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Research Article

Genetic Diversity Based on Cluster and Principal Component Analyses for Quantitative Traits in Field pea (Pisum sativum L.) Genotypes at Arsi Highlands of Ethiopia

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ABSTRACT

In Ethiopia, field pea (Pisum sativum L.) is the major source of protein for resource poor farmers. 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 and Asasa in 2019 cropping season. The first three principal component axis (PCA), PCA1, PCA2 and PCA3 accounted 38.12, 28.3 and 14.1%, respectively, and a total of 80.5% of the total variation. The cluster analysis grouped the 49 genotypes into six clusters. Cluster I and Cluster II consisted of each 13 genotypes and Cluster III consisted of 10 genotypes and the three clusters consisted of 73.47% of the total genotypes. The inter-cluster distances between Cluster VI and other five clusters were high of which the inter-cluster distance between Cluster VI and Cluster I Cluster I, Cluster VI and Cluster II was the inter-cluster distances between Cluster VI and Cluster and Cluster VI and Cluster II were 5567 and 5055, respectively, which was higher than other inter-cluster distances. Cluster I and II had higher intra-cluster distance of 580 and 533, respectively.

Key words: Genetic diversity, Cluster analyses, Principal component, Eigen value, (Pisum sativum).

INTRODUCTION

Field pea (Pisum sativum L.) is self-pollinated an annual herbaceous legume crop that belongs to family Leguminosae and genus Pisum (Duke, 1981). It is a diploid species (2n=2x=14 chromosomes) and has determinate (bush or dwarf) or indeterminate (climbing) growth habit (majority of pea plants) (Zohary and Hopf, 2002). The center of origin for field pea is considered the Mediterranean to central Asia as well as the highlands of Ethiopia (Davies, 1976). Field pea is cultivated since ancient time in Ethiopia (Tadasse *et al.,* 1994) 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).

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A Field pea has a dual advantage in fixing atmospheric nitrogen and serves as a "break crop" (Gemechu *et al.*, 2016). The national field pea program conducted research activities and released about 40 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
- 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 are 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 x 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:

$$\text{Harvest index (HI)} = \frac{\text{Seed yield per plot(g)}}{\text{Biomass per plot(g)}} \text{X}100$$

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.

Table 1: Description of Field pea accessions

Acc. code	Genotype name	Acc. Code	Genotype name	Acc.code	Genotype name
G-1	Bursa	G-18	EH 010004-1	G-35	EK 08024-4
G-2	Burkitu	G-19	EH 07006-5	G-36	EK 08017-3
G-3	EH 05048-5	G-20	EH 010009-1	G-37	PDFPT p-313-050
G-4	EH 08034-2	G-21	EH 08042-2	G-38	PDFPT p-313-015
G-5	EH 010006-2	G-22	EH 07007-5	G-39	PDFPT p-313-017
G-6	EH 08021-1	G-23	EH 08041-4	G-40	PDFPT p-313-26
G-7	EH 09021-5	G-24	EH 08042-4	G-41	PDFPT p-313-020
G-8	EH 08003-2	G-25	EH 08041-1	G-42	PDFPT p-313-052
G-9	EH 08036-4	G-26	EH 010009-2	G-43	PDFPT p-313-062
G-10	EH 010005-2	G-27	EH 08003-1	G-44	PDFPT p-313-098
G-11	EH 08027-2	G-28	EK 08023-5	G-45	PDFPT p-313-022
G-12	EH 08036-1	G-29	EH 08016-2	G-46	GIZ 02019 – 1
G-13	EH 08041-3	G-30	EH 08027-1	G-47	GIZ 02019 – 2
G-14	EH 07005-1	G-31	EH 08027-3	G-48	PDFPT p-313-028
G-15	EH 010011-3	G-32	EK 08017-5	G-49	PDFPT p-313-065
G-16	EH 07002-1	G-33	EK 08016-4		
G-17	EH 08021-4	G-34	EH 08003-7		

Seed Source: Kulumsa and Holeta Agricultural Research Centers.

