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Genetic Divergence and Cluster Analysis for Bulb Yield and Related Traits in Garlic Genotypes at Fogera, Northwestern, Ethiopia

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Abstract: Identifying morphological variations among garlic genotypes is crucial for enhancing garlic breeding schemes. This study aimed to assess genetic diversity based on morphological traits of garlic genotypes. The experiment was conducted at the Fogera National Rice Research and Training Center, utilizing a simple lattice design with two replications for planting the genotypes. A total of 49 Ethiopian garlic genotypes were included in the study. Multivariate analysis methods were employed to assess approximately sixteen morphological characteristics. The genotypes were classified into five distinct clusters based on divergence analysis. Clusters 5 and 4 exhibited the highest inter-cluster distance (29.448), indicating genetic diversity. Conversely, clusters 3 and 1 showed the minimum inter-cluster distance, suggesting genetic similarity. Cluster 5 had the highest mean genotypes, indicating its potential for increasing genetic gain through heterosis breeding. Plant height contributed the most to variance, followed by leaf length, number of cloves per bulb, and total soluble solids. The first two principal components explained 74% of the overall variance. Therefore, this study underscores significant diversity in genotypes based on phenotypic characteristics, which could be valuable for future heterosis breeding programs.

Keywords: *Allium Sativum*, Distance, Inter Cluster, Intra Cluster, Principal Component.

Research Paper

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1. INTRODUCTION

Garlic, a widely used spice and vegetable crop, belongs to the Allium genus and the Alliaceae family, existing as a diploid species with a genetic makeup of 2n=2x=16 (Daniel *et al*., 2022). It thrives as a cool season crop in temperate climate regions across the globe (Ayed *et al*., 2019). Known for its health benefits, garlic contains organosulfur and phenolic compounds (Barboza *et al*., 2020), and has been utilized in both traditional and clinical medicine for the prevention and treatment of various diseases (Akan, 2022). Garlic bulbs exhibit diverse characteristics such as flavor, pungency, bolting, and fertility (Goldstein and Shemesh, 2022). Though sterile, cultivated garlic displays genetic variability due to somaclonal variation, mutation breeding, and molecular techniques (Solanki *et al*., 2023), with clonal selection being crucial for its cultivation (Helmy and Ragheb, 2021).

Multivariate analyses like principal component analysis (PCA) and clustering are commonly employed to elucidate genetic variation (Hair *et al*., 1995). Morphophysiological traits serve as key indicators for identifying genetic diversity within and among populations, as well as for assessing genetic similarities and differences (Hunter, 1993). Addressing diversity in plant breeding is essential, especially considering geographical isolation and genetic barriers (Rauf *et al*., 2010). The cultivation of appropriate garlic varieties under various agro-climatic conditions is vital for both domestic consumption and exportation (Singh *et al*., 2013). Genetic divergence analysis, utilizing techniques like Euclidean distance, aids in grouping genotypes to ascertain their degree of variation (Bhati *et al*., 2015).

Cluster analysis categorizes genotypes based on measured variables, grouping similar ones together (Gernot *et al*., 2013). Clustering involves segregating genotypes into clusters with strong internal associations and weak associations between different clusters (Crossa and Franco, 2004). Principal component analysis identifies variables and characterizes traits along differentiation axes (Sharma *et al*., 2016), assisting in the examination of genetic divergence, identification of promising cultivars, and evaluation of trait importance in total genotype variation (Jollife, 1986).

Garlic's susceptibility to diverse agro-climatic and environmental conditions impacts its productivity, exacerbated by a lack of high-yielding cultivars (Tesfaye *et al*., 2021). Genetic diversity in garlic is influenced by

factors such as soil type, humidity, latitude, altitude, and cultivation practices. Despite this, research on the correlation between garlic traits remains limited. Hence, this study aimed to assess genetic diversity among garlic genotypes through diversity analysis, facilitating the selection of suitable genotypes for breeding programs and providing insights into the nature of variability and trait grouping.

