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Exploring Genetic Diversity for Yield and Yield Attributing Traits in Pea (*Pisum sativum* L.) through D^2 and Principal Component Analysis

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Abstract

An experiment was conducted during the *rabi* season of November, 2019–April, 2020 at JNKVV, Jabalpur, Madhya Pradesh (482 004), India to scrutinize the genetic diversity among different Pea genotypes. Using Mahalanobis D^2 Statistics, 52 genotypes were grouped into 8 clusters. Cluster I (32 genotypes), cluster II (12 genotypes), and cluster VI (3 genotypes) were found to be poly-genotypic, while the rest of the clusters were mono-genotypic. Notably, the genotypes of cluster II exhibited the highest inter-cluster distance with the genotype of cluster V, indicating significant potential for widening the genetic base of pea. Furthermore, the highest intra-cluster distance was found in cluster VI. Principal Component Analysis demonstrated that five principal components (PCs) exhibited more than 1.00 Eigen value, accounting for approximately 80.62% variability among the traits studied. PC1 demonstrated the highest variability at 36.18%, followed by PC2 (15.55%), PC3 (13.33%), PC4 (8.27%), and PC5 (7.28%). The PC1 loaded with yield traits including plant height, number of nodes plant^{-1}, pod-bearing length, number of pods plant^{-1}, effective pods plant^{-1}, seeds plant^{-1}, biological yield, and seed yield plant^{-1}. The PC2 predominantly represented phenological traits such as days to first flower, days to 50% flowering, and days to maturity. The PC3 encompassed the harvest index, while PC4 focused on 100 seed weight. In contrast, PC5 is linked to pod length and seeds per pod. Additionally, based on PCA, the genotypes FP 14–21, JP 180, VRP 5, AMAN, HVP–2 and FP 14–17 were identified as potential lines.

Keywords: Genetic diversity, mahalanobis D^2 statistics, pea, principal component analysis

1. Introduction

Pea, scientifically known as *Pisum sativum* L. (2n=2x=14), is one of the world's oldest crops, which belongs to the family *Leguminosae* and is grown in all temperate countries and the most tropical highlands (Choudhury et al., 2007, Singh et al., 2021). It has been grown for several thousand years in India. Its genetic diversity indicates four main centres of origin: Central Asia, the Near East, Abyssinia (Ethiopia), and the Mediterranean (Smykal et al., 2014). The last common ancestor for the genera *Vicia*, *Lathyrus*, and *Pisum* is believed to be the ancestor of *Vavilovia formosa*. From this ancestor, an extinct perennial and later an annual *Pisum* ancestor evolved (Smýkal et al., 2011). The *Pisum* genus consists of the wild species *P. fulvum*, found in Jordan, Syria, Lebanon, and Israel, and the widely cultivated species *P. sativum*, used globally.

Generally, two types of pea are grown in India: the field pea (*Pisum sativum*(L.) var *arvense*) and the garden pea (*Pisum sativum* (L.) var *hortens*) (Khan et al., 2017, Anand et al., 2024a). The garden pea is typically used for table purposes and harvested in green pod conditions, while the field pea is utilized in various food preparations such as dry, whole or split dal or flour (Besan). Canada is the biggest producer of peas worldwide, followed by China, Russia, and India (Raghunathan et al., 2017, Wu et al., 2023).

Nutritional profile of peaincludes 17–22 g carbohydrates, 20–50 g starch, 14–26 g dietary fibre, 6.2–6.5 g protein, 0.4 g fat, 1.0 g ash per 100 g, with 9–10 mg calcium, 3–5 mg sodium, 97–99 mg potassium per 100 g, and vitamins like 0.7 mg riboflavin, 5–6 mg thiamine, and 0.54 mg folate per kg (Dhaliwal, 2017, Goswami and Shukla, 2019, Anand et al., 2024b). Low saturated fats, cholesterol, and sodium levels, making them a wholesome addition to a balanced diet (Gao et al., 2022, Chen et al., 2023).

