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**RESEARCH PAPER** 

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Evaluation of genetic diversity in pea (Pisum sativum) based on morpho-agronomic characteristics for yield and vield associated traits

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#### **Abstract**

The genetic diversity in 128 exotic pea accessions from diverse origin was determined for four qualitative traits flower color (FC), testa color (TC), cotyledon color (CC) and pod shape (PS) and eleven quantitative attributes i.e., plant height (PH), total pod number (TPN), number of pods per plant (NOPPP), total pod weight (TPW), pod weight per plant (PEPP), average pod length (APL), average pod width (APW), average pod thickness (APT), seed weight (SW), seed weight per plant (SWPP) and seed index (SI) through statistical software using a nonhierarchical, PCA. The projection of attributes on PC1 and PC2 revealed that average pod thickness, average pod length and average pod width are positively correlated to weight per pod. The projection pattern of the attributes on first two PCs depicted that key pod weight contributing attributes were pod thickness, length and width while the cotyledon color was opposite to weight per pod and other yield contributing traits on PC1, therefore, it had negative correlation with all other traits. The projection of accessions on first two PCs was useful to identify diverse groups of parents for better transgressive segregation. Promising accessions showing the variation in the desired parameters can be utilized in the future breeding programs.

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#### Introduction

The pea (Pisum sativum L., 2n = 14) member of Papillonacea family is an annual cool season nitrogen fixing crop having high percentage of digestible protein (23-33%) along with other biomolecules such as carbohydrates and vitamins (Gatti et al., 2011). It is an important legume crop which is thought to be originated from Southwest Asia (Hulse, 1994). Being a self pollinated crop, main focus of breeding in recent times was to develop pure lines which led to narrow genetic diversity among pea varieties (Baranger et al., 2004). But a considerable level of genetic variance among peas of diverse origin has also be reported by some breeders (Nisar et al., 2011).

In order to produce transgressive segregants, genetically different parents must to be selected for recombination breeding in self pollinated crops. The characterization of genetic diversity in crop species has long been based on morphological attributes, however, morphological variation is often found to be of limited use because the expression morphological attributes may be affected by environmental conditions, thereby constraining the analysis of genetic variation (Nisar et al., 2008). On the basis of morphological data, genetic diversity assessments needs a high precision of field experiments through recommended design and analysis so that the germplasm may be exploited to develop better genotypes for the upcoming scenario (Sajjad et al., 2011). Knowledge of genetic diversity is a useful tool in gene bank management and in planning experiments, as it facilitates efficient sampling and utilization of germplasm by identifying and/or eliminating duplicates in the gene stock, and helps in the establishment of core collections (Ghafoor et al., 2005).

In order to maintain, evaluate and utilize germplasm efficiently and effectively, it is important to investigate the extent of genetic diversity it contains (Smith et al., 1990). Many of the landraces and wild species are maintained in the world as genetic resources for crop improvement, but their use for breeding is still limited and we are challenged as to how to use this biodiversity for practical crop improvement (Ali et al., 2007). Broad based plant genetic resources are imperative for sound and successful crop improvement program (Jatoi et al., 2011). Unfortunately, the yield of pea is low in Pakistan as compared to the world average yield owing to the narrow genetic base and limited variability used in the development of local varieties. Crop improvement depends largely on the availability of diverse germplasm and their judicial utilization (Sardana et al., 2007).

If we are dealing with a large number of genotypes, multivariate analytical techniques i.e., principal component analysis (PCA), principal coordinate analysis and cluster analysis at present are mostly utilized to asses' genetic diversity present in the data set whether the data set comprises of biochemical, morphological or molecular data. Usually a nonhierarchical multivariate analysis technique, PCA, is employed by the plant breeders to check the pattern of variation in the given data. Therefore, the present study was undertaken to study the genetic diversity in exotic germplasm of pea accession of diverse origin and for the determination of extent of variation in the given accession for four qualitative traits and eleven quantitative attributes through statistical software using a nonhierarchical, PCA.

#### Material and method

#### Study area

The experiment was conducted during autumn season in glass house condition in Plant Genetic Resource Institute (PGRI), National Agriculture Research Center (NARC), Islamabad (30° 42′ N and 37° 08′ E) Pakistan.

### Planting Material

Pea germplasm used in this study consisting of 128 exotic accessions obtained from ICARDA gene bank. Origin of material was from various Western countries. Recommended plant production measures were adopted to raise the crop. Plant to plant distance was maintained at 6 inches (Table 1).

Table 1. List of accessions and their origin.

