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Genetic Diversity and Cluster Analysis of Ethiopian Hot Pepper Genotypes Using Multivariate Approaches

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Abstract: ***Hot peppers are widely used as a spice for flavour enhancement, colouration, and seasoning, with increasing global demand. This study, conducted during the 2022/2023 cropping season, aimed to evaluate genetic variability among landrace accessions and their trait associations using multivariate analysis under semi-irrigation conditions. A total of 19 landrace genotypes and 1 improved variety were assessed across 12 quantitative and 9 qualitative traits. A randomized complete block design (RCBD) with three replications was employed. Cluster analysis categorized the genotypes into three groups: Cluster I (10 genotypes), Cluster III (6 genotypes), and Cluster II (4 genotypes). The highest genetic divergence was observed between Cluster I and Cluster II (74.20), followed by Cluster II and Cluster III (44.87) and Cluster I and Cluster III (31.86). The considerable genetic distance among these clusters suggests that they could be used in breeding programs to develop improved varieties. Principal component analysis (PCA) identified five significant principal components (PCs), with eigenvalues ranging from 2.51 to 1.44, accounting for 77.74% of the total variance. The first four PCs contributed 20.93%, 20.29%, 14.75%, and 12.02%, respectively. This study highlights the broad genetic diversity among Ethiopian hot pepper genotypes, providing valuable insights for future breeding programs and germplasm conservation.***

Keywords: ***Genetic variability, Cluster Analysis, Principal Component Analysis, Multivariate Analysis, Hot Pepper, Ethiopia***

Nomenclature:
EBI: Ethiopian Biodiversity Institute
RCBD: Randomized Complete Block Design
ED: Euclidean Distance
UPGMA: Unweighted Pair Group Method with Arithmetic Means
PCA: Principal Component Analysis

I. INTRODUCTION

Hot pepper (Capsicum annuum L.) is the second most important vegetable globally after tomatoes, and is widely cultivated for its culinary, medicinal, and nutritional benefits [19]. It is a key spice used for flavour enhancement, colouring, and seasoning, and also serves as a rich source of vitamins A and C, minerals, and antioxidants.

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Native to Mexico, it was introduced to India by Portuguese traders before 1785 AD [22] and has since become an essential crop in tropical regions worldwide [16].

The genus Capsicum belongs to the family Solanaceae [9] and is commonly known as red chilli, hot pepper, chilli pepper, tabasco, paprika, cayenne, and others [2]. The two widely recognized species are Capsicum annuum L. and Capsicum frutescens L. [18]. Morphological similarities among Capsicum annuum, Capsicum chinense, and Capsicum frutescens often lead to classification challenges [24].

Hot pepper was introduced to Ethiopia between 1520 and 1770 by Portuguese explorers and has since become an integral part of Ethiopian cuisine and culture. It is primarily grown by smallholder farmers, covering an estimated 246,000 hectares, making Ethiopia one of the largest producers [7]. On average, Ethiopians consume 15 grams of chilli per person per day, which contributes significantly to their daily vitamin intake.

Genetic diversity is crucial for crop improvement and breeding programs, as greater genetic variation enhances the likelihood of developing superior varieties with desirable traits [12]. Assessing genetic divergence within populations enables breeders to select genetically distant parents, thereby improving hybrid vigour and reducing the number of required crosses [11].

Multivariate statistical tools such as Principal Component Analysis (PCA) and Cluster Analysis are widely used to evaluate genetic diversity in self-pollinated crops [8] and [17]. These methods help identify trait associations, genetic relationships, and distinct clusters of genotypes, facilitating effective germplasm selection and breeding strategies [3].

Cluster analysis groups genotypes based on their morphological and genetic similarities, forming a dendrogram that visually represents these relationships [3]. PCA reduces complex datasets to fewer principal components, allowing breeders to focus on key traits that influence genetic variation [23] and [4].

