1.32	6.53

V: Vigor; PLH: Plant height; SHL: Sheath length; NL: No. leaf plant⁻¹; LW: Leaf width; LL: Leaf length; NC: No. clove bulb⁻¹; WC: Weight of clove; CL: Clove length; CD: Clove diameter; Y: Yield ha⁻¹

clusters could be targeted for further breeding programs to develop high-yielding garlic varieties. Harnet and Yibrah (2015) reported that the clusters mean showed difference for the seven characters of garlic accessions. Similar result was reported by Mulat (2024).

3.2. Estimation of inter-cluster D^2

The studies on genetic divergence among one hundred twenty genotypes of garlic were carried out by using Mahalanobis D^2 statistics'. Genetic improvement through crossing and selection depends on the level of genetic diversity between the parents. The paired D^2 value was calculated based on the pooled average of the accessions. Cluster-wise t-tests showed that there were statistically significant differences between paired clusters (Table 2).

The chi-square (X^2) tests for the six clusters indicate that there are statistically significant differences between all the clusters. The highest inter-cluster distance was recorded between cluster III and VIII ($D^2=10.90$) followed by between cluster III and VII ($D^2=10.55$), between cluster II and III ($D^2=9.18$). The lowest inter-cluster distance was recorded between cluster VII and cluster IX ($D=5.73$) followed by between cluster I and VI ($D^2=5.77$), between cluster I and XI ($D^2=5.86$) (Table 2). Azene (2022) reported that the relative divergence of each from another cluster (i.e., inter-cluster distance) indicated greater divergence between Cluster I and Cluster IV ($D^2=4.15$). The higher distance between groups indicated greater genetic divergence between the genotypes of these groups (Dejen et al., 2021)

Table 2: Average inter-cluster squared distance (D^2) between clusters based on 12 characters of garlic genotypes tested at during the main cropping season of 2020/2021

CLUSTERS	I	II	III	IV	V	VI	VII	VIII	IX	X	XI
I	0	6.26**	9.11**	6.32**	7.05**	5.77**	7.19**	6.58**	6.33**	6.13**	5.86**
II		0	9.18**	6.7**	7.79**	6.53**	8.45	7.91	7.01**	7.17**	7.03**
III			0	8.64**	7.91**	8.72**	10.55	10.9	9.17**	8.7**	9.37**
IV				0	7.85**	6.56**	6.58**	6.82**	6.44**	6.62**	8.04**
V					0	7.21**	9.24**	8.77**	8.17**	8.05**	7.97**
VI						0	6.95**	6.99**	6.13**	6.1**	6.71**
VII							0	6.06**	5.73**	7.1**	7.42**
VIII								0	6.11**	6.39**	7.31**
IX									0	6.06**	7.04**
X										0	6.84**
XI											0

Significance of clusters were computed at 5% and 1% probability level. From chi square table ($X^2=19.675\%$ and 24.725% at 5% and 1% probability level) respectively

3.3. Distribution of genotypes in different clusters

In present investigation one hundred twenty genotypes of garlic were grouped in eleven distinct non-overlapping clusters. This indicates presence of considerable diversity in the genotypes. The genotypes of same origin or geographic region were also found to be grouped together in the same cluster. The instances of grouping of genotypes of different origin or geographic region in same cluster were frequently observed. The highest number of genotypes appeared in cluster I which contained twenty six genotypes, followed by cluster III having twenty one genotypes, cluster X having thirteen genotypes and cluster VIII having twelve genotypes, cluster II having ten genotypes followed by cluster VII and IX nine four genotypes and cluster V and XI having six genotypes followed by cluster VI and IV, which have five and three genotype respectively among all the clusters (Table 3 and Figure 1). Azene (2022) reported

that 28 garlic accessions were classified into four major clusters based on the genetic distance calculated on eleven agro-morphological traits. Similar results were reported by Mulat (2024) garlic forty nine genotypes were clustered (grouped) in to five distinct clusters.