2. MATERIAL AND METHODS

The study analyzed the genetic divergence and clustering of 49 garlic genotypes sourced from various Ethiopian agro-climatic regions (referenced as Table 1). Conducted during the primary rainy season, the field experiment took place at the Fogera National Rice Research and Training Center, situated at an elevation of 1819 meters with coordinates of 11° 58′ N and 37° 41′ E.

The experimental layout followed a simple lattice design, featuring plots sized at 1.8 × 1 meters (equivalent to 1.8 square meters). The spacing between double rows was set at 40 centimeters, while there was a 20-centimeter gap between individual rows and 10-centimeter spacing between plants within rows.

Observations were recorded for various parameters, including plant height, leaf number, leaf length, pseudo stem height, leaf width, plant neck thickness, bulb diameter, bulb length, bulb yield, clove number, clove length, clove weight, clove diameter, and total soluble solids.

For cluster analysis, the study employed the unweighted pair group method with arithmetic average (UPGMA) algorithm, alongside sequential, agglomeration, hierarchic, and non-overlapping methods, as outlined by Ran *et al*., (2023). Euclidean distance matrices were clustered using the average linkage method.

Principal component analysis (PCA) was conducted using Pearson correlation coefficients to identify the major traits contributing to variability. PCA was executed utilizing XLSTAT software (Addinsoft, 2018).

Table 1: Description of forty-nine (49) garlic accessions with their sources

Accession code	Collection site	Accession code	Collection site	Accession code	Collection site
G-067	Debrezeit, DZARC	091/04	Debrezeit, DZARC	G-070	Debrezeit, DZARC
G40-1	Angot, FNRRTC	G31-1	Farta, FNRRTC	025/02	Debrezeit, DZARC
G38-2	Ginaza, FNRRTC	G11-1	Angot, FNRRTC	G14-2	Angot, FNRRTC
017/09	Farta, FNRRTC	G24-1	Angot, FNRRTC	G16-2	Debrezeit, DZARC
G34-1	Maksegnit, FNRRTC	G-007	Debrezeit, DZARC	G20-1	Maksegnit, FNRRTC
G16-1	Angot, FNRRTC	G33-2	Farta, FNRRTC	005/09	Farta, FNRRTC
G10-1	Alember, FNRRTC	G36-1	Alember, FNRRTC	HL	Debrezeit, DZARC
G-028	Debrezeit, DZARC	G44-1	Ginaza, FNRRTC	G-044	Debrezeit, DZARC
G-52	Debrezeit, DZARC	G22-2	Ginaza, FNRRTC	G13-3	Ginaza, DZARC
G3-1	Farta, FNRRTC	G17-1	Ginaza, FNRRTC	G42-1	Maksegnit, FNRRTC
G50-1	Farta, FNRRTC	G45-2	Ginaza, FNRRTC	G29-1	Makesgnit, FNRRTC
G4-2	Maksegnit, FNRRTC	G37-3	Angot, FNRRTC	G35-1	Maksegnit, FNRRTC
009/04	Debrezeit, DZARC	G3-2	Maksegnit, FNRRTC	G39-2	Angot, FNRRTC
G-061	Debrezeit, DZARC	G5-2	Maksegnit, FNRRTC	G10-2	Angot, FNRRTC
G30-3	Angot, FNRRTC	027/06	Debrezeit, DZARC	G1-1	Alember, FNRRTC
G44-2	Angot, FNRRTC	G-011	Debrezeit, DZARC	G18-2	Alember, FNRRTC
				G14-1	Ginaza, FNRRTC

Note: DZARC, Debre Zeit Agricultural Research Center, Oromia, Ethiopia; and FNRRTC, Fogera National Rice Research and Training Center, Amhara, Ethiopia

2.1. Cluster Analysis and Genetic Divergence

The SAS Proc cluster procedure was utilized for genotype clustering; employing Mahalanobis' D square statistics (D^2) to classify genotypes into distinct groups. The average intra- and inter-cluster D^2 values were computed using the formulas $(\sum D^2 i)/n$, where $\sum D^2 i$ represents the total distance between all possible genotype combinations within a cluster (Sikder *et al*., 2022).