The limited availability of diverse genetic resources and the confined genetic diversity present in cultivated germplasm can hinder the effective utilization of traditional breeding methods

and the advancement and implementation of genomic tools.Genetic diversity is crucial for hybridization programs aimed at yield improvement, especially in self-pollinated crops. Information about germplasm diversity and genetic relatedness among elite breeding material is fundamental in breeding the desired plant type (Uhlarik et al., 2022, Kumawat et al., 2024). Crossing among parental lines is the most potent and assured method for creating variability. However, the selection of divergent parents is most important, as the greater the genetic divergence among the parents for the characters, the better the chances of releasing the variability (Sanwal et al., 2015, Sinha et al., 2020).

The limited availability of diverse genetic resources and the confined genetic diversity present in cultivated germplasm could hindered the effective utilization of traditional breeding methods and the advancement and implementation of genomic tools. The Mahalanobis D^2 statistic is helpful in measuring genetic divergence between genotypes and linking clustering patterns to geographic origin (Khan et al., 2017, Baria et al., 2024).

Principal component analysis (PCA) is a set of methods for simplifying high-dimensional data by identifying relationships between variables without losing any information. It helps to capture the most significant variations along each axis of differentiation (Hanci et al., 2019, Sanwal et al., 2024). It helps select the best genotypes based on PC scores for yield traits and quality. The present study explores the magnitude of genetic divergence and aims to identify more diverse parents for pea genetic improvement.

2. Materials and Methods

During the *rabi* season of November 2019–April, 2020, an experiment was conducted at the Seed Breeding Farm, Department of Plant Breeding and Genetics, JNKVV, Jabalpur, Madhya Pradesh (482 004), India. Randomized Complete Block Design was used to scrutinize fifty-two diverse pea genotypes. All the genotypes were sown in three replications with a two-row pattern, with 30 cm row-to-row and 10 cm plant-to-plant distance. Mahalanobis generalized distance D^2 (1936) was utilized to analyze the data obtained on various traits. Tocher's approach, as described by Rao (1952), was employed to group the populations into clusters.

Similarly, Ward's approach was used to create tree diagrams based on Euclidean distances, and cluster analysis was conducted using clustering. Principal Component Analysis, a well-known dimension reduction method (Massy, 1965; Jolliffe, 1986), was used to extract the principal components. These components were ordered based on the variation in the actual data, with the first principal component having a considerable substantial sample variance and each subsequent component representing combinations with the highest possible uncorrelated variance compared to those taken earlier.

3. Results and Discussion

3.1. Mahalanobis D^2 analysis

The Pea genotypes revealed highly significant differences for all 18 characters studied, as revealed by the analysis of variance. The trait pod-bearing length (45.93%) contributed most towards genetic divergence, followed by 100 seed weight (22.32%), days to first flower opening (9.50%), days to maturity (6.86%), number of nodes plant^{-1} (5.73%), number of effective nodes plant^{-1} (3.47%) and number of pods plant^{-1}(2.49%). In contrast, the magnitude of genetic divergence was less than one per cent for biological yield plant^{-1} (0.98%), seed yield plant^{-1}(0.90%) and harvest index (0.23%) (Table 1). Khan et al. (2017), Singh et al. (2017), Bijalwan et al. (2018), and Hanci (2019) reported similar findings in their investigation.

Table 1: contribution of different characters towards clustering in Pea germplasm

Sl. No.	Traits	Times ranked 1st	Contribution towards divergence (%)
1.	Days to first flower opening (DFFO)	126	9.50
2.	Days to 50% flowering (DFF)	0	0.00
3.	Days to maturity (DM)	91	6.86
4.	No. of primary branches plant^{-1} (NPBPP)	0	0.00
5.	No. of secondary branches plant^{-1} NSBPP	0	0.00
6.	Plant height (PH)	9	.68
7.	No. of nodes plant^{-1} (NNPP)	76	5.73
8.	No. of effective nodes plant^{-1} NENPP	46	3.47
9.	Pod bearing Length (PBL)	609	45.93
10.	No. of pods plant^{-1} (NPPP)	33	2.49
11.	No. of effective pods plant^{-1} (NEPPP)	8	0.60
12.	Pod length (PL)	0	0.00
13.	No. of seeds pod^{-1} (NSPP)	4	0.30
14.	No. of seeds plant^{-1} (NSPPlt)	0	0.00
15.	100 Seed weight (SW)	296	22.32
16.	Biological yield plant^{-1} (BYPP)	13	0.98
17.	Harvest index (HI)	3	0.23
18.	Seed yield plant^{-1} (SYPP)	12	0.90