Number of pods per plant (PPP): Average number of mature pods, counted at harvest on five randomly taken plants.

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 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:

$D2 = \Sigma x\text{-}1\ v\text{-}1x$

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.

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

RESULTS AND DISCUSSION

Genetic Diversity

Clustering of Genotypes

The Euclidean distance matrix of field pea genotypes estimated from eight quantitative traits was used to construct dendrograms based on the Unweighted Pair-group methods with Arithmetic Means (UPGMA). Accordingly, the 49 field pea genotypes were grouped into six distinct clusters (Table 2). The two clusters, Cluster I and Cluster II was the highest clusters consisted of each 13 genotypes that account 53.06 % of the total genotypes followed by the Cluster III consisted of 10 genotypes and comprise 20.41% of the total genotypes under this study. Besides the minimum number of genotypes found in Cluster VI and contain only one genotypes (2.04 %). (Santalla M, *et al.,* 2001) Classified fifty-five field pea genotypes in to six clusters which make them moderately divergent. (Habtamu S *et al.,*2013) Studied sixteen field pea genotypes and classified in to five clusters. (Tamene, 2017) Grouped 25 advanced elite breeding field pea materials into five distinct classes.

Distance Analysis between Clusters

The average intra and inter-cluster D2 values with their corresponding intra and inter-cluster distance are presented in (Table 3). The maximum distances were recorded between cluster I and VI followed by cluster VI and II and Cluster VI and Cluster III. 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 (D2 = 696) was observed between clusters III and clusters IV followed by cluster II and III (D2 = 763). These results were in accordance with the result of (Singh *et al.,* 2019) 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.

Table 2: Clusters of 49 field pea genotypes over two locations

Clusters	Percent (%)	No- of genotypes	Genotypes
I	26.53	13	G-1, G-2, G-3, G-8, G-15, G-17, G-18, G-20, G-22, G-25, G-29, G-36, G-42
II	26.53	13	G-9, G-10, G-11, G-12, G-13, G-14, G-16, G-23, G-24, G-27, G-30, G-31, G-39
III	20.41	10	G-4, G-5, G-19, G-21, G-26, G-32, G-33, G-34, G-45, G-48
IV	18.37	9	G-7, G-28, G-35, G-37, G-41, G-43, G-44, G-47, G-49
V	6.12	3	G-6, G-38, G-40
VI	2.04	1	G-46

Note: G- = Genotype

Table 3: Average intra (bold) diagonal and inter cluster (off diagonal) divergence (D2) values in 49 field pea genotypes for combined data

Cluster	Cluster 1	Cluster 2	Cluster 3	Cluster 4	Cluster 5	Cluster 6
Cluster 1	**580****	979**	1228**	1478**	2638**	5567**
Cluster 2		**533****	763**	1294**	2183**	5055**
Cluster 3			**354****	696**	1518**	4428**
Cluster 4				**344****	1251**	4157**
Cluster 5					**540****	2937**
Cluster 6						**0**

Table 4: Mean values of eight traits of the six clusters of 49 field pea genotypes for combined data

Trait	Cluster I	Cluster II	Cluster III	Cluster IV	Cluster V	Cluster VI
DTF	69.9	73.1	70.6	67.4	67.7	69.1
DTM	142.3	143.7	143.2	140.4	141.0	142.5
PHT	182.4	187.0	182.7	159.1	147.9	201.5
GFP	72.3	70.5	72.6	73.0	73.4	73.5
HI	27.1	21.2	23.5	28.3	25.6	22.2
GY	4082.7	3197.0	3064.6	3277.9	2297.4	3027.9
TSW	198.2	178.4	192.6	176.8	172.8	204.3
BM	4882.7	4946.0	4254.1	3678.0	2908.4	4357.4

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.

Table 5: First three principal components and total variance explained for field pea genotypes for combined analysis.

