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CLUSTERING ON PRINCIPAL COMPONENT ANALYSISFOR QUANTITATIVE TRAITS IN FIELD PEA (PISUMSATIVUM L.) GENOTYPES AT ARSI HIGHLANDS OF ETHIOPIA

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ABSTRACT

In Ethiopia, field pea (Pisumsativum 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. Forty-nine field pea genotypes were evaluated in simple lattice design at Asasa in 2019 cropping season. Therefore, this study was conducted to assess genetic diversity by cluster and principal component (PCA) analyses of field pea genotypes.The first three principal component axis (PCA), PCA1, PCA2 and PCA3 accounted 35.4, 27.4 and 13.3%, respectively, and a total of 76.1% of the total variation.The cluster analysis grouped the 49 genotypes into six clusters. Cluster II and Cluster IV consisted of each 10 genotypes and Cluster VI consisted of 11 genotypes and the three clusters consisted of 76.1% of the total genotypes.The inter-cluster distances between Cluster V and VI were high of which the inter-cluster distance between Cluster VI and Cluster I, Cluster VI and Cluster II and Cluster VI and Cluster III.The inter-cluster distances between Cluster VI and Cluster I and cluster 5 and I were 3501.7 and 3341.9, respectively, which was higher than other inter-cluster distances. Cluster I and VI had higher intra-cluster distance of 1469.6 and 503.7, respectively. The study showed the existence of reasonable genetic variability among the field pea genotypes that could be exploited in breeding programs.

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INTRODUCTION

Field pea (*Pisumsativum* 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). In Ethiopia field pea is cultivated since ancient time in Ethiopia (Dawit*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. Field pea grow around the world for its fresh green seeds, tender green pods, dried seeds, and soil restorative purposes (McPhee, 2003). Field pea ranked as fourth largest in the world in volume of production in 2014 with 17.4 and 11.2 million tons of green and dry peas respectively, after soybean, groundnut and common bean. In Ethiopia, (*Pisumsativumvar.sativum)* is grown in high altitude area (1800-3200) m.a.s.l (HaddisYirga*et al*., 2013).

Among the highland pulse crops Field pea is the third most important staple food legume crop in Ethiopia next to faba bean and common bean, among the highland pulses. Field pea covers about 216,786.33 hectares of arable lands with a total production of 3,608,112.40 quintals with average yield of 1.664 t ha^{-1}. It constitutes 12.73% of the total area covered by pulses (CSA, 2019). In Ethiopia, field pea is mainly used to prepare "*shiro wet*", a stew eaten with local bread made of teff, i.e. "*Injera*". The crop is commonly grown in association with faba bean (*Viciafaba*), and is important food, cash and "hunger break" crop in highlands of the country. Field pea supplies 344 calories, 20.1 g protein and 64.8 g carbohydrates/100g edible portion (Asfaw*et l*., 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). A Field pea has a dual advantage in fixing atmospheric nitrogen and serves as a "break crop" (Gemechu*et al*., 2016). Despite the importance of field pea in Ethiopia, the major yield-limiting constraints in field pea production in Ethiopia are aphids, low yielding local varieties, lodging, diseases (ascochyta blight, powdery mildew), and pod shattering (Yirga *et al.,* 2019).

This fungus spread locally with air currents, whereas rain controls the disease by washing off spores and making them burst instead of germinating (Hargedorn, 1991). The most preferable management measure against the pathogen is developing resistant varieties (Sharma, 1995). The high diversity of the field pea accession associated with the robust representation of its center of domestication, that is, the Near East and Mediterranean (Warkentin*et al*., 2015) and other centers of diversity, including Central Asia and Ethiopia (Van der Maesen *et al*., 1988). The existence of wide range of field pea germplasm in Ethiopia makes the country the secondary center of genetic diversity (Gemechu*et al.,* 2012). Some scholars also considered the high elevation of Ethiopia within the range of the center of origin of the crop. This indicates that has Ethiopia the potential for improving field pea for desired traits either through selection and/or hybridization breeding programs. Genetic variability is the key factor for the success of any breeding program. In field pea, studies showed that the landraces and accessions in the breeding programs are focused on selection and evaluation from the existing diversity (Smýkal*et al*., 2011; Burstin*et al*., 2015). That indicates the great potential for the breeding program. Even selection among a diverse population provide a certain amount of success in the breeding program, crossing will be essential to combine to different contrasting genotypes to produce a hybrid that combine the trait of interest and produce heterosis (Arunachalam and Bandyopadhyay, 1984; Reddy, 1988; Singh, 1990; Wallace and Yan 1998; Chahal and Gosal, 2002).