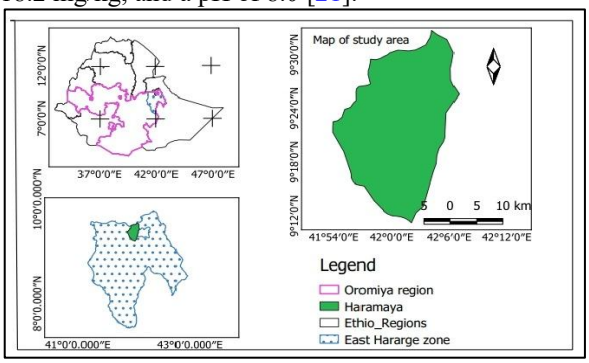
This study aims to assess the genetic diversity, cluster relationships, and principal component contributions among Ethiopian hot pepper genotypes, providing valuable insights for future breeding and conservation efforts.

II. METHODOLOGY

A. Description of Study Area

This study was conducted at Haramaya University, located in Haramaya Woreda, East Hararghe Zone, Oromia Regional State, Ethiopia (Figure 1). The research site lies at 9°26′ N latitude and 42°03′ E longitude, with an altitude of 1,980 meters above

sea level (m.a.s.l.). The area experiences a mean annual rainfall of 760 mm, an average temperature of 16°C, and a relative humidity range of 20%-81% [5]. The soil type is alluvial, with an organic carbon content of 1.15%, a total nitrogen content of 0.11%, an available phosphorus content of 18.2 mg/kg, and a pH of 8.0 [21].



[Fig.1: Map of Study Area]

B. Planting Material and Experimental Design

The experiment involved 20 hot pepper genotypes, comprising 19 landraces maintained at the Ethiopian Biodiversity Institute (EBI) and 1 improved variety (Marako Fana), obtained from the Fedis Agricultural Research Centre, as shown in Table 1.

The study was arranged in a randomized complete block design (RCBD) with three replications. Seeds were first sown in seedbeds measuring 7.8m × 2m, and seedlings were transplanted 40 days after sowing, once they reached 20–25 cm in height. Each experimental plot measured 1.5m × 2m (3m² total area), with 50 cm spacing between plants and 70 cm between rows. The distance between plots and replications was 0.7m and 1m, respectively.

Table I: The Passport of Experimental Material Used for this Study was Obtained From EBI

Accession No	Region/Region	Zone/Zone	Woreda	Latitude	Longitude	Altitude
20206	Oromiya	Misrak Wellega	Sasiga	09-12-47-N	36-25-52-E	1667
20207	Oromiya	Misrak Wellega	Wama hagelo	08-48-31-N	36-55-12-E	1597
20212	Oromiya	Illubabor	Metu	08-20-07-N	35-35-08-E	1678
20213	Oromiya	Illubabor	Ale	08-11-36-N	35-32-27-E	1758
20214	Oromiya	Illubabor	Chora	08-21-53-N	36-03-13-E	1652
27845	SNNP	Bench maji	Gurra farda	06-46-37-N	35-11-50-E	1408
27846	SNNP	Bench maji	Gurra farad	06-90-46-N	35-18-32-E	1121
28334	Oromiya	Illubabor	Harrumu	08-21-36-N	35-43-39-E	1657
28336	Oromiya	Illubabor	Durame	08-26-45-N	35-52-54-E	1868
28337	Oromiya	Illubabor	Durame	08-25-43-N	35-52-48-E	1877
28338	Oromiya	Illubabor	Algae	08-84-43-N	35-45-60-E	1831
229694	Benishangul Gumuz	Metekel	Dibate	-	-	1640
229695	Benishangul Gumuz	Metekel	Dibate	-	-	1650
229696	Benishangul Gumuz	Metekel	Dibate			1700
229697	Benishangul Gumuz	Metekel	Dibate	-	-	1440
229698	Benishangul Gumuz	Metekel	Dibate	-	-	1520
229699	Amara	Misrak gojam	Bibugn	11-07-00-N	37-44-00-E	1850
229700	Amara	Misrak gojam	Bibugn	11-06-00-N	37-44-00-E	1830
229701	Amara	Misrak gojam	Hulet ej enese	11-05-00-N	37-46-00-E	1940
M/fana						

