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# ASSESSMENT OF GENETIC DIVERGENCE BASED ON CLUSTER AND PRINCIPAL **COMPONENT ANALYSES FOR YIELD AND ITS CONTRIBUTING CHARACTERS** IN FIELD PEA (PISUM SATIVUM L.) GENOTYPES AT BEKOJI **SOUTH EASTERN OF ETHIOPIA**

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The pea (Pisum sativum L.) is an annual grain legume of the Papillonaceae family. It is thought to be

originated in the Ethiopia, part of Europe and Asia. It is one of the four most important cultivated

legumes alongside soybean, groundnut and beans. The development of varieties for yield and disease

resistance is one of the important activities to support farmers and improve the productivity of the crop.

Therefore, this study was conducted to assess genetic diversity by cluster and principal component

(PCA) analyses of field pea genotypes. Forty-nine field pea genotypes were evaluated in simple lattice design at Bekoji in 2019 cropping season. The first three principal component axis (PCA), PCA1,

PCA2 and PCA3 accounted 37.2, 27.5 and 13.5%, respectively, and a total of 78.2% of the total variation. The cluster analysis grouped the 49 genotypes into eight clusters. Cluster III consisted of 10

genotypes and Cluster I and cluster VI each consisted of 9 genotypes and the three clusters consisted of

57.15% of the total genotypes. The inter-cluster distances between Cluster VIII and other seven clusters were high of which the inter-cluster distance between Cluster VIII and Cluster II, Cluster VIII and Cluster IV was the inter-cluster distances between Cluster VIII and Cluster II were 4420 and 4161,

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### ABSTRACT

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Cluster, Principal component, Field pea, Divergence, Genotype.

respectively, which was higher than other inter-cluster distances. Cluster VII and VI had higher intra-\*Corresponding author: Temesgen Abo, cluster distance of 1291 and 1057.3 respectively.

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# INTRODUCTION

Field pea (Pisum sativum L.) is a cool season legume crop which is an important source of Protein for humans in the developing world and a major fodder crop in developed countries. The pea is very important in nutrition of domestic animals due to its high content of protein, calcium and stimulating substances. The center of origin for field pea is considered the Mediterranean to central Asia as well as the highlands of Ethiopia (Davies, 1976). It is a good preceding crop for all field crops, except for annual and perennial legumes. Due to its symbiosis with root nodule bacteria, it leaves significant amounts of nitrogen in the soil for the subsequent crop and a Field pea has a dual advantage in fixing atmospheric nitrogen and serves as a "break crop" (Gemechu etal., 2013). After the pea is harvested, the soil retains a favorable structure (Stenvovic et al., 2005). The Pea plant has two pairs of chromosomes (2n= 2x=14) and can grow in a bush or dwarf form or a climbing form (the majority of peas) Temesgen (2021). Historically, field pea has been grown in the Mediterranean and central Asian regions, as well as in the Ethiopian highlands.

The main field pea producing countries include Canada, Russia, China, India and France. Ethiopia ranks first in Africa and number six in the world in field pea production FAOSTAT (2012). Field pea is the second major pulse cultivated in Ethiopia next to faba bean (Vicia faba L.). Extensive areas of the central and northern highlands of Ethiopia are cultivated with field pea Temesgen (2022). Historically, field pea has been grown in the Mediterranean and central Asian regions, as well as in the Ethiopian highlands. The fact that Ethiopia has a variety of field peas makes it one of the centers of field pea diversity Hagedorn (1991). Field pea is cultivated since ancient time in Ethiopia and its wild and primitive forms of the species was concealed in the highlands of Ethiopia. Due to this fact Ethiopia considered as one of the centers of diversity for field pea Hagedorn DJ (1991). Field pea grow around the world for its fresh green seeds, tender green pods, dried seeds, and soil restorative purposes (Haddis et al., 2013). In Ethiopia, Pisum sativum var. sativum is grown in high altitude area (1800-3200) M.A.S.L (Haddis et al., 2013). Field pea is the third best essential chief food legume crop in Ethiopia next to Faba bean and common bean, amongst the highland pulse crops. Field pea covers about 223657.49 hectares of arable lands with a total

