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#### **Research Note**



### **Genetic diversity and character association studies for agro-morphological and quality traits of advanced breeding lines in field pea (***Pisum sativum* **L.)**

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#### **Abstract**

The present study analysed the trait associations and the genetic diversity for morphological and qualitative traits among the 23 field pea genotypes using multivariate analysis. The correlation analysis revealed that traits like primary branches per plant, pod length, seeds per pod, harvest index and biological seed yield per plant showed a noteworthy positive association at both genotypic and phenotypic levels. Path analysis revealed the correlation of pod length, harvest index and biological yield per plant was due to direct positive effects. The principal component analysis indicated that the fiseven PCs together accounted for 86.56% of the total diversity. The cluster analysis classified genotypes into five different clusters with cluster 1 comprising 18 genotypes, cluster 2 with 2 genotypes, and the remaining three mono-genotypic clusters. The selection of diverse genotypes superior in traits with positive influence on yield can result in greater genetic gains and aid in crop improvement.

**Keywords:** Genetic diversity, correlation, path analysis, PCA

Field pea is a diploid annual cool season legume whose cultivation extendes from semi-arid to temperate regions (Sharma *et al.,* 2023)**.** Globally it is cultivated in an area of 7.15 M ha with a productivity of 1.9 tons/ha and production of 12.4 M tons. The Russian Federation (1.61 M ha), Canada (1.34 M ha) and China (0.93 M ha) are the three major countries in-terms of area and Russian Federation 3.6 (M tons) and Burundi (5.5 tons/ha) occupy top position in-terms of production (*FAOSTAT*, 2024). During the year 2022, the cultivation of field pea across India occupied an area of 0.76 M. ha with a production of 1.0 M tonnes and productivity of 1.3 tons/ha (*FAOSTAT*, 2024). Further, it also has several advantages in terms of nutritional supremacy and aid in combatting hidden hunger (Amarakoon *et al.,* 2012). It also has nutraceutical features because of its anti-oxidant, anti-inflammatory, and immunomodulation properties (Clemente & Del Carmen Arques, 2014; Ndiaye *et al*., 2012)**.** Apart from these, it possesses several other advantages in-terms

of its forage potential (Strydhorst *et al.,* 2008; Tamiru *et al*., 2023) and suitability for rice fallow cultivation (Das *et al.,* 2017). The current yield potential of field pea in India is low when compared to the global productivity. The enhanced production levels of field pea coupled with large number of nutraceutical properties can aid combatting with malnutrition.

Genetic variability being the key to crop improvement enhances the essentiality of diversity studies. Several studies were carried to assess genetic diversity using morphological traits and molecular markers that provides better understanding regarding genotypes which is essential for hybridisation between diverse genotypes in sweet pea (Ahmad *et al.,* 2012; Negisho *et al.,* 2017; Rispail *et al.,* 2023; Sharma *et al*., 2022)**.** But there is need to estimate the diversity among newly developed genotypes and the ones in breeding pipeline as advanced lines to tap their existing potential and enhancing their

utility in breeding programmes. In addition, as yield is a quantitative trait which required strenuous efforts for improvement. The multivariate approaches like cluster analysis and principial component analysis provides the view of existing diversity and aid selection of diverse genotypes. In addition, the understanding of complex relationships among yield and its contributing characters can be an asset for carrying forward the breeding programs. In order to address the above concerns, the current study was taken with the objective of estimating genetic divergence using multivariate analysis and character association studies of field pea. The outcome of the experiment can contribute significantly to the improvement of field pea.

The current study was carried out during the postrainy season of 2020-21 and the materials utilized for the study consists of 23 genotypes sourced from 12 research stations across the country, provided by AICRP-MULLARP (Mung bean, Urd bean, Lentil, Lathyrus, Rajmash and Pea) unit, Department of Genetics and Plant Breeding, Birsa Agricultural University, Ranchi, Jharkhand (**Table 1**). The 23 genotypes including three checks *i.e*., zonal, local, and national were evaluated in Randomized Block Design with three replications. The lines were sown during November, 2020 at BAU college farm, Kanke, Ranchi. Each genotype was grown in a six



ow plot with a row length of four meter. The row-to-row and plant-to-plant spacing were 45cm and 15cm respectively. All the recommended crop management practices were followed. The observations on quantitative traits namely, days to 50% flowering and days to maturity have been recorded on plot basis and the traits plant height, primary branches per plant, secondary branches per plant, pods per plant, seeds for the pod, pod length and biological yield per plant, 100 seed weight, seed yield per plant and harvest index were recorded based on average of five random plants from each replication. The protein content was estimated using Near Infrared Spectroscopy (NIRS) while swelling capacity and swelling index were estimated as per the formula proposed by (Williams *et al*., 1983)**.**