C. Crop Management Practices

i. *Soil Preparation:* The land was ploughed and levelled, incorporating decomposed cow dung and recommended fertilisers before transplanting.

ii. *Fertilization:* 200 kg/ha of DAP was applied at transplanting, followed by 100 kg/ha of UREA, divided into two applications—half at transplanting and half 15 days later [10].

iii. *Irrigation:* A semi-irrigation system was used, with supplementary watering as needed.

iv. *Weeding and Mulching:* Regular weeding, hoeing, and mulching were conducted to reduce competition and improve soil structure.

v. *Harvesting:* Fruit harvesting began at 75 days after planting (DAP) and continued every 25 days until the final harvest.

D. Data Collection

Quantitative (12) morphological data were collected according to the descriptor for Capsicum [14]. At harvest, 10 guarded plants were randomly taken from each plot to measure quantitative morphological characters. Some of the characters were measured before harvest. The sampling was designed to avoid border effects. For this purpose, the outer two lines and the end of the middle rows were excluded. The following quantitative morphological data were collected:

i. *Quantitative Characters Measured*

- **Plant Height (PH):** Length in centimetres of the central axis of the stem, measured from the soil surface up to the tip of the stem and the average was recorded. Recorded when in 50% of the plants the first fruit has begun to ripen
- **Days to 50% Flowering (DFL):** Number of days from transplanting to when 50% of plants in a plot open the flower.
- **Days to 50% Fruiting (DF):** Number of days from transplanting until 50% of the plants bear mature fruits at the first and second bifurcation. Recorded on mature fruits.
- **Number of Flowers Per Axil (NFLA):** the number of

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flowers counted per axil recorded on a fully open flower.

- **Days to First Harvest (DH):** Number of days from transplanting to first harvest.
- **Number of Fruits Per Plant (NFP):** Average number of chilli fruits, counted at harvest on 10 sample plants of each plot
- **Fruit Length (FL):** The average length of five chilli fruits was measured in centimetres on 10 plants of each plot.
- **Fruit Width (FW):** Measured at the widest point. Average fruit width of 10 ripe fruits.
- **Fruit Weight (FWT):** Average fruit weight of 10 ripe fruits of the second harvest
- **Number of Seeds Per Fruit (NSF):** Average of at least 10 fruits selected from 10 random plants.
- **1000-Seed Weight [g] (TSW):** The weight of 1000 seeds measured for each plot.
- **Yield Per Plot [Kg] (FYPP):** The weights of total fruits harvested in each plot from all central row plants were recorded to estimate yield per plot.

$$EDjk = \sqrt{\sum_{i=1}^{n}(Xij-Xik)^2}$$

E. Data Analysis

i. Cluster Analysis

Cluster analysis is a set of multivariate techniques whose primary purpose is to group objects (e.g., respondents, products, or other units) based on their characteristics. It is a means of grouping genotypes based upon attributes that make them similar.

Euclidean distance (ED) was computed from all data collected for hot Pepper accessions after standardization (subtracting the mean value and dividing it by the standard deviation) as: Where EDjk = distance between accessions j and k; xij and xik = phenotype trait values of the ith character for genotypes j and k, respectively; and n = number of phenotype traits used to calculate the distance. The distance matrix derived from phenotypic traits was used to construct dendrograms using the Unweighted Pair Group Method with Arithmetic Means (UPGMA). The results of cluster analysis were presented as dendrograms. In addition, the mean ED was calculated for each accession by averaging the ED across the 28 genotypes. The calculated average distance (ED) was used to estimate which genotype(s) are closest or most distant from others.

ii. Principal Component Analysis

Principal component analysis was performed to identify the variables that contributed most to the total variance, using the correlation matrix in SAS. Principal component analysis (PCA) was used to identify the characters that accounted for the greatest proportion of the total variation. Only principal components with eigenvalues greater than one are considered in this analysis, according to [6].