production of 3,905,635.50 quintals with average yield of 1.75 t ha-1. It constitutes 12.16% of the total area covered by pulses CSA (2020). In Ethiopia, field pea is mainly used to prepare "shiro wet", a stew eaten with local bread made of tef, i.e. "Injera". The crop is commonly grown in association with faba bean (Vicia faba) and is important food, cash and "hunger break" crop in highlands of the country. Field pea supplies 344 calories, 20.1g protein and 64.8g carbohydrates/100g edible portion (Telaye et al., 1994). It is known as poor man's meat in the developing world since it provides valuable cheap protein. In combination with wheat, rice and other cereals it provides a balanced diet (Santalla et al., 2001) though pea protein is deficient in sulphur- containing amino acids (Cysteine and methionine) McPhee (2003). The national field pea program conducted research activities and released about 43 varieties, still now these varieties did not address the production constraints of field pea in the country. (Plant variety release, protection and seed quality control directorate crop variety register, 2016). Besides to plan appropriate selection method understanding the association among traits and its effect on the target trait (like yield) will be important. Yield it is highly affected by different yield component traits that required a clear understanding how these traits affect yield and designing a selection procedure. This indicates sometimes direct selection for the target trait (grain yield) which is a polygenic trait may not be effective in unless yield contributing traits are considered during selection (Srivastava et al., 2017). So, to have a successful breeding program, the breeder should study the genetic variability of the base population, understand the nature of inheritance of the traits and understand the interrelationship among traits of interest to design the breeding strategy. Despite the large number of filed pea accessions held in the gene bank of Ethiopia, limited information available on the magnitude and pattern of genetic variability for these materials. Therefore, this study was conducted in the field pea populations of the breeding program with the following specific objectives.

#### Objectives

- To cluster genotypes into their genetically divergent groups and there by estimate the genetic difference (distance) between clusters
- To assess the extent of association among agronomic characters of field pea genotypes.

# **MATERIALS AND METHODS**

Description of the Study Area: The experiments were conducted at Bekoji and Asasa research sites of Kulumsa Agricultural Research Center during 2019 main cropping season. Bekoji is located 39°14'46"E longitude and 07o31'22"N latitude with an altitude of 2780 m.a.s.l. It receives an average annual rainfall of 1020 mm with the average annual minimum and maximum temperatures of 7.9°C and 16.6°C, respectively. The soil type of the trial site is eutric niti sols with a good drainage system. It contains 5.5% organic matter, 0.25% nitrogen and its pH is 5.35 Tamene TT, (2017). Asasa is located at 07°06'12"N latitude and 38°11'32"E longitude with an altitude of 2340 m.a.s.l. The site receives an average annual rainfall of 620 mm with the average annual minimum and maximum temperatures of 5.8°C and 23.6°C, respectively. The soil type of Asasa is gleisoil and its pH is 6.25 light sandy soil with low water holding capacity (Kulumsa Agricultural Research Center meteorology station unpublished paper).

*Experimental Materials and Design:* Forty-nine field pea genotypes obtained from Kulumsa and Holeta Agricultural Research Centers was used for this study. The list and description of the materials used for the study are presented in Table 1. A plot size of  $4m \ge 0.8m$  (3.2m2) was used in this study where each plot was consisted of four rows with 80 plants within each row, with an inter-row spacing of 20 cm and 5 cm between plants within the row. The spacing between plots and blocks distances was 1m and 1.5m, respectively. The experiment was laid out in 7 x 7 simple lattice designs at each

location and each genotype was assigned randomly in blocks of each replication.

**Data Collection:** Data on agronomic and morphological traits were collected on plot and individual plant basis. In this experiment the following data was recorded based on the standard of Ethiopian Institute of Agricultural Research (EIAR) data collection guidelines.

#### Data Collected on Plot Basis

*Days to 50% flowering (DTF):* The number of days from the date of sowing to the date at which about 50% of the plants in a plot showed blooming on about 50% of their flower buds.

**Days to 90% Maturity (DTM):** The number of days from the date of sowing to a stage when 90% of plants have reached their physiological maturity was assessed by yellowish foliage color and shedding start on the lower stem, pods and seeds hardened.