The crossing among the highly divergent parents can produce varieties with broad genetic base (Russell, 1978; Chandel and Joshi, 1983; Singh, 1990; Gemechu*et al*., 1997) and raises the yield ceilings imposed by a narrow genetic base (Chandel and Joshi, 1983). The national field pea program conducted research activities and released about 45 varieties, still now these varieties did not address the production constraints of field pea in the country (MOANR, 2016). So, to design appropriate breeding strategy assessing the genetic variability and estimating the genetic parameters (heritability of traits) in the base population will be prerequisite since it is the base to get high yielding; biotic and abiotic stress tolerant varieties. In addition, assessing the genotype x environment interaction will be crucial since most of the traits are governed through polygenic inheritance that affected mostly by the environment (Legesse, 2015; Benti and Yohannis, 2017). 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 indicate sometimes direct selection for the target trait (grain yield) which is a polygenic trait may not be effective in a 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 Asasa research sites of Kulumsa Agricultural Research Center during 2019 main cropping season. 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 gleysol 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 (Table1). 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. All agronomic management practices were applied equally and properly as per the recommendations of Kulumsa Agricultural Research Center for each location.

Data Collection: Data on agronomic and morphological traits were collected on plot and individual plant basis. In this experiment the following data was recorded in plot and average plant basis.

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 the 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 andweighted to get the biologicalyield perplotin 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:

$$\text{Harvest index (HI)} = \frac{\text{Seed yield per plot (g)}}{\text{Biomass per plot (g)}} \text{ X100}$$

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. Discription of Field peaaccetions

Acc.code	Genotype name	Seed Source	Acc.code	Genotype name	Seed Source
G-1	Bursa	Breeder seed	G-26	EH 010009-2	PVT 2018
G-2	Burkitu	Breeder seed	G-27	EH 08003-1	NVT 2018
G-3	EH 05048-5	NVT 2018	G-28	EK 08023-5	NVT 2018
G-4	EH 08034-2	NVT 2018	G-29	EH 08016-2	NVT 2018
G-5	EH 010006-2	PVT 2018	G-30	EH 08027-1	NVT 2018
G-6	EH 08021-1	NVT 2018	G-31	EH 08027-3	NVT 2018
G-7	EH 09021-5	NVT 2018	G-32	EK 08017-5	NVT 2018
G-8	EH 08003-2	NVT 2018	G-33	EK 08016-4	NVT 2018
G-9	EH 08036-4	NVT 2018	G-34	EH 08003-7	NVT 2018
G-10	EH 010005-2	PVT 2018	G-35	EK 08024-4	NVT 2018
G-11	EH 08027-2	NVT 2018	G-36	EK 08017-3	NVT 2018
G-12	EH 08036-1	NVT 2018	G-37	PDFPT p-313-050	ICARDA
G-13	EH 08041-3	NVT 2018	G-38	PDFPT p-313-015	ICARDA
G-14	EH 07005-1	NVT 2018	G-39	PDFPT p-313-017	ICARDA
G-15	EH 010011-3	PVT 2018	G-40	PDFPT p-313-26	ICARDA
G-16	EH 07002-1	NVT 2018	G-41	PDFPT p-313-020	ICARDA
G-17	EH 08021-4	NVT 2018	G-42	PDFPT p-313-052	ICARDA
G-18	EH 010004-1	PVT 2018	G-43	PDFPT p-313-062	ICARDA
G-19	EH 07006-5	NVT 2018	G-44	PDFPT p-313-098	ICARDA
G-20	EH 010009-1	PVT 2018	G-45	PDFPT p-313-022	ICARDA
G-21	EH 08042-2	NVT 2018	G-46	GIZ 02019 – 1	GERMANY
G-22	EH 07007-5	NVT 2018	G-47	GIZ 02019 – 2	GERMANY
G-23	EH 08041-4	NVT 2018	G-48	PDFPT p-313-028	ICARDA
G-24	EH 08042-4	NVT 2018	G-49	PDFPT p-313-065	ICARDA
G-25	EH 08041-1	NVT 2018			