The data were standardised to a mean of zero and unit variance before performing principal component analysis. Principal components were calculated in SAS using the following formula.

The linear combination of the variables X1 and X2 yields the first PCA value (Y1)... Xp

Y1 = a11X1 +a12X2 +...+a1pXp

The second principal component is calculated in the same way,

Y2 = a21X1 +a22X2 +...+a2pXp

This continues until a total of p principal components have been calculated, equal to the original number of variables. At this point, the sum of the variances of all of the principal components was equal to the sum of the variances of all of the variables.

III. RESULT

A. Multivariate Analysis of 12 Quantitative Characters

Multivariate analysis was used to assess genetic variability and the contributions of different traits to total variation among 20 hot pepper genotypes. Cluster analysis and Principal Component Analysis (PCA) were performed to identify genetic relationships and key traits influencing diversity.

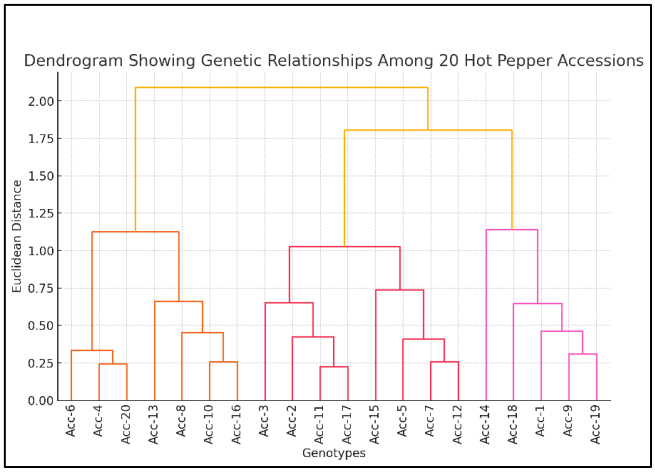
i. Genetic Divergence and Cluster Analysis

Cluster analysis grouped the 20 genotypes into three distinct clusters using Mahalanobis's D^2 statistics (Table 2). The resulting dendrogram (Figure 2) visually represents the genetic relationships among the genotypes. Cluster I contained 10 genotypes, Cluster III had 6 genotypes, and Cluster II included 4 genotypes. Genotypes within the same cluster exhibited lower genetic distances than those in different clusters.

The highest inter-cluster genetic distance was observed between Cluster I and Cluster II (74.20), followed by Cluster II and Cluster III (44.87) and Cluster I and Cluster III (31.86) (Table 3). This wide genetic divergence suggests that crossing genotypes from different clusters could result in improved hybrid varieties. These findings are consistent with previous studies conducted in Ethiopia and other regions. A study by [1] also identified three distinct clusters among Ethiopian hot pepper accessions, with significant genetic distances, findings that align with this study's results. Also, Shiferaw [20] reported a similar clustering pattern, confirming the genetic differentiation observed among Ethiopian landraces. Studies from India [15] and [3] also revealed three major clusters in their analyses of hot pepper diversity, reinforcing the reliability of the current clustering approach.

Table II: Distribution of 20 Genotypes of Hot Pepper into Different Clusters

Cluster No.	Accession Number	No. of Population
I	Acc-20206, Acc-20207, Acc-0213, Acc-229699, Acc-27846, Acc-28338, Acc-229696, Acc-229700, Acc-229698, M/fana	10
II	Acc-2012, Acc-20214, Acc-229694, Acc-28334	4
III	Acc-28337, Acc-229695, Acc-229697, Acc-28336, Acc-27845, Acc-229701	6
Total		20



[Fig.2: Dendrograms Showing Genetic Relationships Among 20 Hot Pepper Accessions Based on 12 Quantitative Traits]