*Thousand Seed Weight (TSW) (g):* The weight in gram of 1000 seeds randomly taken from each plot.

*Grain Yield (g/plot):* the net plot grain yield in gram per plot Gy (g/plot).

*Grain Yield per Hectare (kg/ha):* The net plot grain yield adjusted at 10.0% moisture content was converted in to yield per hectare in a kilogram.

*Grain Filling Period (GFP):* The number of days from days to 50% flowering to days to 90% physiological maturity.

*Above Ground Total Biomass per Plot (TBPP):* The mean weight of above ground parts sun dried and weighted to get the biological yield per plot in grams.

*Harvest Index (HI):* ratio of grain yield which is oven dried over total biomass of oven dried.

This was calculated by the following formula:

Harvest index (HI) = <u>Seed yield per plot(g) X100</u> Biomass per plot (g)

#### **Data Collected on Plant Basis**

**Plant Height (PH):** Average height of five randomly selected plants in each plot measured (cm) from the ground surface to the top of the main stem at physiological maturity (where the color of their pods changed from green to lemon yellow).

**Pod length (PL):** Average length of 25 fully matured pods randomly taken from each five sample plants per each test genotype was measured from the pod apex to the peduncle in centimeters.

*Number of Seeds Per Pod (SPP):* Average number of seeds per pod, counted at harvest on five randomly taken plants, in five randomly taken pods per plant.

#### The Analysis of Genetic Divergence

*Cluster:* The genetic distance can be estimated to study the pattern and level of genetic diversity of a given population. The genetic divergence of two varieties is a function of their ancestry, geographic separation and adaptation to differing environments (Moll *et al.*, 1965). Genetic similarity is the converse of genetic distances, as it is refers to the extent of gene similarities among cultivars (Smith, 1984). Nisar M (2008) defined the genetic distance as the extent of gene differences between cultivars as measured by allele frequencies. Genetic distances are measures of the average genetic divergence between cultivars or populations (Souza and Sorrells, 1991). The Mahalanobis D2 genetic distance Rao CR (1952) was estimated by

considering the mean data and the variance covariance matrix of the traits using the bio tools package of R. Based on the estimated distance, the Hierarchical cluster analysis was employed to cluster the field pea genotypes using the UPGMA clustering method using the R base function hclust. After the appropriate number of clusters determined based on the above analysis the intra and inter genetic distance within and among the cluster groups were estimated using clv package of R, (Jyoti Thakur, *et al.*, 2020), respectively.

The manhalobis genetic distance among the 49 field pea genotypes was estimated as follow:

 $D2 = \Sigma x - 1 v - 1x$ 

Where D2 is the Mnahlobis genetic distance between genotype i and j, X the mean performance of the genotypes of the traits, V is the variance covariance matrix of the traits under consideration.

component based on correlation matrix was calculated using SAS software version 9.0 SAS Institute (2002).

## **RESULTS AND DISCUSSION**

**Clustering of Genotypes:** The Euclidean distance matrix of field pea genotypes estimated from eight quantitative traits was used to construct dendrograms based on the Unweight Pair-group methods with Arithmetic Means (UPGMA). Accordingly, the 49 field pea genotypes were grouped into eight distinct clusters (Table 1). Cluster III was the highest clusters consisted of 10 genotypes that account 20.41 % of the total genotypes followed by the Cluster I consisted of 9 genotypes and comprise 18.37% of the total genotypes under this study. Besides the minimum number of genotypes found in Cluster VII and contain only one genotypes in to six clusters which make

Table 1. Clusters of	of 49	field pea	genotypes	at Bel	coji
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Clusters	Percent (%)	No- of genotypes	Genotypes
Ι	18.37	9	G-15, G-36, G-3, G-1, G-20, G-17, G-18, G-11, G-26
Π	12.24	6	G-24, G-23, G-31, G-10, G-25, G-9
Ш	20.41	10	G-2, G-29, G-48, G-42, G-6, G-32, G-39, G-27, G-22, G-4
IV	10.21	5	G-13, G-21, G-12, G-16, G-30
V	10.21	5	G-34, G-41, G-47, G-37, G-44
VI	18.37	9	G-33, G-45, G-8, G-14, G-19, G-28, G-35, G-5, G-7
VII	2.04	1	G-46
VIII	8.16	4	G-38, G-43, G-40, G-49



The distance matrix from phenotype traits were used to construct dendrogram based on the Unweighted Pair-group Method with Arithmetic Means (UPGMA). The results of cluster analysis are presented in the form of dendrogram. Using the mean data, the principal component analysis was conducted to see the distribution of the genotypes in two dimensional plots using the princomp" package of R, R Core Team (2019).