The genotypic and phenotypic correlation and phenotypic and genotypic path analysis was carried out using R package variability (Version 0.1.0)(Popat *et al*., 2020). Bartlett's test for sphericity and Kaiser -Meyer-Olkin (KMO) measure of sampling adequacy was used for evaluating



the suitability of data for carrying out principal component analysis using the SPSS for Windows, Version 16.0. The principal component analysis was carried out using the R package facto-extra (Version 1.0.7)(Kassambara & Mundt, 2017). The D<sup>2</sup> cluster analysis was carried out using the R package bio tools (version 4.2)(da Silva & da Silva, 2017) and cluster means and the inter and intra cluster distances were calculated.

The phenotypic correlation coefficient varied from 0.001 to 0.80 and the genotypic correlation coefficient ranged from 0.001 to 0.88 (**table 2**) which are almost similar to the study carried out by (Jeberson *et al.,* 2016)**.** The traits primary branches per plant, seeds for pod, pod length, biological yield per plant and harvest index showed a positive association with seed yield per plant at both phenotypic and genotypic levels. The significant positive impact of 100 seed weight at the phenotypic level and non-significant association at genotypic level indicates the impact of environment on this trait. Days to 50% flowering recorded positive association with days to pod initiation and a negative association with pods per plant and protein percentage at both genotypic and phenotypic levels, but its association with plant height is significant at phenotypic level only. This indicates that the days to flowering was influenced by the environment. The positive association between primary branches and pods per plant, pod length, seeds per pod and biological yield was remarkable at both phenotypic and genotypic level which is in agreement with earlier reports (Lal & Singh, 2019)**.** The note-worthy positive relation at both phenotypic and genotypic level was observed between days to pod initiation and days to 50% flowering and it was negative with pod length, pods per plant, harvest index and biological yield. The character pod length showed positive

significant relation with seeds per pod which were similar to the findings of Nawab *et al.,* (2008) and (Meena *et al.,* 2022)**.** The trait pods per plant showed significant positive correlations at both genotypic and phenotypic level with primary branches per plant (Parihar *et al.,* 2014)**)** and negative correlation with days to 50% flowering, pod initiation respectively. The positive association between pods per plant and swelling capacity was significant at genotypic level and phenotypically non-significant where as it is inverse in the case of biological yield. Among the traits primary branches per plant, pod length, seeds per pod, 100 seed weight, biological yield per plant and harvest index, except 100 seed weight all of them showed noteworthy positive relationship with seed yield per plant at both phenotypic and genotypic level. These results were similar results of earlier studies ( Sharma *et al.,* 2017; Singh *et al.,* 2018; Uhlarik *et al.,* 2022). These positively related traits can be used as indirect measure for selection of genotypes for genetic improvement of yield.

Path analysis:The direct and indirect effects at genotypic and phenotypic level are furnished in **tables 3** and **4** respectively. Most of the traits at phenotypic level showed positive direct effects on seed yield per plant except number of secondary branches per plant, days to pod initiation, seeds per pod and swelling capacity, but at the genotypic level a greater number of traits *i.e*., days to maturity, primary branches per plant, seed per pod, swelling capacity, swelling index and protein content had negative direct impact on seed yield per plant which were in agreement with previous reports (Toppo *et al.,* 2017)**.** The traits biological yield per plant, harvest index and pod length had most significant impact on yield at genotypic level in the form of positive direct effects, whereas

**Table 2. Phenotypic path coefficients indicating direct and indirect effects of various traits on seed yield of field pea**







**Table 4. Statical tests for testing the suitability of data for principal component analysis**



at the phenotypic level it was identified that harvest index, swelling index and 100 seed weight contributed positively with larger direct effects on seed yield. This is in agreement with the reports of (Jeberson *et al.*, 2016; Kumar *et al.,* 2021; Nawab *et al.,* 2008)**.** Among the several traits with significant associations with seed yield per plant at phenotypic level, most of them are due to direct effects. The trait swelling index recorded positive directs on seed yield per plant but showed negative significant association with it whereas, it was vice versa in the case of seeds per pod. Though the traits had positive direct effects, the correlation with seed yield per plant was negative and *vice versa*, which was due to the greater contribution of negative and positive indirect effects of several traits respectively towards correlation. The path analysis at genotypic level identified that primary branches and seeds per pod recorded positive correlation with seed yield per plant but recorded negative direct influence whereas it was *vice-versa* in case of number of days to pod initiation. The residual value of (0.06) and (0.1426) at genotypic and phenotypic levels was observed. The phenotypic and genotypic path analysis has revealed that intensity of residual value was low at genotypic and phenotypic levels (Lenka & Mishra, 1973)**,** which is a measure of unaccounted traits and necessary characters that contributing towards yield. The low above

values indicates the characters considered as sufficient in case for considering the breeding program.

