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RESEARCHARTICLE

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Evaluating Morphological Diversity of Pea Genotypes using Multivariate Analysis

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Abstract
Pea (*Pisum sativum* L.) is the second most economically essential winter legume in Pakistan. The yield potential of this crop is low in Pakistan contrary to other countries and little genetic diversity in the germplasm is the reason for it. This research was planned to determine the genetic diversity in pea genotypes. Twenty pea accessions collected from NARC and AARI were sown following the RCBD design in October 2022 at the Department of Plant Breeding and Genetics. Data were collected for days to 50% flowering, pod length (cm), pod width (cm), yield per plant (g), plant height (cm), number of seeds per pod, hundred seeds weight (g), seed diameter (mm) and number of pods per plant. For estimating genetic diversity, analysis of variance and cluster analysis. Significant variation was exhibited by all the studied genotypes through analysis of variance. 8 clusters were formed during cluster analysis. The most diverse clusters were 1 and 8 for observed traits exhibiting that their enclosed genotypes showed maximum genetic diversity and these accessions could be resourceful for pea crop improvement. The least divergent clusters were 7 and 8, displaying that their members had very little genetic diversity. Through this study, it is viewed that a great range of genetic diversity is present in assembled accessions which may be used in the breeding of high-yielding pea cultivars.

KEYWORDS
Genetic diversity, Crop improvement, Morphological traits, Multivariate analysis

1 | INTRODUCTION
Peas are a vital food and feed legume that are cultivated worldwide in various temperate zones, especially from Asia to Europe and North America (Wu et al. 2017). Peas are widely employed as commercial protein due to their ample production, affordability, and high availability. Pea protein and its hydrolysates are responsible for a variety of its functional qualities, including its ability to hold water and oil, foam, dissolve, gel, and emulsify, as well as several health benefits like antihypertensive, modifying the activities of gut flora, and antioxidant.

The primary challenges facing breeders are producing high-yielding cultivars with additional improved traits like sufficient photosynthesis, early maturing types, protein content, essential amino acid ratios, organic matter accumulation during the early growth phases, and the ability to develop resistance to diseases and climate change (Chattha et al. 2021; Bilal et al. 2022). It has been established that genetic divergence is a crucial instrument for enhancing each of these traits. Utilizing biometric techniques to quantify genetic variability aids breeders in selecting unique parents, which in turn guarantees the success of hybridization programs (Zafar et al., 2023). Additionally, evaluating genetic variability is important for determining the gene for a given parameter among the variety of germplasm that is accessible. A deeper comprehension of genetic variability and similarity may be beneficial for extending selection gain (Chowdhury & Sharma, 2003; Chattha et al. 2017). To make predictions regarding the significance of genetic diversity for a given population, it is necessary to evaluate the relationship between fitness and genetic variability. The main factors influencing the degree of genetic variety in a population are its evolutionary history, level of environmental homogeneity, mating system, and population history (Zafar et al., 2024).

It is advantageous to find pea genetic resources by looking at the genetic variability of peas in different environmental situations, since this will have an impact on pea productivity and breeding (Zhao et al. 2020). Peas have a high potential for hereditary performance, but because of a decline in output, their average yield is not up to par. However, this can be corrected by inter crossing genotypes that are genetically different from one another. Finding genetically separate lines of descent became essential as a result. (Lal et al. 2018).

Numerous techniques are currently accessible for examining the genetic diversity of populations, breeding lines, and germplasm accessions (Ahmad et al. 2019; Zafar et al. 2022; Zafar et al. 2023). These techniques rely on morphological, genetic, agronomic performance, pedigree, and biochemical data. Path analysis revealed that the height of the plant after days to maturity in peas contributed the most to genetic difference (Singh et al. 2017). The aim of the present investigation is to obtain genetic variability through physical features.