**Principal Component Analysis:** Principal component analysis (PCA) was computed to find out the characters, which accounted more to the total variation. The data was standardized to mean zero and variance of one before computing principal component analysis. The principal

them moderately divergent. Habtamu and Million (2013) studied sixteen field pea genotypes and classified in to five clusters. Singh *et al.* (2019) studied 55 field pea genotypes and classified into six clusters. Kefyalew *et al.* (2017) studied 142 field pea germplasm and clustered into seven distinct groups. Tamene (2017) grouped 25 advanced elite breeding field pea materials into five distinct classes.

**Distance Analysis between Clusters:** The average intra and intercluster D2 values with their corresponding intra and inter-cluster distance are presented in (Table 2). The maximum distance was recorded for cluster III and followed by cluster I and VI. The minimum distance was recorded for cluster VII. 65823 Temesgen Abo et al., Assessment of Genetic Divergence based on cluster and Principal Component Analyses for yield and ITS Contributing Characters in field pea (Pisum Sativum L.) Genotypes at Bekoji South Eastern of Ethiopia

Table 2. Average intra (bold	) diagonal and inter cluster	· (off diagonal) divergence	(D2) values in 49 field pe	a genotypes at Bekoji
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Cluster	C1	C2	C3	C4	C5	C6	C7	C8
C1	753.5**							
C2	791.77**	717.9**						
C3	839.5**	922.4**	1033.3**					
C4	720.4**	726.9**	871.2**	819.0**				
C5	708.9**	951.**	876.1**	730.3**	392.6**			
C6	868.6**	991.7**	1010**	888.7**	795.3**	1057.3**		
C7	1058**	1305.3**	1167.6**	1104.7**	827.2**	1140.8**	1291.**	
C8	4131**	4420.9**	4012**	4161.8**	3753.9**	4137.4**	3430**	0.0

C= cluster

Table 3. Mean values of eight traits of eight clusters of 49 field pea genotypes for Bekoji

Traits	C1	C2	C3	C4	C5	C6	C7	C8
DTF	64.8	70.38	65.16	66.36	64.25	64.96	61.42	64.05
DTM	132.82	133	132.4	132.95	132.04	132.61	131.62	130.21
PHT	218.86	211.97	204.27	223.77	196.83	215.66	175.58	112.47
GFP	67.86	63.08	67.13	66.84	67.53	67.61	69.45	65.3
GY	4031.5	3865.9	3964.9	3926.2	3958.9	4123.4	3752.0	1658.9
TSW	187.6	161.96	179.3	184.31	172.94	182.95	163.82	187.75
TBM	4983.6	5427.6	4886.9	5086.9	4567.4	4920.0	4325.2	1613.6
HI	26.16	23.31	26.18	24.91	27.91	27.16	27.4	22.84

DTM = Days to maturity, DTF = Days to % flowering, PHT=plant height, GFP= grain filling period, HI= Harvest index, GY= grain yield, TSW=thousand seed weight and TBM= total biomass, C=clusters.

