



## RESEARCH PAPER

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

Hafiz Muhammad Imran Umar<sup>1</sup>, Shoaib-Ur-Rehman<sup>1</sup>, Muhammad Bilal<sup>1</sup>, Syed Atif Hasan Naqvi<sup>2</sup>, Syed Amir Manzoor<sup>3</sup>, Abdul Ghafoor<sup>4</sup>, Muhammad Khalid<sup>4</sup>, Muhammad Tayyab Iqbal<sup>1</sup>, Abdul Qayyum<sup>1</sup>, Farah Ahmad<sup>5</sup>, Malik Ahsan Irshad<sup>1</sup>

<sup>1</sup>Department of Plant Breeding and Genetics, FAST, Bahauddin Zakariya University Multan, Pakistan.

<sup>2</sup>Department of Plant Pathology, FAST, Bahauddin Zakariya University Multan, Pakistan

<sup>3</sup>Department of Forestry, FAST, Bahauddin Zakariya University Multan, Pakistan

<sup>4</sup>Institute of Agri Biotechnology and Genetic Resources, NARC, Islamabad, Pakistan

<sup>5</sup>Department of Biotechnology, Islamia University Bahawalpur, Pakistan

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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.

\*Corresponding Author: Syed Amir Manzoor ✉ [amir.kzd@gmail.com](mailto:amir.kzd@gmail.com)

## Introduction

The pea (*Pisum sativum* L.,  $2n = 14$ ) member of Papilionacea 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 been 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 of 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 judicious 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 assess 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            |
| IG 50625    | 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 | Range   |         |
|----------|----------|----------------|--------------------|-----------------|---------|---------|
|          |          |                |                    |                 | Minimum | 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 |        |        |        |        |
|--------------------------|--------------|--------|--------|--------|--------|
|                          | PC1          | PC2    | PC3    | PC4    | PC5    |
| Weight/ Pod              | 0.931        | -0.077 | 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     | <b>Flower Color</b>    |           |             |
|       | i) White               | 121       | 94.5        |
|       | ii) Purple             | 7         | 5.5         |
| 2     | <b>Testa Color</b>     |           |             |
|       | i) Green               | 104       | 81.2        |
|       | ii) Creamy             | 24        | 18.8        |
| 3     | <b>Cotyledon color</b> |           |             |
|       | i) Yellow              | 55        | 43          |
|       | ii) Green              | 73        | 57          |
| 4     | <b>Pod Shape</b>       |           |             |
|       | i) Straight            | 105       | 82          |
|       | ii) Curved             | 23        | 18          |



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