Accession #	Origin country	Accession #	Origin country	Accession #	Origin country	Accession #	Origin country
IG 50545	DEU	IG 50754	NLD	IG 50937	GBR	IG 123040	ITA
IG 50547	ETH	IG 50777	SWE	IG 50946	FRA	IG 123091	ITA
IG 50556	NLD	IG 50782	NLD	IG 50953 AS	GBR	IG 123092	ITA
IG 50564	FRA	IG 50785	NLD	IG 50953 BR	GBR	IG 123094	ITA
IG 50566	DEU	IG 50788	BEL	IG 50954	GBR	IG 123131	COL
IG 50580	RUS	IG 50792	ETH	IG 50955	USA	IG 123208	CHN
IG 50581	DEU	IG 50805	FRA	IG 50957	DEU	IG 123308	KAZ
IG 50591	UNK	IG 50810	FRA	IG 50958	USA	IG 123317	PRT
IG 50602	NLD	IG 50818	DEU	IG 50959	DEU	IG 123336	MAR
IG 50604	DEU	IG 50820	DEU	IG 50963	DEU	IG 123349	TUR
IG 50611	DEU	IG 50821	USA	IG 50978	POL	IG 123356	CHL
IG 50613	UKR	IG 50828	GBR	IG 50982	DEU	IG 123451	ITA
IG 50615	FRA	IG 50830	BEL	IG 50987	DEU	IG 125371	RUS
IG 50622	NLD	IG 50836	DEU	IG 51004	GBR	IG 125379	BLR
IG 50623	NLD	IG 50837	DEU	IG 51008	DEU	IG 125407	BLR
IG 50624	NLD	IG 50838	DEU	IG 51009	DEU	IG 125410	SWE
IG50625	NLD	IG 50840	DEU	IG 51016	USA	IG 125413	RUS
IG 50626	NLD	IG 50845	PRT	IG 51017	USA	IG 125421	RUS
IG 50632	DEU	IG 50863	USA	IG 51019	NLD	IG 125439	ITA
IG 50634	DEU	IG 50870	DEU	IG 51025	GBR	IG 125448	RUS
IG 50638	DEU	IG 50871	DEU	IG 51029	DEU	IG 125461	RUS
IG 50646	NLD	IG 50878	GRC	IG 51069	GBR	IG 125470	GEO
IG 50662	ROM	IG 50898	GRC	IG 51072	FRA	IG 125525	AFG
IG 50663	FRA	IG 50903	DEU	IG 51077	CHN	IG 125556	ITA
IG 50686	DEU	IG 50905	GBR	IG 51083	DEU	IG 125593	ITA
IG 50689	DEU	IG 50907	DEU	IG 51085	DEU	IG 125614	RUS
IG 50694	ETH	IG 50910	DEU	IG 51094	GBR	IG 128865	CYP
IG 50712	DEU	IG 50920	USA	IG 51097	CAN	IG 128911	MNG
IG 50730	CZE	IG 50923	FRA	IG 51100	SWE	IG 129819	RUS
IG 50733	GBR	IG 50924	DEU	IG 51103	UNK	IG 128950	CHL
IG 50734	SWE	IG 50932	DEU	IG 51128	FIN	IG 129052	FIN
IG 50735	BGR	IG 50933	CZE	IG 51133	FRA	IG 1254116	Unknown

# Qualitative and quantitative attributes

Observations were recorded on both qualitative attributes i.e., flower color (FC), testa color (TC), cotyledon color (CC) and pod shape (PS) and quantitative attributes i.e., plant height (PH), total pod number (TPN), number of pods per plant

(NOPPP), total pod weight (TPW), pod weight per plant (PEPP), average pod length (APL), average pod width (APW), average pod thickness (APT), seed weight (SW), seed weight per plant (SWPP) and seed index (SI). Five samples of pod of each accession were taken for the data recording.

#### Statistical analysis

Mean data of each parameter viz., Mean (M), Variance (Var), and Standard deviation (Std. D) for average plant height (APH), number of pod (NOP), number of pod per plant (NOPPP), pod weight (PW), pod weight per plant (PWPP), seed weight (SW), seed weight per plant (SWPP), average pod length (APL), average pod width (APW), average pod thickness (APT), seed index (SI), was calculated and the data was subjected to analysis of variance (ANOVA) for simple statistics through SPSS (Version 6.0) PAST and Microsoft Excel 2007. Principal component analysis was carried out through Microsoft Excel 2007, SPSS v16.0 and PAST software to find the pattern of variation in the data.