3.4. Principal component's analysis

Principal component analysis (PCA) was used to examine the variability among the 120 garlic genotypes. To validate the clustering (grouping) observed by the cluster analysis (Table 3), principal components analysis (PCA) was executed using the 12 quantitative characters. From the 12 principal components extracted, the first four principal components (PCs) within an eigenvalue value ≥ 1 explained 65.8% of the total variation among the examined genotypes (Figure 2). With eigenvalues of 4.48%, 1.40%, 1.10% and 0.90%, respectively, the overall variances of the

Table 3: The Wards clustering using Euclidean distance method classified 120 garlic genotypes into eleven distinct clusters

Clusters	No. of gen.	By %	Name of genotypes
I	26	21.67%	G.001/2011, G.003/2011, G.022/2011, G.024/2011, G.026/2011, G.028/2012, G.029/2012, G.038/2012, G.040/2012, G.041/2012, G.047/2012, G.044/2012, G.043/2012, G.056/2012, G.059/2012, G.066/2012, G.067/2012, G.070/2012, G.071/2012, G.075/2012, G.083/2012, G.088/2012, G.092/2012, Holeta Local, Tseday, Kuriftu
II	10	8.33%	G.002/2011, G.016/2011, G.037/2012, G.045/2012, G.050/2012, G.064/2012, G.065/2012, G.081/2012, G.085/2012, G.093/2013,
III	21	17.5%	G.004/2011, G.006/2011, G.007/2011, G.008/2011, G.009/2011, G.010/2011, G.011/2011, G.013/2011, G.019/2011, G.020/2011, G.025/2011, G.027/2011, G.030/2012, G.031/2012, G.033/2012, G.035/2012, G.048/2012, G.063/2012, G.079/2012, G.086/2012, G.091/2012
IV	3	2.5%	G.005/2011, G.046/2012, G.061/2012
V	6	4.17%	G.012/2011, G.052/2012, G.053/2012, G.055/2012, G.062/2012, G.090/2012
VI	5	4.17%	G.014/2011, G.021/2011, G.095/2013, G.098/2013, G.105/2013
VII	9	7.5%	G.015/2011, G.017/2011, G.039/2012, G.049/2012, G.051/2012, G.057/2012, G.073/2012, G.077/2012, G.078/2012
VIII	12	10%	G.018/2011, G.042/2012, G.060/2012, G.068/2012, G.069/2012, G.072/2012, G.074/2012, G.082/2012, G.084/2012, G.087/2012, G.089/2012, G.114/2013
IX	9	7.5%	G.023/2011, G.032/2012, G.034/2012, G.036/2012, G.054/2012, G.058/2012, G.076/2012, G.080/2012, Chefe
X	13	10.83%	G.094/2013, G.096/2013, G.097/2013, G.099/2013, G.100/2013, G.101/2013, G.103/2013, G.104/2013, G.106/2013, G.107/2013, G.108/2013, G.115/2013, G.116/2013
XI	6	5%	G.102/2013, G.109/2013, G.110/2013, G.111/2013, G.112/2013, G.113/2013

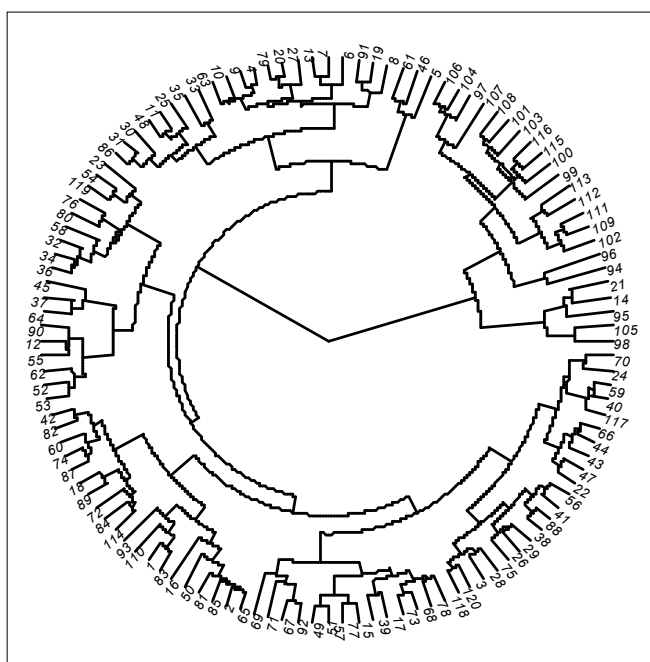


Figure 1: Cluster dendrogram of 120 garlic germplasm based on phenotype traits

twelve variables evaluated among the 120 garlic genotypes, 37.37% and 11.67% were contributed from PC1 and PC2, respectively (Table 4). Harnet and Yibrah (2015) reported that the three principal components captured 81% of the total variation in the data set. According to Mulat (2024) stated that in the result the first two principal components play a significant role in influencing cultivar phenotypes, collectively explaining 74% of the diversity observed in the 49 genotypes, with Eigenvalues exceeding 1.0.