Table III: Inter-Cluster Distance Between Each Cluster

Cluster	1	2	3
1		74.208	31.856
2			44.871
3			

ii. Characterization Cluster Based on 12 Characters

The cluster means for 12 characters across 28 chilli genotypes are presented in Table 4. The purpose of the cluster mean for each character was to characterise each cluster. From the range and mean values of all clusters for the respective character, the data were categorised into low (L), intermediate (I), and high (H) classes. About plant height cluster I has a cluster mean of (H=53.2), cluster II has a cluster mean of (I=50.7), and cluster III has a cluster mean of (L=49.6). About the number of fruits per plant cluster, cluster I has a cluster mean of (H=27.6), cluster II has a cluster mean of (H=23.8), and cluster III has a cluster mean of (H=22.0). For days to flowering cluster II has (H=70.6), cluster I has (I=68.8), and cluster III has a cluster mean of (L=67.3). For fruit length, the highest cluster mean was cluster I (H=7.4), intermediate cluster means (I=7.3), and the lowest cluster mean was cluster III (L=6.8). For fruit weight, cluster III (H=18.4), cluster I (I=17.0), and cluster II (14.5). For fruit yield per plot cluster, cluster I and cluster II have equal cluster means (0.6), and cluster III has (0.5) cluster mean. This variation aligns with [13], who identified fruit traits as major contributors to genetic divergence in Ethiopian hot pepper accessions.

The highest cluster mean in cluster II for fruit number per plant and the lowest cluster mean in cluster I for the same trait indicate the maximum distance between clusters II and I for this trait.

Table IV: Cluster Mean Values of 12 Different Characters of 20 Genotypes

Character	Cluster I	Cluster II	Cluster II
PH	53.2	50.7	49.6
DFL	68.8	70.6	67.3
DF	94.3	96	96.1
NFLA	60.9	61.3	54
DH	157.7	153.6	160.4
FL	7.4	7.3	6.8
FW	13.3	22.9	19.1
FWT	17	14.5	18.4
NFP	22	27.6	23.8
NSF	126.3	199.4	156.3
TSW	6.1	5.3	5.2
YPP	0.6	0.6	0.5

PH: Plant height, DFL: Days to 50% flowering, DF: Days to 50% Fruiting, NFLA: Number of flower per axil, DH: Days to first harvest, FBP: Fruit bearing period, NFP: Number of fruits per plant, FL: Fruit length, FW: Fruit width, FWT: Fruit weight, NSF: Number of seed per Fruit, 1000 SW

iii. Principal Component Analysis.

PCA was performed to determine the primary traits contributing to genetic diversity. The first five principal components (PCs) had eigenvalues ranging from 2.51 to 1.44, accounting for 77.74% of the total variance (Table 5). PC1 (20.93%) was strongly associated with fruit length, fruit width, and number of flowers per axil. PC2 (20.29%) highlighted fruit weight and number of seeds per fruit as key distinguishing factors. PC3 (14.75%) reflected variation in days to flowering and plant height. These results agree with international studies [15]. In India and [24] in China, similar PCA patterns were observed, with fruit traits as the primary contributors to total variation. The study by [3] also confirmed that PCA effectively differentiates hot pepper genotypes, supporting the approach used in this study.

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Table V: The Principal Component Values of five Principal Components from 12 Quantitative Traits for 20 Hot Pepper Genotypes Evaluated at Haramaya