2 METERIAL AND METHOD

Twenty genotypes of peas (*Pisum sativum* L.) were used in the experiment. In two replications, experimentation accession seeds were seeded using a randomized complete block design (RCBD). A 10 cm gap between plants and a 30 cm gap between rows was used for seeding. Reasonable growth was achieved by using appropriate agronomic methods. Each replication produced four germinations, which were tagged to provide information about the following morphological characters:

Data Collection

Days to 50% flowering were recorded by noting the number of days from planting the crop until the point where 50% of the plants began to produce flowers in each replication. At the complete pod development stage, the average length of the fruiting pod from the first reproductive node was measured in centimeters for four distinct plants in each replication. At full maturity, the average width of four distinct pods was measured in centimeters for each replication. The number of seeds was counted from the four largest pods, and an average was calculated. The height of the selected plant was measured in centimetres from the ground to the tip using a meter rod after it reached maturity. Reading proficiency was considered to be average across all genotypes. Pod numbers were counted from four distinct plants in each replication and an average was calculated. At full seed maturity, the average diameter of four seeds was measured in millimetres from both replications. The weight of one hundred seeds was measured in grams when the seeds' moisture content decreased to 12% or less through sun drying, using an electronic balance. The total output of the chosen plant from each repetition was determined using an electronic balance. At maturity, the yield per plant of selected plants was measured in grams using a weighing scale. Reading proficiency was considered to be average across all genotypes.

Statistical analysis

Ronald Fisher's analysis of variance (ANOVA) and LSD test were utilized to determine statistical significance using Statistics 8.1. Cluster Analysis (CA) was employed to identify the interaction among the pea genotypes. Cluster analysis was conducted using Minitab 17, and a tree diagram based on elucidation distances was created using the Linkage technique. The D2 statistic was computed following Rao's (1952) method.

3 RESULTS AND DISCUSSION

The selection for the superior genotypes with desirable traits in a diverse germplasm is essential because all the succeeding genetic improvement is depending upon selection. Genetic variability is the variation in the genetic information content of individuals and within a species, community, and population. Variation generates either by differences in genetic makeup of the individual of a population or as a result of the environment differences. Selection is most productive where there is a significant amount of genetic variability is present in individuals within a population.

Analysis of variance (ANOVA) practiced as stated by steel et al. (1997) was applied with the intention of studying the significance of all the characters between 20 pea genotypes under study. It was performed for all the studied morphological traits to examine significant difference between the accessions. Sharma et al. (2003) determined that genetic diversity is necessary for crop improvement and the degree of variability available for a given character determines how far genetic improvement of that character may exhibit.

Plant Height (cm)

Analysis of variance revealed that genotype differences were highly significant at the 5% level of probability (Table 1).

The degree to which hereditary factors influence plant height variation is presented (Table 2). The PCV was found to be more than GCV which means that height of plant has some interaction with environment. Results from this study exhibited that GCV and PCV assessed for height of plant, which were further noted by Singh et al. (2012). Plant height showed heritability score (0.85), indicating a significant genetic influence on the development of this trait. The genotypic variance

Table 1: Analysis of variance for plant height (cm)

SOV	DF	PH	D to 50% flowering	PL	PW	HSW	SP	SD	Y/P	NPP
Replication	1	13.39	60.02	0.04	0.00013	0.72	0.35	24.21	24.21	28.47
Genotypes	19	284.03**	36.25**	1.07**	0.03**	93.04**	1.64**	119.79**	119.8**	77.4**
Error	19	22.13	0.86	0.05	0.001	22.06	0.11	22.49	22.49	15.71

** = highly significant, *=significant, ns= non-significant

Table 2: Basic statistics and genetic components of variation for plant height (cm)

SOV	PH	D to 50% flowering	PL	PW	HSW	SP	SD	Y/P	NPP
Maximum	64.87	83	8	1.11	38.37	6.08	0.85	31.75	28.87
Minimum	24.45	70.5	5	0.64	16.75	2.45	0.62	4.6	4.25
Grand Mean	41.08	76.62	5.94	0.95	27.47	4.12	0.73	17.47	14.59
Standard Error of Mean	3.32	0.65	0.17	0.02	3.32	0.24	0.03	3.35	2.80
Critical Difference (CD) 5%	9.84	1.94	0.5	0.06	9.83	0.72	0.12	9.92	8.29
Critical Difference (CD) 1%	13.47	2.66	0.69	0.09	13.43	0.99	0.14	13.56	11.33
Environmental Variance	22.12	0.86	0.05	0.001	22.06	0.12	0.03	22.49	15.71
Genotypic Variance	130.93	17.69	0.5	0.01	35.49	0.76	0.08	48.64	30.84
Phenotypic Variance	153.07	18.56	0.56	0.02	57.55	0.88	0.05	71.14	46.55
Environmental Coefficient of Variance	11.45	1.21	4.06	3.31	17.09	8.39	7.15	27.14	27.15
Genotypic Coefficient of Variance	27.85	5.48	11.99	13.34	21.68	21.20	5.94	39.92	38.05
Phenotypic Coefficient of Variance	30.11	5.62	12.66	13.74	27.60	22.80	9.25	48.27	46.75
Heritability (Broad Sense) (%)	0.85	0.95	0.89	0.94	0.62	0.86	0.41	0.68	0.66
Genetic Advance	21.80	8.46	1.39	0.25	9.64	1.67	0.06	11.88	9.31
Genetic Advance as percentage of mean	53.07	11.04	23.4	26.67	35.07	40.61	7.87	68.00	63.81