Based on D^2 values, 52 genotypes were grouped into 8 clusters using Tocher's method (Figure 1). Cluster I (32), cluster II (12) and Cluster VI (3) were polygenotypic, while the remaining five clusters were monogenotypic (only one genotype in each

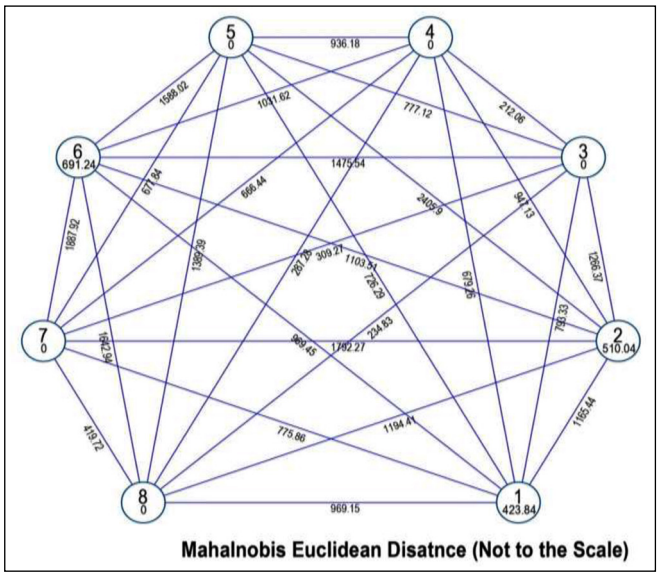

Figure 1: Cluster Diagram using Tocher's method

cluster). These results were in agreement with Upadhyay et al. (2022), Parihar et al. (2014), Khan et al. (2017) and Assen et al. (2020). Cluster VI showed a maximum intra-cluster D^2 value (691.24). The highest inter-cluster divergence was observed between genotypes of Cluster II and Cluster V (2405.90), followed by Cluster VI and Cluster VII (1887.92), Cluster II and VII (1792.27).

The lowest inter-cluster distance was reported between cluster III and cluster IV (212.06), as given in Table 2. Similar findings have been reported by Bijalwan et al. (2018), Ertiro (2021) and Singh et al. (2021). These above results indicated that genotypes included in this investigation represent sufficient genetic diversity between clusters and within clusters.

The genotypes of cluster II (KPMR 402, FP 14–27, FP 14–21, IPF 99–25, PP 14–27, JM 6, KPMR 585, Aman, HVP 2, Jayanti, FP 14–33, DDR 54) showed higher inter-cluster distance with cluster V (JP 885) followed by cluster VI (PP 155, B 22, JP 180) and cluster VII (FP 75–62), Cluster II(KPMR 402, FP 14–27, FP 14–21,IPF 99–25, PP 14–27, JM 6, KPMR 585, Aman, HVP 2, Jayanti, FP 14–33, DDR 54) and VII (FP 75–62). The lowest inter-cluster distance was reported between cluster III (FP 14–56) and cluster IV (PP 155, B 22, JP 180), as shown in Table 3.