Principal Component Analysis: It is one of the most common multivariate techniques which is used to simplify large data sets and further used for analysis. From the correlation analysis, it is evident that most of the variables are correlated with seed yield per plant and with each other. This enhanced the need for dimension reduction of the data which can explain larger variability with less data. The value of Bartlett's sphericity test and KMO test **(Table 4)** confirmed the suitability of data set for dimensionality reduction. The variation among the 23 genotypes and the association among the traits under consideration was studied using principal component analysis. The analysis resulted in a total of 16 principal components. Based on the eigen value and percent of variation explained by individual components, only seven principal components were considered as significant. The seven significant principal components which accounted greater than 5% variance accounted for about 86.56% of the total variability, of which the PC1 itself contributed around one fourth of total variability, which was in agreement with the study carried out by (Hedau *et al*., 2018) and PC2 contribute 16.5% **(Table 5).** The biplot analysis **(Fig. 1)** revealed the trait performance and

individual trait associations of genotypes. The genotypes which are most adjacent to a particular trait vector are promising for the particular trait in terms of performance. The angle of less than 45 degree between vectors of traits indicate the noteworthy relationship among them. From the biplot, strong association was evident among the traits primary branches per plant, pod initiation, seeds per pod, 100 seed weight and biological yield per plant with seed yield per plant which were in agreement with study of (Nath *et al.,* 2023) . Seed yield per plant was negatively associated with swelling index, days to pod initiation and plant height. Noteworthy relationship among the traits harvest index, swelling capacity, protein content and swelling index and similarly between days to maturity and pods per plant was identified. The RFP 12-08 genotype was very proximate to trait vectors of days to pod initiation, 100 seed weight and biological yield, whereas genotype HFP-1607 was very close to primary branches per plant and seeds per pod which is indicating the diversity in trait performance of genotypes. The nearness of the genotypes IPF 20-17 and TRCP 11 with harvest index, VL 72 with days to maturity, Pant P 498, RFPG 180 and KPMR 937 with plant height made them distinct from other genotypes in-terms of those traits respectively. The vector









length of a particular trait is a measure for its contribution towards diversity among the material being evaluated. From the **Fig. 1**, it is inferred that, seed yield per plant, biological yield per plant, pod length, primary branches per plant, pods per plant, seeds per pod and days to 50% flowering are the most contributing traits towards genetic diversity among the 23 genotypes. Among the several variables considered, most significant contribution of PC1 (26.8%) diversity was made by biological yield per plant (18.5% 0f PC1) followed by pod length (16.4% of PC1) and seed yield per plant (16.3% of PC1). Similarly, the large portion of diversity due to primary branches and seeds per pod is accounted by PC11 and PC9 respectively. The PC16 represented the greater portion of diversity due to biological yield and seed yield per plant. From the **Fig. 2**, it is inferred that there are some other variables whose diversity is majorly represented by single principal component. Among the genotypes considered for diversity study, the genotypes HUDP-15, Dantewada-FP-1 and SKNP-04-09 made a major contribution of the diversity accounted by PC1. From the figure 5, we can observe the other genotypes which are major contributers towards diveristy of PC1.(**Fig.3**)

**pea** was highest in case of cluster 5 and cluster 3 and lowest Cluster Analysis: The cluster analysis using Mahalanobis D<sup>2</sup> method classified the 23 genotypes into five clusters, with the maximum number of genotypes grouped under cluster 1 (18 genotypes) followed by cluster 2 (2 genotypes) and the remaining three clusters with one genotype each. It was observed that number of days to 50% flowering (76.67) and days to maturity (111.67)