Genetic divergence was further assessed using generalized Mahalanobis' statistics, where $D^2 ij$

represents the distance between two groups, i and j. The formula for D^2ij is as follows: $D^2ij = (Xi - Xj) S (Xi - Xj)$, where Xi and Xj denote the mean vectors of the i^{th} and j^{th} genotypes, respectively, and S is the inverse of the pooled divergence matrix.

Chi-square ($\chi 2$) values for cluster pairs were evaluated for significance at 1% and 5% probability levels by comparing them to tabular values for the 'P' degree of freedom (Singh and Chaudhary, 1985).

Optimal clusters were determined through the examination of Cubic Clustering Criteria (CCC), Pseudo F statistic, and pseudo t^2 statistic using SAS software (Pacáková, 2013).

Principal Component Analysis

The principal components were estimated using XLSTAT statistical software 2018 and the SAS PRINCOMP procedure, both based on pre-standardized original data.

In the PCA analysis, the first principal component (Y1) is calculated by linearly combining the original variables (X1, X2... Xp) using the formula:

$Y1 = a11X1 + a12X2 + ... + a1pXp$

Similarly, the second principal component (Y2) is computed using the same formula:

$Y2 = a21X1 + a22X2 + ... + a2pXp$

This process allows for manipulation of the number of major components to be equal to the initial variables. However, this discussion primarily focuses on identifying significant components with Eigenvalues exceeding one.

3. RESULTS AND DISCUSSION

3.1. Genetic Divergence and Cluster Analysis

The D^2 analysis examined 49 genotypes based on morphological traits, utilizing the Mahalanobis Euclidean Distance technique to classify them into five clusters. Cluster 1 contained the highest number of genotypes, followed by Cluster 3, while Cluster 4 comprised the fewest genotypes (refer to Table 4). The largest cluster accounted for 38.78% of the total variability, whereas the smallest clusters, Cluster 1 and Cluster 4, represented 2.04% each. This result was in line with Mishra *et al*., (2018).