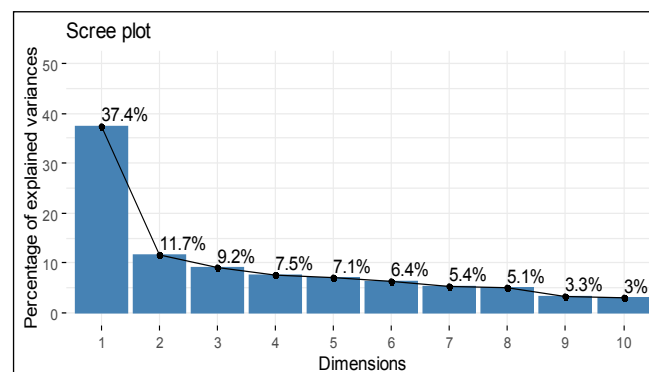


Figure 2: Screen plot

Table 4: Eigen vectors, proportion and cumulative percentage of variation explained by the first four principal components (PC) for morphological and bulb characters of 120 garlic genotypes evaluated at KARC in, 2020/2021

	Principal components			
	PC1	PC2	PC3	PC4
Eigen values	4.48	1.40	1.10	0.90
Proportion of variance (%)	37.37	11.67	9.19	7.53
Cumulative variance (%)	37.37	49.04	58.23	65.76
Characters	Eigenvectors			
Vigor	0.26	0.16	-0.14	-0.14
Plant height	0.33H	-0.06	0.40H	0.28
Neck thickness	0.28	0.29	-0.10	0.30H
Shaft length/ Pseudostem	0.27	-0.22L	0.29	0.55
Number of leaves	0.02L	0.58H	0.34	0.12
Leaf width	0.29	0.21	0.25	-0.45L
Leaf Length	0.32	0.12	0.33	-0.39
Number of cloves	0.04	0.51	-0.45L	0.27
Weight of cloves	0.38H	-0.08	-0.28	0.01
Clove height	0.35	-0.19	-0.30	-0.23
Cloves diameter	0.25	-0.37	-0.02	0.10
Total yield	0.40	0.04	-0.25	0.01

L: Stands for lowest, H: Stands for highest

The total yield ($t\ ha^{-1}$), weight of clove, plant height and clove height were the main contributing features to higher loading effects in the pc1 and number of leaf and number of clove bulb⁻¹ are the highest contributors to the total variation in the pc 2 (Figures 3 and 4). The maximum variability (variable PCA) was seen from parameters which were displayed in the right two quadrants (Figure 4) and genotypes, which are extremes from the origin (PCA-Biplot), signify phenotypic diversity of the genotypes compared with those near to the origin (Figure 5). The higher and lower contributors of genotypes for the total variation were G.096/2013 and G.088/2012, respectively (Figure 6) in the first two pcs. The angle between traits approximate the relationship among them the lower the angle the positive and strong correlation the traits had between them however, if the angle is wider the reverse is true (Yan et al., 2007). Mulat (2024) in his study identified that several primary contributing traits, including clove weight, diameter, and number, pseudo-stem height, leaf width, clove length, bulb weight, diameter, total bulb yield,

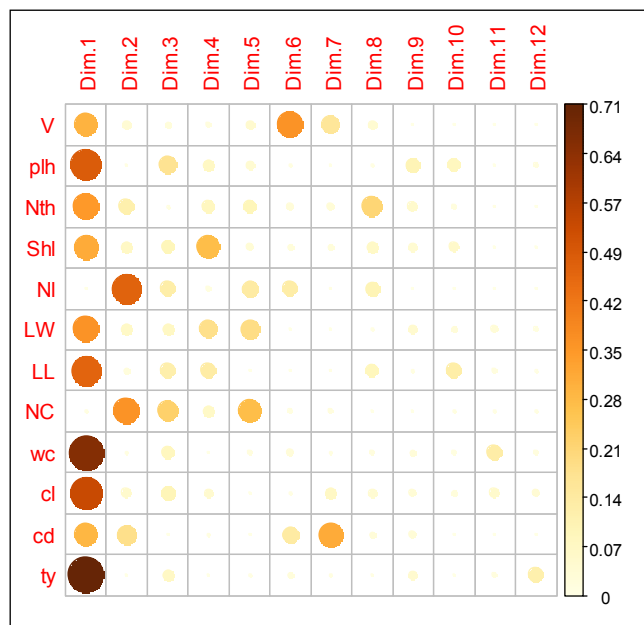


Figure 3: The contributors of the variables in the first two pcs with their correlation