Trait	PCA1	PCA2	PCA3
Days to 50% flowering	0.451	0.35	0.171
Days to maturity	0.479	-0.124	-0.29
Plant height	0.429	-0.217	-0.292
Grain filling period	-0.173	-0.494	-0.428
Harvest index	-0.27	-0.41	0.469
Grain yield	0.231	-0.502	0.472
Thousand seed weight	-0.097	-0.3	-0.365
Total biomass	0.466	-0.247	0.204
Eigenvalues	1.746	1.504	1.063
Proportion%	0.3812	0.283	0.141
Cumulative	0.3812	0.664	0.805

Mean values of the Clusters

The mean performances of six clusters were presented in (Table 4). The mean value of traits in each cluster showed that cluster I recorded the high mean value for grain yield kg/ha that reach about 4082 kg/ha. Whereas the lowest mean grain yield was observed in cluster V. Therefore, the genotypes in Cluster I and Cluster IV 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 VI (204.3g) 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 I, II and VI. That indicate the genotypes in this cluster can be used as a source gene to improve the biomass yield in field pea. The lowest mean value was recorded harvest index by cluster II. Filed pea researchers in the past also analyzed the genetic diversity from the Ethiopian field pea gene pool and found high genetic variability and identified different cluster group with variable cluster mean Habtamu S *et al.,* (2013).

Principal Component Analysis

Principal component analysis results of eight traits for 49 field pea genotypes evaluated over two locations are presented in Table 5. The principal component analysis showed that the first three principal components have Eigenvalues greater than one (1) explained about (80.5%) of the total variation among 49 field pea genotypes (Table 5). The first principal component accounts (38.12%) of the total variation of genotypes. PCA-1 showed positive association towards plant height, days to maturity, grain yield, days to flowering and total biomass yield had high contributions for the variation in first principal components. (Habtamu S *et al.,* 2013) Reported 89% of the total variation was explained by the first four principal components and 40.26% of the total variations were explained by the first principal components.

The second principal component accounted about (28.3%) of the total variation of the genotypes. Whereas, PCA-2 positively associated with days to flowering only. Similar results were reported by (Tamene TT, 2017) , the first four principal components accounted for 88.7% of the total variation in the field pea genotypes, of which about 63.6% was contributed by the first two principal components. The third components accounted 14.1% of total variation among genotypes.

Summary and Conclusions

This study was conducted to assess the extent of genetic variability for grain yield and yield related traits in field pea. Analysis of variances ANOVA for each character