The significant inter-cluster distances suggested a promising avenue for the future of pea research. Hybridization between genotypes from these diverse clusters could potentially broaden the genetic base of Pea. Such hybridization was likely to enhance heterosis and yield superior recombinants

Table 2: Inter and intra cluster D^2 values for different clusters

Cluster No.	Cluster I	Cluster II	Cluster III	Cluster IV	Cluster V	Cluster VI	Cluster VII	Cluster VIII
Cluster I	423.84	1165.44	793.33	679.26	726.29	969.45	775.86	969.15
Cluster II		510.04	1266.37	947.13	2405.90	1103.51	1792.27	1194.41
Cluster III			0.00	212.06	777.12	1475.54	309.27	234.83
Cluster IV				0.00	936.18	1031.62	666.44	287.28
Cluster V					0.00	1588.02	677.84	1389.39
Cluster VI						691.24	1887.92	1642.94
Cluster VII							0.00	419.72
Cluster VIII								0.00

Table 3: Grouping of germplasm into various clusters

Cluster no.	No. of genotypes	Name of the genotypes
Cluster I	32	P 3, FP 14-56, HFP 94-13, FP 14-46, KPMR 30, FP 9-539, FP 14-82, RP 3, KPMR 302, DDR 55, KPMR 327, NDVP 4, KPMR 502, VL 3, FP 14-13, Rachana, FP 7-596, Choti Safed (Anju), FP 18-30, FP 14-8, Matar Rangpur, FP 94-12, VRP-5, PSM 3, FP 13-30, GS 10, FP 14-86, Arka Sampurna, Arkel, DDR 27, FP 14-15, Pusa Pragati
Cluster II	12	KPMR 402, FP 14-27, FP 14-21,IPF 99-25, PP 14-27, JM 6, KPMR 585, Aman, HVP 2, Jayanti, FP 14-33, DDR 54
Cluster III	1	DDR 52
Cluster IV	1	Safed Batra (Gudda)
Cluster V	1	JP 885
Cluster VI	3	PP 155, B 22, JP 180
Cluster VII	1	FP 7562
Cluster VIII	1	Gol Batra Tendua

in segregating generations, benefiting hybrid development programs to achieve higher yield and quality. The intra-cluster distance was highest in Cluster VI, suggesting that hybridization among genotypes within this cluster could also yield good recombinants.

Cluster II recorded high mean values for days to first flower opening, days to 50% flowering, plant height, number of nodes Plant^{-1} and pod-bearing length. Cluster IV recorded a high mean value for the number of primary branches Plant^{-1}, the number of secondary branches Plant^{-1}, and the number of seeds pod^{-1}. Cluster V recorded a high mean value for pod length and harvest index. Cluster VI recorded the number of effective nodes plant^{-1}, number of pods plant^{-1}, number of effective pods Plant^{-1}, number of seeds plant^{-1}, biological yield plant^{-1} and seed yield plant^{-1}. Cluster VII recorded a high mean value for 100 seed weight. Cluster VIII recorded a high mean value for days to maturity (Table 4). The high mean value for the particular traits in different clusters underscores the good diversity present in these clusters, and the selection of genotypes from these clusters would be potent for improvement in specific traits dominated in them.

Table 4: Cluster mean for yield and its component traits of pea lines: Tocher's method

Cluster	DFFO	DFF	DM	NPBPP	NSBPP	PH	NNPP	NENPP	PBL
1	57.39	63.55	94.99	1.83	3.57	72.23	43.89	10.51	40.97
2	59.06	65.42	94.53	2.20	3.96	128.01	63.58	13.82	76.65
3	41.00	47.33	81.00	1.87	3.47	74.29	36.75	9.61	43.35
4	36.33	43.33	85.33	3.32	5.48	102.44	45.55	7.37	51.93
5	47.00	52.00	80.00	1.75	2.79	39.40	27.78	8.31	17.35
6	51.78	58.66	91.89	2.31	4.20	111.42	63.20	23.86	58.61
7	50.67	57.33	88.00	1.83	3.94	49.21	32.93	7.29	32.42
8	40.67	46.67	95.33	2.38	4.38	85.46	44.37	8.69	51.15

Table 4: Continue...