#### **Results**

### Morphological traits

Morphological characteristics of 128 pea genotypes were determined on five randomly selected pods per genotype. Variability was observed among the accessions studied for qualitative attributes i.e., flower color (white and purple), testa color (green and creamy), cotyledon color (yellow and green) and pod shape (straight and curved). Traits frequency distributions revealed that accessions were

predominantly vigorous. Frequency distribution of 128 pea germplasm for qualitative attributes is shown in (Table 5). High variation along with range was observed for most of the characters that indicated the scope of simple selection. The result of analysis of variance (ANOVA) revealed that the accession included in this study had significant variation for most of the traits as illustrated in (Table 3). Maximum variations were observed for cotyledon color, testa color and pod shape whereas minimum variations were recorded for flower color. Basic statistics including mean, standard deviation and variance have been presented in the. The extent of range for average plant height was 14.39-245.53 cm. Total number of pods per accession ranged from 2-210. The range of number of pods per plant was 1-70. Pod weight of accessions ranged from 0.7-107 g while pod weight of each plant ranged from 0.45-47.63 g. The extent of range per seed weight accession was 0.4-90 g while seed weight per plant ranged from 0.3-70.1 g. The range for average pod length, average pod width and average pod thickness was recorded from 1.73-8.55 cm, 0.2-0.9 cm and 0.2-0.8 cm respectively. The range for seed index was recorded from 8-31.3 (Table 2).

**Table 2.** Basic statistical analysis of 11 quantitative traits of *Pisum sativum* germplasm

	Mean	Standard error	Standard deviation	Sample variance		nge Maximum
APH (cm)	93.04302	4.783274	54.11657	2928.603	14.39	245.53
TPN	26.63281	2.59459	29.35444	861.683	2	210
NOPPP	15.85677	1.233895	13.95993	194.8796	1	70
PW (g)	18.75086	1.735965	19.6402	385.7375	0.71	107
PWPP (g)	11.27113	0.933075	10.55654	111.4405	0.45	47.63
SW (g)	15.49203	1.489706	16.8541	284.0608	0.44	90
SWPP (g)	9.466419	0.899909	10.18131	103.6591	0.31	70.12
APL (cm)	4.917459	0.101251	1.145529	1.312236	1.72	8.55
APW (cm)	0.49478	0.009917	0.112195	0.012588	0.17	0.85
APT (cm)	0.612728	0.009267	0.104842	0.010992	0.20	0.81
SI	17.90914	0.465629	5.267987	27.75168	8	31.3

Table 3. Analysis of variance (ANONA)

Source of Variation	SS	Df	MS	F	P-value	F crit
Between Groups	973650.3	14	69546.45	213.7604	0	1.697004
Within Groups	614907.2	1890	325.3477			
Total	1588558	1904				

#### Principal component analysis

Out of 15 principal components (PCs), first 5 exhibited eigenvalue greater than 1 (significant) while the rest of 10 PCs exhibited non significant variation so they were not worth interpreting (Table 4). The first 5 PCs showed 79.406% variation in the accession. The first PC accounted 40.298% variation, second PC showed 15.027% variation, third PC showed 9.664% variation, fourth PC accounted for 6.925% variation while fifth PC accounted for 5.987% variation (Figure 1). The first PC was highly related to weight per pod, seed weight, pod weight, seed weight per pod, number of pods per plant and total pod number, average pod thickness, average pod length and average width. This implies that PC1 is a weighted average of these nine attributes. The traits of significant importance in PC2 were average pod thickness, average pod length, average width and seed index. PC3 was related to cotyledon color and testa color. The fourth PC was related to average plant height and pod shape while the fifth PC was related to flower color (Table 5). The projection of attributes on PC1 and PC2 revealed that average pod thickness, average pod length and average pod width are positively correlated to weight per pod. The projection pattern of the attributes on first two PCs depicted that key pod weight contributing attributes were pod thickness, length and width. Cotyledon color was opposite to weight per pod and other yield contributing traits on PC1, therefore, it had negative correlation with all other attributes. The projection of accessions on first two PCs was useful to identify diverse groups of parents for better transgressive segregation (Figure 2). The projection of accessions on first 2 PCs showed population structure and illustrated the identified hetrotic accessions. The accession IG\_50547 was most distinct and variant among rest of all. Accession IG 51094, IG 123451 and IG\_50566 were opposite to accession IG\_51128, IG-123040 and IG\_125448. The genotype IG\_123208 was contrasted to accession IG\_125439. IG\_128950 was opposite to IG\_125470 etc. On the basis of this analysis, promising genotypes have been identified and these are suggested to use in genetic improvement through selection or involving these in hybrid program for crop improvement (Figure 3).