Seed Source: Kulumsa and Holeta Agricultural Research Centers

Table 1. Clusters of 49 field pea genotypes at Asasa

Clusters	Percent (%)	No- of genotypes	Genotypes
I	6.12	3	G-6, G-38, G-46
II	20.41	10	G-7, G-4, G-35, G-40, G-26, G-45, G-33, G-21, G-19, G-34
III	18.37	9	G-9, G-16, G-5, G-32, G-24, G-13, G-23, G-48, G-37
IV	20.41	10	G-17, G-36, G-2, G-20, G-18, G-28, G-44, G-41, G-47, G-49,
V	12.24	6	G-1, G-30, G-27, G-12, G-11, G-31
VI	22.45	11	G-8, G-15, G-29, G-22, G-39, G-43, G-3, G-42, G-10, G-25, G-14

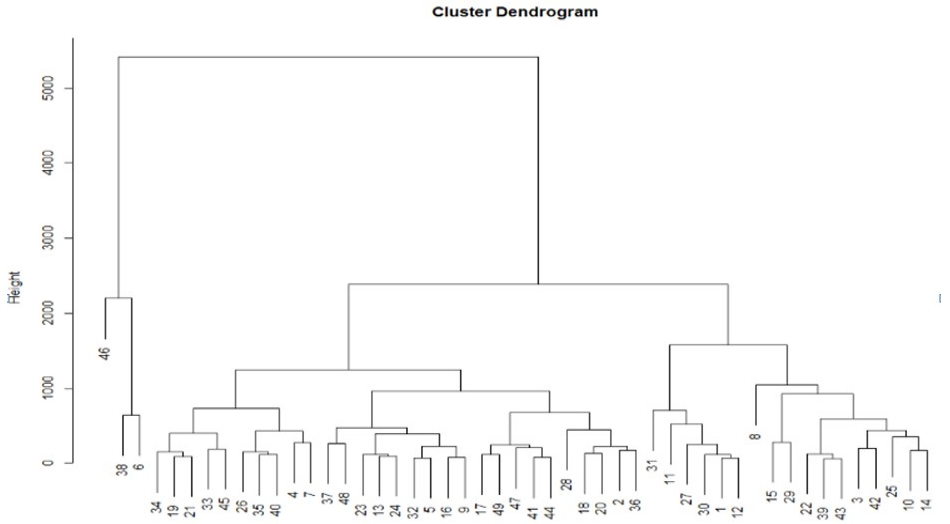

Figure 1. Thedendrogram of the 49 tested genotyeps evaluated for Asasa

Table 2. Average intra (bold) diagonal and inter cluster (off diagonal) divergence (D2) values in 49 field pea genotypes for Asasa

Cluster	C1	C2	C3	C4	C5	C6
C1	1469.6**					
C2	2189.5**	421.0**				
C3	2850.9**	723.7**	257.4**			
C4	2726.5**	731.9**	585.9**	345.3**		
C5	3341.9**	1247.1**	627.4**	1082.6**	402.6**	
C6	3501.7**	1364.8**	752.6**	888.5**	719.7**	503.7**

C= cluster

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.

DATA ANALYSIS

Genetic divergence: The Mahalanobis D^2 genetic distance (Rao, 1952) was estimated by considering the mean data and the variance covariance matrix of the traits using the bio tools package of R (da Silva, 2017). 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 (Nieweglowski, 2020), respectively.

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

$$D2 = \sum X^{-1} V^{-1} X$$

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 un-weighted 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, 2000).

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 Un-weighted Pair-group methods with Arithmetic Means (UPGMA). Accordingly, the 49 field pea genotypes were grouped into six distinct clusters (Table 2). The three clusters, Cluster II, Cluster IV and cluster VI was the highest clusters consisted of each 10, 10 and 11 genotypes that account 63.27 % of the total genotypes followed by the Cluster III consisted of 9 genotypes and comprise18.37% of the total genotypes under this study. Besides the minimum number of genotypes found in Cluster I and contain three genotypes (6.12%). Shalini*et al.* (2019) classified fifty five field pea genotypes in to six clusters which make them moderately divergent. Habtamu and Million (2013) studied sixteen field pea genotypes and classified into five clusters.Singh *et al.* (2019) studied 55 fieldpea 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 fieldpea 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 distance were recorded between cluster I andVI followed by cluster V and I and Cluster I 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 (D^2 = 585.9) was observed between clusters III and clusters IV followed by cluster V and III (D^2 = 627.4).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 six clusters were presented in (Table 4). The mean value of traits in each cluster showed that cluster VI recorded the high mean value for grain yield that reach about 4431.8kg/ha and cluster V recorded the high mean value for Biological yieldthat reach about 5620.7kg/ha. Whereas the lowest mean grain yield was observed in cluster I. Therefore, the genotypes in Cluster IV and Cluster VI can be used as asource 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 (188.2g) 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 IV, VI, and III. 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 V. Filed pea researchers in the past 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).