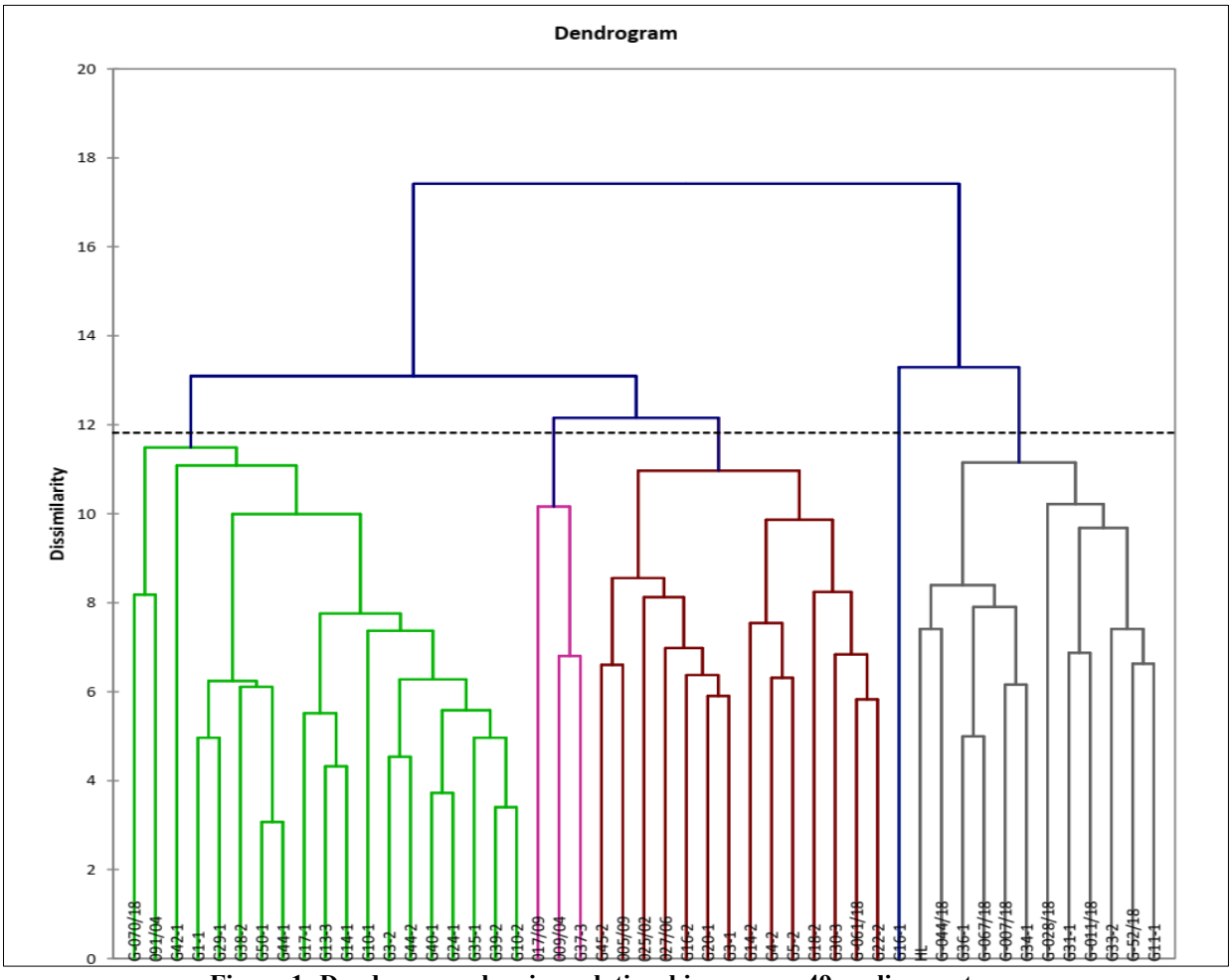

Figure 1: Dendrogram showing relationships among 49 garlic genotypes

Table 2: The distribution of 49 garlic genotypes into five clusters based on Euclidean distance

Cluster Numbers	Number of Genotypes	List of Genotypes in a Group
1	19	G50-1, G38-2, G44-1, G1-1, G39-2, G3-2, G13-3, G44-2, G29-1, G-070/18, G14-1, 091/04, G35-1, G40-1, G24-1, G10-2, G17-1, G42-1 and G10-1
2	12	G-52/18, HL, G-007/18, G36-1, G33-2, G34-1, G-067/18, G-028/18, G31-1, G11-1, G-044/18 and G-011/18
3	14	G14-2, G30-3, G-061/18, G45-2, G22-2, G20-1, G4-2, G5-2, 005/09, G3-1, 027/06, G18-2, G16-2 and 025/02
4	1	G16-1
5	3	017/09, 009/04 and G37-3

3.2. Cluster Means of 49 Garlic Genotypes

Significant differences were noted in 16 garlic traits across different clusters. Cluster 1 exhibited the highest mean values for plant height, maturity days, bulb diameter, bulb length, total soluble solids, leaf length, and clove length, whereas Cluster 5 displayed the highest mean values for the same traits.

Similarly, Singh *et al*., (2014) and Islam *et al*., (2020) reported the highest mean values for maturity days, plant height, bulb diameter, bulb length, total soluble solids, clove length, leaf length, and pseudo-stem height across five clusters comprising nineteen genotypes of garlic.

Table 3: Cluster means for sixteen traits of 49 garlic genotypes

Traits	Cluster 1	Cluster 2	Cluster 3	Cluster 4	Cluster 5
LN	8.3	8.43	8.96	8.7	10.63
LW	1.05	0.99	1.16	0.94	1.3
LL	24.91	23.68	27.75	19.63	30.69
PSH	20.61	14.79	22.56	15.07	20.17
PH	44.67	43.65	49.31	36.2	50.6
PND	0.64	0.71	0.76	0.47	0.98
MD	106.32	112.46	108.29	107	113.5
BL	33.34	30.21	34.17	28.36	38.29
BD	35.15	30.49	37.08	28.63	39.78
CL	25.03	22.79	27.74	19.81	28.86
CD	17.31	14.46	18.04	13.77	18.97
CN	7.43	10.23	8.83	6.8	9.47
TSS	28.66	25.52	27.62	27.34	26.54
BW	13	9.97	17.19	8.4	18.96
CW	0.36	0.23	0.44	0.22	0.39
TBY	3.65	2.48	4.74	2.13	4.4

Note: LN = leaf number; LW = leaf width; LL = leaf length; PSH = pseudo stem height; PH = plant height; ND = neck diameter; MD = maturity date; BL = bulb length; BD = bulb diameter; CL= clove length; CD = clove diameter; CN = clove number; TSS = total soluble solid; BW = bulb weight; CW = clove weight; TBY = total bulb yield.