Table 4	. First three	principal	components and	l total	variance ex	plained 1	for fiel	l pea	genotype	s at Beko	oji
			1			1			- vi		

Trait	PCA1	PCA2	PCA3
Days to 50% flowering	0.291	0.518	0.242
Days to maturity	0.504	0.058	-0.286
Plant height	0.401	0.145	-0.127
Grain filling period	0.237	-0.43	-0.519
Harvest index	-0.067	-0.503	0.517
Grain yield	0.405	-0.369	0.414
Thousand seed weight	-0.064	-0.355	-0.322
Total biomass	0.522	-0.082	0.179
Eigenvalues	1.725	1.484	1.04
Proportion%	0.372	0.275	0.135
Cumulative	0.372	0.647	0.783

PCA= Principal component analysis



Figure 2. Plots of the first two principal components of 8 traits for 49 field pea genotypes at Bekoji

This showed the genotypes with maximum genetic diversity can be used in the future crossing program to develop varieties with diverse genetic background. While a minimum distance ( $D^2 = 708.9$ ) was observed between clusters V and clusters I followed by cluster IV and I ( $D^2 = 720.4$ ). The maximum distance ( $D^2 = 4420.9$ ) was observed between clusters VIII and clusters II followed by cluster VIII and IV ( $D^2 = 4161.8$ ). These results were in accordance with the result of (Sksanwal *et al.*,2015) who reported that indicate high genetic variability. Similarly Tamene (2017) reported maximum distance among cluster groups of the field pea genotypes in his study. Therefore, the genetic divergence observed in this study give a first insight for the breeder to utilize the existing genetic variability for the improvement field pea in the country.

Mean values of the Clusters: The mean performances of eight clusters were presented in (Table 2). The mean value of traits in each cluster showed that cluster VI recorded the high mean value for grain yield kg/ha that reach about 4123.4 kg/ha. Whereas the lowest mean grain yield was observed in cluster VIII. Therefore, the genotypes in Cluster I and Cluster VI can be used as a source to improve grain yield in field pea breeding program. Besides the same cluster groups has the second highest TSW that has direct impact on grain yield. The highest TSW was observed in Cluster VIII (187.75g) and the genotypes in this group also can be used as parental material in the crossing program to improve grain yield and thousand seed weight in the field pea breeding program. The high mean value of biomass was recorded by Cluster II, IV, and I. That indicate the genotypes in this cluster can be used as a source gene to improve the biomass yield in field pea. Also analysed the genetic diversity from the Ethiopian field pea gene pool and found high genetic variability and identified different cluster group with variable cluster mean Kedir (2020).

Principal component analysis: The principal component analysis at Bekoji showed that the first three principal components have Eigenvalues greater than 1 explained about 78.3% of the total variation among forty-nine field pea genotypes evaluated for nine quantitative traits. The three principal components had eigenvalues 1.725, 1.484 and 1.04 respectively. The first principal component accounts 37.2% of the total variation of genotypes. Days to 50% flowering, days to maturity, plant height, grain filling period, grin yield and total biomass had high positive contributions for the variation in first principal components; those imply that they contribute significantly to the discrimination among the genotypes. The second principal component accounted about 27.5% of the total variation of the genotypes. Days to maturity, days to flowering and plant height had high positive contributions for the total variation. The third principal component analysis accounted 13.5% of total variation by days to flowering, harvest index, grain yield and total biomass (Table 4). Singh et al. (2019) studied 55 field pea genotypes and reported that the first five principal components explained about 86.7% of the total variation, the first component accounted about 35% from the total variation. Desalegn (2023) reported that 60.84% of the total variation was explained by the first three principal components.

## SUMMARY AND CONCLUSIONS

This study was conducted to assess the extent of genetic variability for grain yield and yield related traits in field pea. The 49 genotypes were grouped in to eight clusters based on UPGMA clustering analysis. The maximum inter-cluster distance was observed between clusters III followed by cluster I and VI and the minimum number of genotypes found in Cluster VII and contain only one genotypes (2.04 %) followed by cluster IV, V and VIII. The first three principal components with eigenvalues greater than one explain about 78.3% of the total variation of Bekoji. Generally the individual trait and multivariate analysis showed the existence of high genetic variability that can be exploited in the future breeding program of field pea. In order to have more concrete result and conclusion the study should be done by including more genotypes and tested across locations. This result being from one location, it is recommended for further testing in diverse environments to identify favorable environments for genotypes. It needs further studies on field pea to identify and select genotypes that have important agronomic properties and use them in direct hybridization. It should be worthwhile to study more available germplasm over years and locations to identify more accessions as well as to confirm the importance of the traits identified as predictors of yield.

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