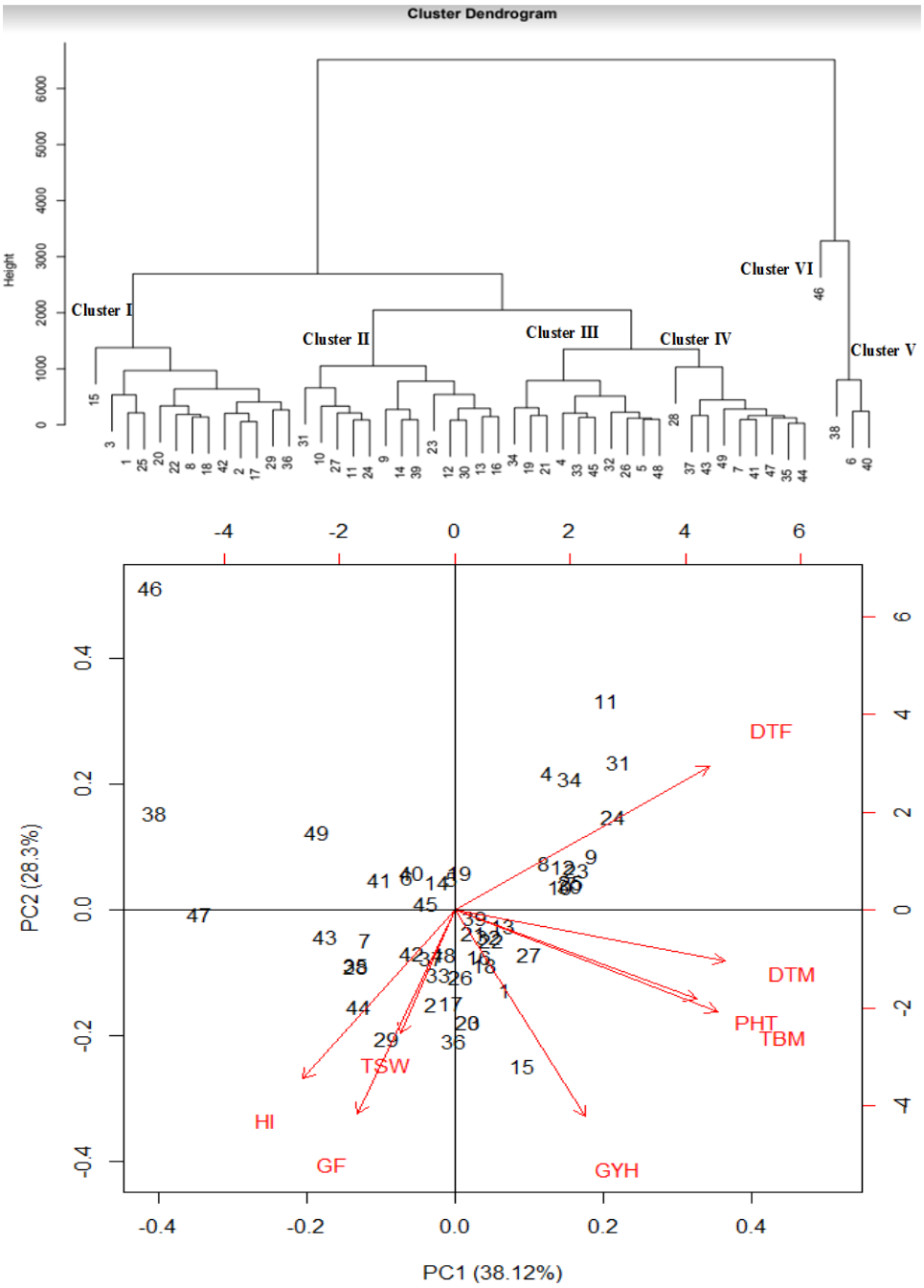

Fig. 1: The dendrogram of the 49 tested genotypes evaluated for combined data.

Fig. 2: Plots of the first two principal components of 8 traits for 49 field pea genotypes both location (combined).

showed the existence of highly significant difference among genotypes ($p<0.01$) at each location and over location. The highest yielding genotypes obtained at both locations was recorded by EH 010011-3 (4498 kg/ha at Bekoji and 5605 kg/ha at Asasa) that showed the potential of this variety to be released in the future. In addition, the study also showed the existence of high genetic variability among the tested field pea genotypes that can be exploited in the breeding program. The genotypes matured early at Asasa than Bekoji that can be due to the high temperature and terminal stress experienced at Asasa. Generally, genotypes performed well at Asasa than at Bekoji that showed the potential of the location for the production of these crops in the area.

The 49 genotypes were grouped in to six clusters based on UPGMA clustering analysis. The maximum inter- cluster cluster distance was observed between clusters I and VI followed by cluster II and VI, cluster III and VI, cluster IV and VI and the minimum between cluster distances was observed between cluster IV and III followed by cluster II and III, cluster I and II. The first three principal components with eigenvalues greater than one explain about 80.5% of the total variation for combined analysis. 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.

The study showed the presence of genetic variability among the genotypes that can be exploited in the breeding program. The traits have positive significant association with grain yield and positive direct effect on grain used as direct and indirect selection criteria in the breeding program. The genetic parameter estimated in this study should be used to design the breeding program of field pea in the country. 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 two locations, 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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