3.3. Intra Cluster and Inter Cluster Distance of 49 Garlic Genotypes

The study found no significant differences in inter-cluster distances but identified significant differences in intra-cluster distances, particularly between clusters 4 and 5. It suggests that genotypes demonstrate greater genetic diversity in clusters with the greatest distances between them, while the smallest distances are observed between clusters 3 and 1.

The findings imply potential for further development through hybridization and clonal selection. Intra-cluster values indicate homogeneity within clusters due to their smaller results. The study proposes that selecting genotypes with large cluster distances, especially clusters 5 and 4, can enhance garlic bulb yield through heterosis breeding.

Table 4: Estimates of average intra (bold diagonal) and inter-cluster distances for 5 clusters in garlic

Clusters	1	2	3	4	5
1	6.27				
2	10.61ns	6.77			
3	9.92ns	13.14ns	6.71		
4	17.27ns	13.58ns	24.01ns	0	

Clusters	1	2	3	4	5
5	15.94ns	19.25ns	8.62ns	29.448*	5.21

Note: *; ns; significance at 5% probability level and ns = non significance at 5% probability level respectively from chi square table ($\chi 2$ = 24.966 and 30.578 at 5% and 1% probability level) respectively.

3.4. Principal Components Analysis

Principal component analysis (PCA) aids in identifying plant factors that significantly contribute to genotype variation, thereby facilitating parental selection for breeding purposes (Ahmadizadeh and Felenji, 2011).

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.

The study identified several primary contributing traits, including clove weight, diameter, and number, pseudo-stem height, leaf width, clove length, bulb weight, diameter, total bulb yield, and clove length. These traits are crucial in characterizing genotype variations and can inform breeding efforts aimed at improving garlic cultivars.

Table 5: Principal component analysis, Eigen value and total variability explained by the six teen traits of garlic genotypes

Traits	Eigen vectors	
	P1	P2
Leaf number (count)	0.2	0.34
Leaf width (cm)	0.28	0.12
Leaf length (cm)	0.3	0.15
Pseudo stem height (cm)	0.28	-0.22
Plant height(cm)	0.29	0.19
Neck diameter (mm)	0.2	0.4
Maturity date (days)	-0.08	0.46
Bulb length(mm)	0.28	-0.03
Bulb diameter (mm)	0.3	-0.08
Clove length (mm)	0.29	-0.03
Clove diameter (mm)	0.26	-0.2
Clove number (count)	-0.03	0.46
Total soluble solid (%)	0.05	-0.3
Bulb weight (kg)	0.31	0.03
Clove weight(kg)	0.27	-0.21
Total bulb yield (t/ha)	0.3	-0.04
Eigenvalue	8.67	3.09
Variability (%)	54.2	19.34
Cumulative %	54.2	73.54

***Note**: LN = leaf number; LW = leaf width; LL = leaf length; PSH = pseudo stem height; PH = plant height; ND = neck diameter; MD = maturity date; BL = bulb length; BD = bulb diameter; CL= clove length; CD = clove diameter; CN = clove number; TSS = total soluble solid; BW = bulb weight; CW = clove weight; TBY = total bulb yield.*

The bi-plot analysis unveiled that genotype diversity is more pronounced among genotypes situated closer to the origin, while genotypes farther from the origin exhibit greater similarity.

Specifically, the study identified genotypes G16-1, G14-2, 017/09, G007/18, and G-011/18 as diverse, clustering closer to the origin. Conversely, genotypes such as G20-1, 091/04, 027/06, G16-2, G40-1, 005/09, G30-3, G5-2, G22-2, G18-2, G4-2, G23-3, and G10-1 were found to be less diverse and could be targeted for bulb yield improvement.