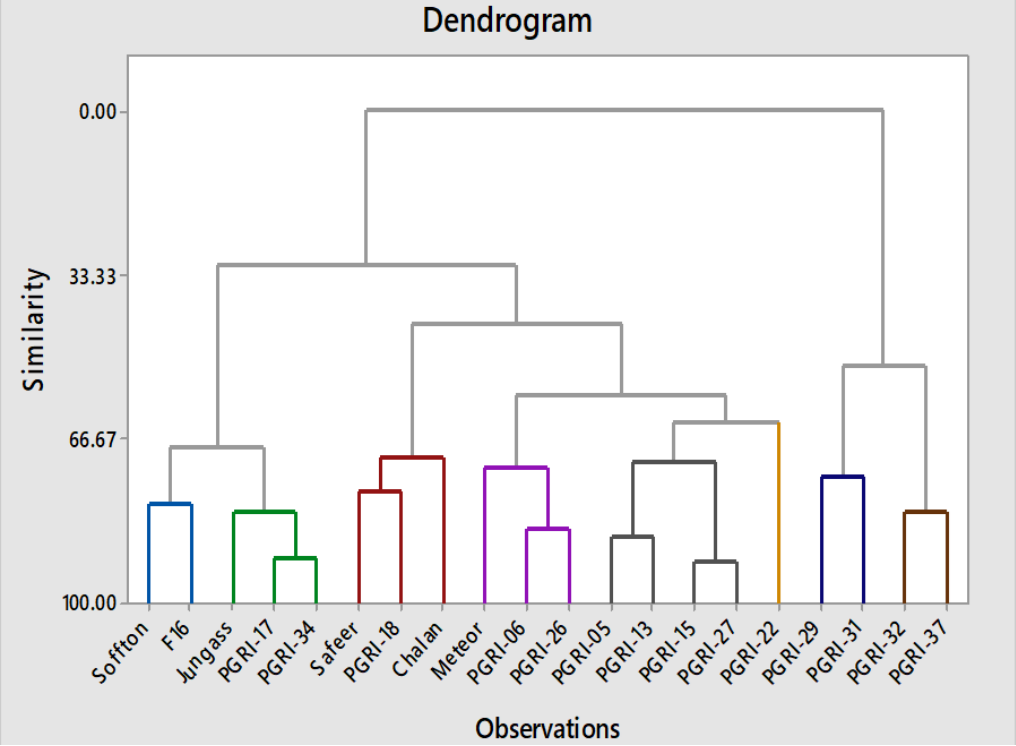

Fig. 1: Dendrogram showing the spatial position of pea genotypes. The numbering of genotypes is shown in the Table 4.19.

was smaller than phenotypic variance but greater than environmental variance indicating higher degree of genetic variability for this trait as previously observed by Georgieva et al. (2016). Genetic advance as a percentage of the mean was (53.07), indicating that additive gene action control plant height and the selection would be helpful for enhancing this character. Jeberson et al. (2016) observed high genetic advance as percent of mean for plant height.

Days to 50% Flowering

The genotypes had significant variations that were high for days to 50% blooming at the 5% probability level (Table 1).

The extent of genetic variation for days to 50% blooming that is shown in (Table 2). The PCV was found to be greater than GCV which means that the trait days to 50% flowering was least influenced by

environment to some extent as observed by Gupta et al. (2018). The genotypic variance was smaller than phenotypic variance but greater than environmental variance indicating higher degree of genetic variability for this trait. Results from this study exhibited that GCV and PCV assessed for days to 50 % flowering were moderate (10-25%) which were also reported by Singh et al. (2017). The heritability observed for this character was high (0.95) which exhibits great extent of genetic effects in determination of this character and should be adopted for pod yield. High genetic advance as percent of mean was 11.04 exhibited by days to 50 % flowering. High genetic advance as a percentage of mean and high heritability concluded that hybridization and selection will be useful for improvement of this trait.