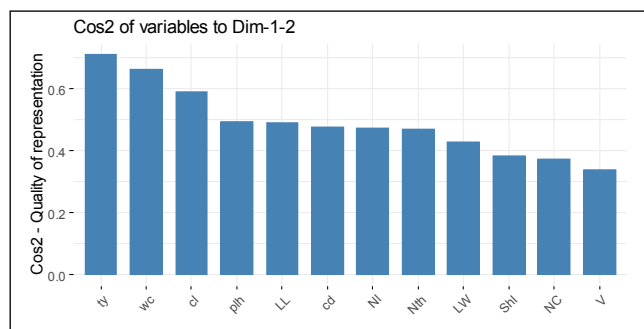


Figure 4: Contribution variables for dimension 1 and 2

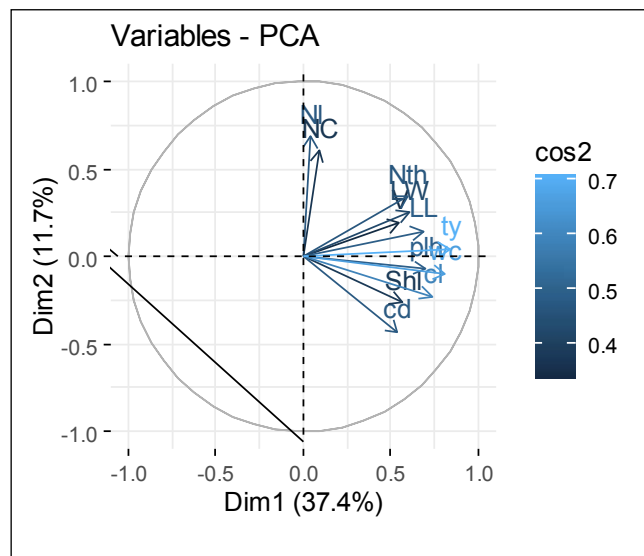


Figure 5: Total contribution of the variables

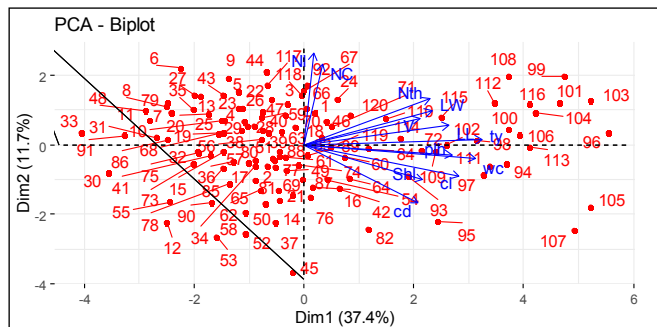


Figure 6: The PCA biplot of both the morphological characters and garlic genotypes

and clove length.

The PC-I showed positive factor loadings for all of the traits while PC-II indicated positive factor loading for all traits except plant height, shaft length, number of cloves, weight of clove, clove height and clove diameter. Traits which contributed positive factor loadings towards PC-III were plant height, shaft length, number of leaves, leaf width and leaf length and PC-IV indicated highest positive factor loading for plant height, neck thickness, shaft length/pseudostem, number of leaves, number of cloves, weight of cloves, cloves diameter and total yield (Table 4). The importance and relationship between variables within a component were determined by the magnitude and direction of factor loadings within a PC (Azeez et al., 2013). The sign of the loading indicates the direction of the relationship between the components and the variable. The greater the loading factors, the higher the contribution of the associated traits to the variance.

4. CONCLUSION

The tested genotypes have a significant level of genetic divergence. The highest inter-cluster distance was recorded between cluster III and VIII followed by cluster III and VII. The highest number of genotypes appeared in cluster I followed by cluster III having twenty one genotypes. The first four principal components explained 65.8% of the total variation. On the basis of present study we can conclude that selecting genotypes for the further breeding programme can be achieved.

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