Moreover, the study uncovered a strong positive association between various traits, including maturity date, clove number, neck diameter, leaf number, plant height, leaf length, bulb weight, bulb diameter, and total soluble solids. These findings provide valuable insights for genotype selection and breeding strategies aimed at enhancing garlic bulb yield.

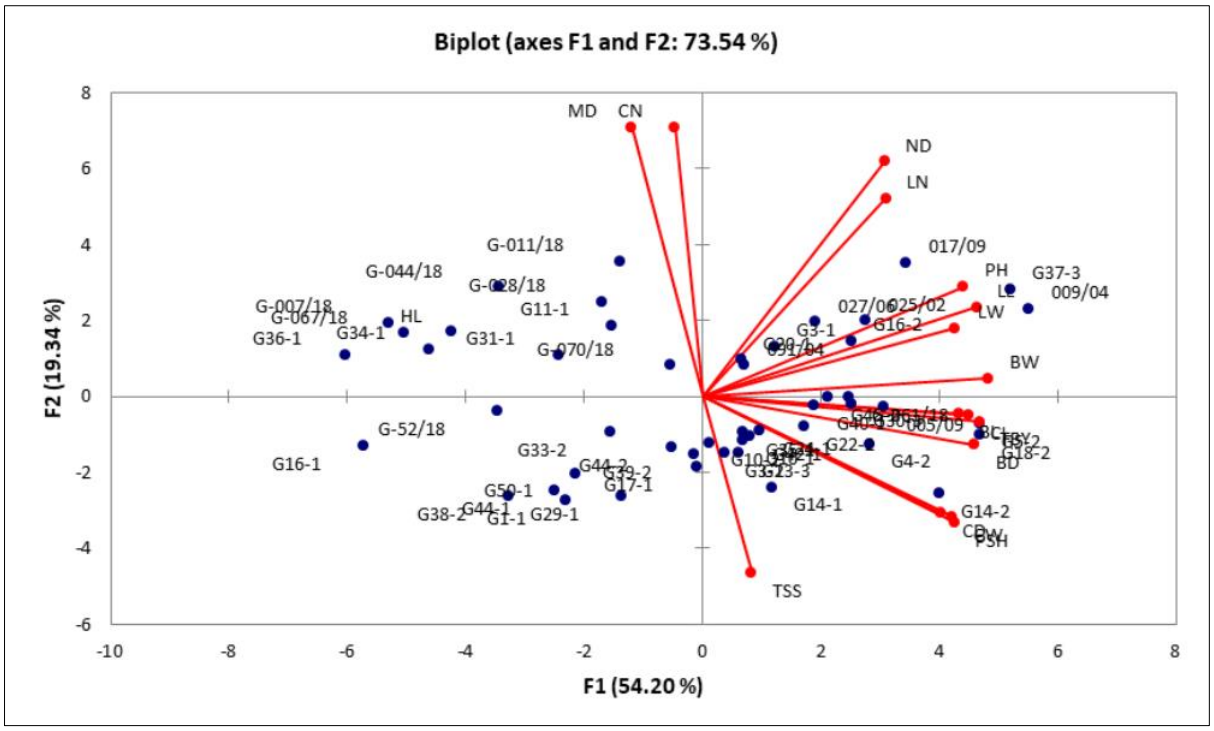

Figure 2: Bi plot of PC1 and PC2 showing relationships of genotypes by traits

Note: The light-black dot color represents genotypes (n=49) and the red color represents the traits under study.

4. CONCLUSIONS

The genetic diversity within a crop like garlic is crucial for its improvement and resilience against pests, diseases, and environmental stresses. By identifying distinct clusters, researchers can identify varieties with unique traits that could be valuable for breeding programs aimed at enhancing yield, flavor, or resistance. Hence, five main clusters were formed from 49 genotypes. Cluster 3 and 1 were determined to be closest, with clusters 5 and 4 having the greatest distance. It's encouraging that clusters with high genetic divergence were identified, as they offer a wealth of variation to draw upon for future breeding efforts. This could potentially lead to the development of garlic varieties better suited to specific growing conditions or culinary preferences.

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