No	Characters	PC 1	PC2	PC 3	PC 4	PC 5
1	PH	0.285305	-0.064592	-0.421341	0.033732	0.128015
2	DFL	0.136971	0.393420	-0.415203	-0.149451	0.224377
3	DF	-0.228784	-0.166237	0.431623	-0.290802	0.230450
4	NFLA	0.474258	0.237643	-0.006651	0.028722	-0.207498
5	DH	-0.453268	-0.125034	-0.234749	0.318310	0.062139
6	FL	0.419365	-0.110828	0.377191	0.185049	-0.138359
7	FW	-0.089277	0.558247	0.197986	0.061300	-0.0206
8	FWT	-0.221598	-0.28556	-0.09328	-0.286952	-0.507202
9	NFP	-0.360459	0.303658	0.012828	0.420849	0.168296
10	NSF	-0.071134	0.359751	0.409801	-0.208788	0.015563
11	TGW	0.199403	-0.31303	0.141931	0.056584	0.694177
12	YPP	0.097106	-0.127363	0.173811	0.667506	-0.214414
	Eigenvalue	2.51	2.43	1.77	1.44	1.71
	difference	0.077	0.66	0.327	0.27	0.306
	contribution to variability	20.93	20.29	14.75	12.02	9.76
	Cumulative contribution %	20.93	41.22	55.96	67.98	77.74

PC: Principal Component, PH: Plant height, DFL: Days to 50% flowering, DF: Days to 50% Fruiting, NFLA: Number of flowers per axil, DH: Days to first harvest, FBP: Fruit bearing period, NFP: Number of fruits per plant, FL: Fruit length, FW: Fruit width, FWT: Fruit weight, NSF: Number of seed per Fruit, 1000 SW: Thousand seed weght

IV. DISCUSSION AND CONCLUSION

A. Genetic Diversity and Breeding Implications

The observed clustering pattern suggests significant genetic divergence among the studied genotypes, supporting their potential for use in breeding programs. The highest genetic distance observed between Cluster I and Cluster II suggests that crossing genotypes from these clusters could enhance hybrid vigour.

The principal component analysis revealed that fruit traits (length, width, weight, and number of seeds per fruit) are the primary contributors to genetic variation. These findings align with similar studies conducted in Ethiopia and other regions, confirming the reliability of clustering and PCA methods in hot pepper genetic research.

B. Conservation Implications

The broad genetic diversity of Ethiopian hot pepper landraces provides a strong foundation for breeding programs to improve yield, fruit quality, and disease resistance. Genetic conservation efforts should prioritise landraces from divergent clusters to preserve valuable traits for future breeding.

C. Future Research Directions

Future studies should integrate molecular markers with morphological and multivariate analyses to further enhance genetic characterization. This approach would provide a more comprehensive understanding of genetic diversity, aiding in the development of improved varieties.

V. CONCLUSION AND RECOMMENDATION

This study assessed the genetic diversity, clustering patterns, and principal component contributions of 20 Ethiopian hot pepper genotypes using multivariate analysis. The findings revealed significant genetic variability, offering valuable insights for breeding, conservation, and crop improvement programs.

A. Key Findings

i. Cluster analysis grouped the genotypes into three distinct clusters, with Cluster I and Cluster II exhibiting the highest genetic distance (74.20). This suggests that crossing genotypes from these clusters could enhance hybrid vigour.

ii. Principal Component Analysis (PCA) identified fruit traits (length, width, weight, and number of seeds per fruit) as the primary contributors to genetic variation, explaining 77.74% of the total diversity.

iii. The results align with similar studies conducted in Ethiopia and other regions, confirming the reliability of clustering and PCA methods in hot pepper genetic research.

B. Implications for Breeding and Conservation

- The wide genetic diversity of Ethiopian hot pepper landraces provides a strong foundation for breeding programs to improve yield, fruit quality, and disease resistance.
- Genetic conservation efforts should prioritise landraces from divergent clusters to preserve valuable traits for future breeding.
- Future research should integrate molecular markers with morphological and multivariate analyses to further enhance genetic characterization.

In conclusion, the study underscores the importance of Ethiopian hot pepper landraces as valuable genetic resources. The significant genetic divergence observed suggests promising potential for hybrid development and sustainable chilli production in Ethiopia.

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As the article's author, I must verify the accuracy of the following information

after aggregating input from all authors.

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- **Author's Contributions:** The authorship of this article is contributed equally to all participating individuals.

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