Pod Length (cm):

At 5% probability level, all the genotypes showed significant variations in pod length (Table 1).

Magnitude of genetic components of variation for pod length is presented in (Table 2). The GCV was lower than the PCV which showed that some environmental variation existed since it as obtained by Gupta et al. (2018). Genetic advance as a percentage of mean was 23.40 and heritability 0.897 indicated very limited improvement through selection as stated by Tezera (2000). Low genetic advance 1.39 was observed suggested that non-additive gene action control this trait. Low values of phenotypic and genotypic variance showed small variability for the trait were the reason for low genetic advance value as previously observed by Fikreselassie, 2012.

Pod Width (mm):

Significant variability for pod width was present within the genotypes at 5% probability level (Table 1).

Magnitude of genetic components of variation for pod width is presented (Table 2). For pod width, GCV was smaller than PCV which exhibited that environmental influence for pod width as suggested by Meena et al. 2017. Low values of phenotypic and genotypic variance showed small variability for the pod width were the reason for low genetic advance value 0.25 as previously observed by Fikreselassie (2012). Low genetic advance and 0.94 heritability were found for the width of pod exhibited non-additive gene action describing little betterment through selection as previously researched by Singh et al. (2012).

Hundred Seeds Weight (g):

When assessed genotypes for hundred seed weight at 5% probability level, significant differences were founded (Table 1).

The magnitude of genetic components of variation for hundred seeds weight is presented (Table 2). In comparison to PCV was greater than indicating higher degree of genetic variability for this trait and it was less influenced by environment as previously studied by Georgieva et al. (2016). Genetic advance as percentage of mean was 35.07 and heritability 0.62 obtained by hundred seeds weight showed that the character governed by non-additive gene action as observed by Singh et al. (2012). Low genetic advance which was 9.63 for hundred seeds weight due to low values of genotypic and phenotypic variances exhibiting low variability for the traits as previously displayed by Fikreselassie (2012).

Number of Seeds per Pod

At 5% probability level, was seen a significant variation in the number of seeds per pod between the pea genotypes (Table 1).

The extent of genetic variability for number of seeds per pod presented in (Table 2). The GCV was smaller than PCV indicated environmental influence on seeds per pod was near to non-significant. Similar conclusion was founded by Lavanya et al. (2010). 0.86 heritability and 1.67 genetic advance as a percentage of mean exhibited by seeds per pod unveiling involvement of no-additive genes as suggested by Jaiswal et al. (2015). Values of phenotypic and genotypic variance showed small variability for seeds per pod because of the low genetic advance value 1.67 as previously observed by Fikreselassie (2012).

Seed Diameter (mm)

Analysis of variance (ANOVA) showed that highly significant difference is present between genotypes at 5% probability level (Table 1).

The estimated broad sense heritability was 0.413 for seed diameter as shown (Table 2). The value of GCV for seed diameter was observed less as compares to the values of PCV. The findings showed similarity with the findings of Gudadinni et al. (2017).

Yield per Plant (g):

Significant variations were observed between the genotypes for yield per plant by analysis of variance (Table 1).

The value of GCV was less than the value of PCV for yield per plant which exhibited that there will be least environmental effects for this character. It also had high value 0.68for estimated heritability (Table 2). The findings showed similarity described by Singh et al. (2017).

Number of Pods per Plant

It was displayed through ANOVA that highly significant difference is present between genotypes at 5 % probability level (Table 1).

Magnitude of genetic components of variation for number of pods per plant is presented (Table 2). The value of GCV for number of pods was observed less as compared to the values of PCV which exhibited that least environmental influence for number of pods per plant suggested by Meena et al. (2017). Low values of phenotypic and genotypic variance showed small variability for the number of pods per plant were the reason for low genetic advance value as previously observed by Fikreselassie (2012). The estimated broad sense heritability was low 0.66 for number of pods per plant exhibited non-additive gene action describing little betterment through selection as previously researched by Singh et al. (2012).