 $\frac{F}{\sigma}$  **Figure 5:**  $\sigma$  and  $\sigma$  binding the relationship  $\frac{F}{\sigma}$  and  $\frac{F}{\sigma}$ in case of cluster 3 (73) and cluster2 (109) respectively (**table 6**). The primary and secondary branches per plant were maximum in case of cluster 4 (2.13) and cluster 2(2.01) and were minimum in cluster 3 (0.6) and cluster 4 (0.23) respectively. The cluster 1 (87.81) and cluster 2 (86.50) recorded the maximum and minimum number of days to pod initiation respectively. In case of pods per plant, the cluster means were maximum and minimum in case of cluster 3(17.06) and cluster 1 (12.63) and plant height was minimum in cluster 5 (114.16) and cluster 2 (51.3). The cluster mean of the trait seeds per pod was maximum in cluster 4(6.39) and minimum with cluster 3 (3.149) and maximum and minimum cluster means for swelling capacity was observed in cluster 3 (0.25) and cluster 5 (0.09) respectively. The genotypes under the cluster 3 and cluster 2 showed maximum (20.547) and minimum (16.818) cluster means for 100 seed weight. The cluster 2 and cluster 5 showed maximum (1.566) and minimum (0.61) values of swelling index, whereas the protein content was maximum in cluster 3(27.76%) and minimum in cluster 5 (24.66). It is the trait harvest index that showed maximum and minimum mean values in cluster 2(0.398) and cluster 4 (0.23). The mean biological yield per plant was maximum in case of cluster 4 (50.97) and minimum in case of cluster 1 (21.13) and it was the cluster 4 (11.5) and cluster 3 (6.56) which recorded maximum and minimum cluster mean for seed yield per plant respectively. The cluster means can be used as criteria for identification of genotypes for improvement of a particular trait *i.e.,* the selection of genotypes from a cluster with superior mean can result in its improvement. (Kumar *et al.,* 2022).



Fig. 2. Corrplot Indicating dimensional contribution of individual variables towards diversity





**Fig. 3. Corrplot Indicating dimensional contribution of genotypes towards diversity**

<b>Trait /Cluster</b>	<b>Cluster 1</b>	<b>Cluster 2</b>	<b>Cluster 3</b>	<b>Cluster 4</b>	<b>Cluster 5</b>
<b>DFF</b>	74.17	74.33	73.00	75.67	76.67
<b>DM</b>	109.39	109.00	113.00	109.67	111.67
PB	0.73	0.98	0.60	2.13	1.70
<b>SB</b>	1.01	2.02	0.57	0.23	1.03
PI	87.83	86.50	87.33	87.67	87.67
<b>PPPL</b>	12.63	17.03	17.07	14.40	13.33
PH	95.64	51.30	94.93	60.53	114.17
PL	5.58	6.14	5.21	7.35	5.33
<b>SPP</b>	4.66	4.95	3.15	6.40	5.98
SW	16.23	16.82	20.55	18.37	18.53
SC	0.14	0.16	0.25	0.15	0.09
<b>SI</b>	1.07	1.06	1.57	1.09	0.62
PC	26.28	25.82	26.77	26.30	24.67
H <sub>II</sub>	0.33	0.40	0.28	0.23	0.36
BY	21.14	24.46	23.50	50.97	30.24
SY	7.02	9.23	6.57	11.50	10.87

**Table 6. Cluster means of 23 field pea genotypes**

The  $D<sup>2</sup>$  cluster analysis revealed that the characters secondary branches per plant (14.1%), plant height (12.6%) and 100 seed wight (10.7%) were the major contributors for diversity. The contribution of biological yield per plant (1.9%), days to pod initiation (2.7%) and pods per plant (2.6%) was very much meagre towards diversity among the genotypes. The contribution of other variables can be observed from the **Fig. 4**. The inter and intra cluster distances are tabulated in the

**table 7.** The traits with superior performance for particular traits among these genotypes can be improved by the selection among the segregating generations. The maximum inter-cluster distance was observed between cluster 2 and cluster 5 and it was minimum in case of cluster 1 and cluster 5. The inclusion of genotypes from most diverse clusters in crossing programmes can generate a huge genetic variability that can be tapped for development of high yielding field pea varieties.

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**Fig. 4. Pie chart indicating the contribution of various traits towards genetic diversity**





The current study identified that the traits pod length, harvest index and biological yield per plant are positively correlated at both phenotypic and genotypic levels with seed vield per plant and had significant direct positive effects on seed yield per plant can be used selection criteria. The first seven principal components explained 86.7% of diversity and the results of correlation analysis agreed with correlation identified using principal component analysis. The principal component analysis identified that traits seed yield per plant, biological yield per plant and pod length are major contributors. The D<sup>2</sup> cluster analysis identified cluster 1 and cluster 5 were the most diverse clusters. The genotype HUDP-15 under cluster 4 possessed maximum cluster mean for biological yield per plant and pod length that positively correlated with seed yield per plant. The genotype HUDP-15 with Dantewada FP-1 from cluster 4 and cluster 2 respectively based on cluster diversity can be the most appropriate parental combination for crossing that can result in maximum heterosis. Further selection for yield in segregating generations can result in development of promising varieties that can augment the field pea productivity in India.

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