Table 4. Mean values of eight traits of six clusters of 49 field pea genotypes for Asasa

Trait	Cluster I	Cluster II	Cluster III	Cluster IV	Cluster V	Cluster VI
DTF	62	65.9	66.4	63.5	68.5	65.2
DTM	130.8	132.6	133.1	132	133.6	132.3
PHT	150.9	222.2	214.1	196.4	214.4	208.3
GFP	68	66.8	67	68	65.6	66.8
GY	2434.6	3616.7	3879.7	4226.3	3821.8	4431.8
TSW	176.1	182.4	173.3	175	166.2	188.2
BM	2599.5	4423.5	5047.3	4648.2	5620.7	5462.4
HI	25.2	25.9	24.9	29.4	22.4	26.5

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.

Principal component analysis: The principal component analysis at Asasa, showed that the first three principal components have Eigenvalues greater than 1 explained about 76.1% of the total variation among forty-nine fieldpea genotypes. The first principal component accounts 35.4% of the total variation of genotypes. Kefyalew*et al.* (2017) reported 90% of the total variations were explained by the first three principal componentsand 76.85% of variations were explained by the first principal components. Days to flowering, days to maturity, plant height, grain 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. Similar results were reported by Tamene (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 14.8% of total variation among genotypes. The second principal component accounted about 27.4% of the total variation of the genotypes. Days to flowering had high positive contributions for the total variation while other traits have a negative contribution for the variation. The third principal component analysis accounted 13.3% of total variation by days to flowering, harvesting index, grain yield, thousand seed weight and total biomass (Table 5).

Table 5. First three principal components and total variance explained for field pea genotypes at Asasa

Trait	PCA1	PCA2	PCA3
Days to 50% flowering	0.451	0.38	0.175
Days to maturity	0.431	-0.177	-0.37
Plant height	0.409	-0.251	-0.283
Grain filling period	-0.298	-0.471	-0.374
Harvest index	-0.285	-0.402	0.419
Grain yield	0.237	-0.522	0.449
Thousand seed weight	-0.008	-0.178	-0.441
Total biomass	0.465	-0.27	0.203
Eigenvalues	1.683	1.481	1.03
Proportion%	0.354	0.274	0.133
Cumulative	0.354	0.628	0.761

PCA=Principal component analysis

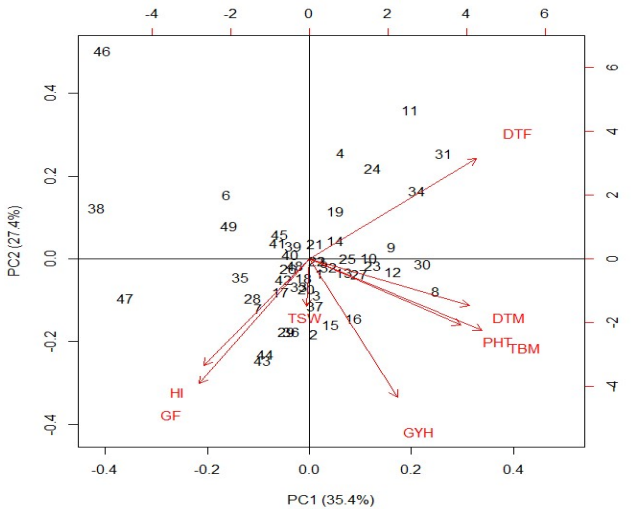

Figure 2. Plots of the first two principal components of 8 traits for 49 fieldpea genotypes at Asasa

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 showed the existence of highly significant difference among genotypes ($p \leq 0.01$) at Asasa. The 49 genotypes were grouped in to six clusters based on UPGMA clustering analysis. The maximum inter-cluster distance was observed between clusters I and VI followed by cluster I and III, and the minimum cluster distances was observed between cluster I and V followed by cluster III. The first two principal components with eigenvalues greater than one explain about 76.1% of the total variation. 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 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 identifiedas predictors of yield.

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