Cluster Analysis and D^2 Statistics

The cluster analysis confirmed that there was significant morphological diversity for various genotypes (Fig. 1). Twenty pea genotypes were grouped into 8 clusters based on different morphological traits (Table 3). Cluster analysis exhibited that cluster 1 enclosed 2 genotypes, while cluster 2, cluster 3 and cluster 4 had 3 genotypes. Cluster 5 comprised of 4 genotypes. Cluster 6 contained 1 genotype, while cluster 7 and cluster 8 had 2 genotypes (Table 3). The cluster mean value of various traits are showed in Table 4. The highest cluster mean values were showed by Cluster 8 containing genotypes PGRI-32 and PGRI-37 for traits plant height (61.83), seed diameter (0.83), hundred seed weight (37.46) and days to 50% flowering (78.50). Cluster 7 showed highest cluster mean values comprising genotypes Jungass, PGRI-17 and PGRI-24 for traits number of pods per plant (27.62), number of seeds per pod (5.93) and yield per plant (26.56). The lowest cluster mean values were estimated in cluster 1 which were assembling genotypes Soffton and F16 for plant height (24.03), number of pods per plant (6.00), yield per plant (3.62) and number of seeds per plant (6.75).

The pairwise Mahalanobis distance (D^2 statistics) among 8 clusters of 20 pea genotypes showed in the Table 5. It was concluded that inter cluster distance was recorded highest between the cluster 1 and cluster 8 (46.31) followed by cluster 8 and cluster 3 (38.68), cluster 8 and cluster 2 (34.83), cluster 8 and cluster 6 (31.45), cluster 4 and cluster 8 (28.21) and cluster 5 and cluster 8 (18.20). The lowest inter cluster distance was noted between the cluster and cluster 8.

Table 3: Cluster numbers with their associated genotypes based on different morphological traits

No. of Clusters	No. of Genotypes	Genotypes
Cluster 1	2	Soffton, F16
Cluster 2	3	Jungass, PGRI-17, PGRI-24
Cluster 3	3	Safeer, PGRI-18, Chalan
Cluster 4	3	Meteor, PGRI-06, PGRI-26
Cluster 5	4	PGRI-05, PGRI-13, PGRI-15, PGRI-27
Cluster 6	1	PGRI-22
Cluster 7	2	PGRI-29, PGRI-31
Cluster 8	2	PGRI-32, PGRI-37

Table 4: Cluster mean values for different morphological traits in pea

Traits	Cluster 1	Cluster 2	Cluster 3	Cluster 4	Cluster 5	Cluster 6	Cluster 7	Cluster 8
PH	24.03	29.21	32.10	45.38	46.45	38.78	52.57	61.83
NPP	6.00	14.37	12.58	12.37	14.28	11.50	27.62	19.00
SD	0.72	0.75	0.68	0.70	0.75	0.69	0.70	0.83
PW	0.83	1.02	0.80	1.02	0.98	1.10	0.94	0.97
NSPP	3.62	4.71	3.65	4.00	3.62	2.46	5.93	4.50
HSW	24.50	27.39	18.52	21.06	33.01	34.75	28.93	37.46
YPP	6.75	25.08	11.66	11.62	19.11	8.87	26.56	26.18
PL	6.41	7.24	5.55	5.46	5.57	5.18	5.89	5.97
DFF	78.50	73.67	79.00	78.50	77.12	69.00	73.75	78.50

Table 5: D^2 statistics among 8 clusters for various pea genotypes

	cluster1	cluster2	cluster3	cluster4	cluster5	cluster6	cluster7	cluster8
cluster1	0.00	21.60	13.02	23.10	28.27	21.23	41.50	46.31
cluster2		0.00	17.40	22.67	19.51	21.16	26.10	34.83
cluster3			0.00	13.54	21.86	20.46	31.75	38.68
cluster4				0.00	14.34	18.23	24.40	28.21
cluster5					0.00	15.55	17.43	18.20
cluster6						0.00	28.84	31.45
cluster7							0.00	16.05
cluster8								0.00

Conclusion

Genetic divergence among 20 genotypes was measured by morphological characteristics in seedlings. With four genotypes, Cluster V has the most, with three each for Clusters II, III, and IV. Clusters I, VII, and VIII each had two genotypes, whereas cluster VI only had one. At 78.50 days to 50% blooming, plant height (61.83), seed diameter (0.83), and hundred seed weight (37.46), Cluster 8 with genotypes PGRI-32 and PGRI-37 had the highest mean values. Among the six sets of twenty pea genotypes, the pairwise Mahalanobis distance (D2 statistics) was greatest between clusters 1 and 8, measuring 46.31. All nine genotype traits showed substantial variation. The results of this research will aid in genetic improvement and diversity conservation of pea crop.

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