Table 4. Eigen value and % variance

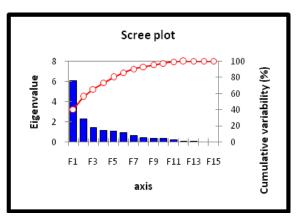
PC	Eigen value	% Variance	PC	Eigen value	% Variance	PC	Eigen value	% Variance
1	6.0447	40.298	6	0.898085	5.9872	11	0.217124	1.4475
2	2.25404	15.027	7	0.678066	4.5204	12	0.0747416	0.49828
3	1.44956	9.6638	8	0.453869	3.0258	13	0.061935	0.4129
4	1.12389	7.4926	9	0.362197	2.4146	14	0.00869331	0.057955
5	1.03875	6.925	10	0.334112	2.2274	15	0.00023018	0.0015345

 Table 5. Principal components for fifteen attributes

Traits		Eigen values						
Traits	PC1	PC2	PC3	PC4	PC5			
Weight/ Pod	0.931	077	0.020	-0.074	-0.102			
Seed Weight	0.920	-0.183	0.192	0.085	-0.048			
Pod Weight	0.915	-0.174	0.254	0.099	-0.015			
Seed weight/ Pod	0.867	-0.085	-0.064	-0.082	-0.150			
Number of Pods per Plant	0.861	-0.323	0.082	-0.166	-0.034			
Pod Number	0.813	-0.366	0.280	0.005	0.071			
Average Pod Thickness	0.557	0.490	-0.247	0.207	0.122			
Average Pod Length	0.572	0.739	-0.010	0.015	-0.007			
Average Pod Width	0.535	0.723	0.021	-0.044	-0.020			
Seed Index	0.332	0.577	-0.265	0.227	0.054			
Testa Color	0.211	-0.327	-0.744	0.087	-0.348			
Cotyledon Color	-0.437	0.311	0.672	0.057	-0.120			
Average Plant Height	-0.017	-0.129	0.278	0.802	-0.201			
Flower Color	0.155	0.171	0.183	-0.536	0.027			
Pod Shape	0.218	-0.218	-0.106	0.172	0.896			

**Table 6.** Frequency distribution of qualitative traits

S. No	Traits	Frequency	Frequency %
1	Flower Color		
	i) White	121	94.5
	ii) Purple	7	5.5
2	Testa Color		
	i) Green	104	81.2
	ii) Creamy	24	18.8
3	Cotyledon color		
	i) Yellow	55	43
	ii) Green	73	57
4	Pod Shape		
	i) Straight	105	82
	ii) Curved	23	18



**Fig. 1.** Scree plot between eigen values and number of principal components

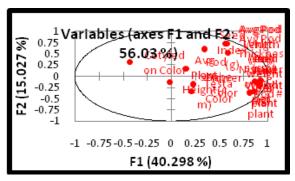
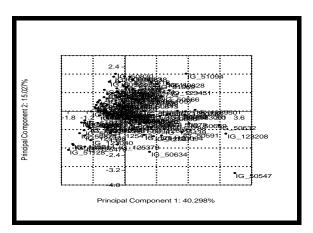


Fig. 2. Principal component biplot of yield traits



**Fig. 3.** Two dimensional ordinations of 128 pea accessions on PC1 and PC2

# Discussion

Phenotypic characterization is the first step towards the classification of crop germplasm (Nisar *et al.*, 2008), the outcome of this study revealed that there is immense diversity in the present exotic pea germplasm. Considerable degree of allelic variation was observed for flower color, cotyledon color and

seed surface as well. All these parameters are of much importance for description of the genotypes. As the quantitative traits are economically more important and are generally used for the improvement of the crop. Similar results were obtained by (Nisar et al., 2008). Analysis showed that high degrees of variations were observed for average plant height, number of pods, number of pods per plant per accession, pod weight per plant, seed weight per accession and seed weight per pod, our results are in agreement with the findings of (Ali et al., 2007) which described pod length showed low degree of variations similarly our results are in agreement with findings of (Ali et al., 2007). Whereas contrasting results were obtained by (Gritton, 1980) and (Duke, 1981) reported that pod length range from 4 to15cm. Pod width and pod thickness showed a very narrow genetic background, our results are in disagreement with the findings of (Ali et al., 2007) which may due to the exotic nature of germplasm in the same climate. Number of pods per plant showed high degree of diversity and can be used directly for the improvement of the crop. The results are in accordance with the findings of (Ali et al., 2007) which observed the consistency of the diversity of the trait in the pea germplasm. 100 seed weight exhibited low degree of variations; our results are in agreement with findings of (Duke, 1981).

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