Cluster	NPPP	NEPPP	PL	NSPP	NSPPlt	100 SW	BY	HI%	SY
1	15.07	13.49	5.57	4.18	53.65	17.41	25.07	37.14	9.19
2	22.52	19.98	5.51	3.75	74.01	18.70	38.28	35.81	13.58
3	15.62	14.63	5.83	3.52	51.54	24.31	33.03	37.92	12.52
4	11.98	9.28	5.89	5.14	47.45	20.18	30.77	31.16	9.57
5	15.36	13.42	6.53	3.50	46.83	17.47	18.43	44.32	8.18
6	33.62	24.33	5.36	4.51	114.04	12.81	38.73	36.63	14.68
7	8.37	6.68	5.72	3.58	23.96	26.33	18.78	33.41	6.30
8	9.37	8.60	4.40	3.71	31.40	26.22	26.57	31.03	8.22

3.2. *Principal component analysis*

The investigation identified the first five principal components as crucial and demonstrated significant total variation among the 52 pea genotypes under study. Out of the total of eighteen, only these five principal components (PCs) exhibited more than 1.00 Eigenvalue, accounting for about 80.62% variability among the traits studied presented in Table 5. Therefore, these five principal components hold substantial importance for further explanation. PC1, with the highest variability (36.18%), was followed by PC2 (15.55%), PC3 (13.33%), PC4 (8.27%) and PC5 (7.28%) for the traits under study (Figure 2). These findings were in agreement with Uhlarik et al. (2022), Sanwal et al., 2024 and Pratap et al. (2024).

In the 52 Pea genotypes, the top principal component scores (PC scores) for all the traits were estimated in these

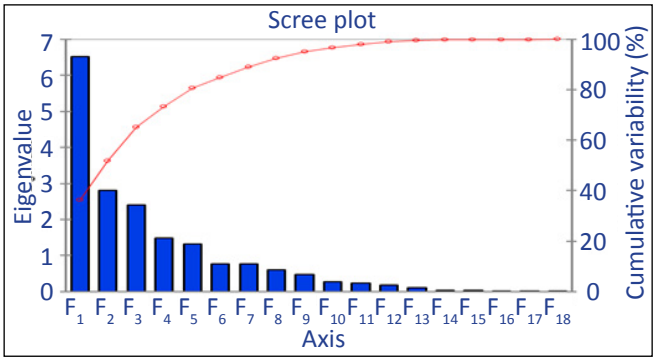

Figure 2: Scree plot

five components and presented in Table 6. These scores, as suggested by Sharma et al. (2022) and Uhlarik et al. (2022), can be effectively utilized to propose precise selection indices. The intensity of these indices can be decided by the

Table 5: Principal components for eighteen yield contributing traits of pea genotypes

Traits	Principal components				
	PC1	PC2	PC3	PC4	PC5
DFFO	0.307	0.735	-0.501	-0.101	0.165
DFF	0.298	0.712	-0.517	-0.140	0.190
DM	0.072	0.428	-0.530	-0.350	-0.106
NPBPP	0.293	-0.687	-0.093	-0.414	-0.163
NSBPP	0.153	-0.691	-0.171	-0.430	0.067
PH	0.733	-0.345	-0.359	0.052	0.199
NNPP	0.829	-0.190	-0.184	-0.069	-0.131
NENPP	0.769	0.031	0.125	-0.039	-0.376
PBL	0.655	-0.226	-0.448	0.205	0.249
NPPP	0.918	0.092	0.209	0.059	-0.249
NEPPP	0.926	0.157	0.207	0.125	-0.108
PL	-0.066	0.226	0.279	-0.517	0.355
NSPP	-0.335	-0.023	0.443	-0.495	0.463
NSPPlt	0.843	0.110	0.420	-0.145	0.112
100 SW	-0.384	-0.295	-0.151	0.577	0.465
BYPP	0.790	-0.193	-0.002	0.072	0.417
HI	0.109	0.433	0.691	0.156	-0.024
SYPP	0.812	0.059	0.383	0.124	0.359

Extraction Method: Principal Component Analysis

Table 6: Interpretation of rotated component matrix for the traits having values>0.3 in each PCs

Characters	PC 1	PC 2	PC 3	PC 4	PC 5
	Plant height	Days to first flower opening	Harvest index	100 seed weight	Pod length
	No. of nodes plant^{-1}	Days to 50% flowering			Seed per pod
	No. of effective nodes Plant^{-1}	Days to maturity			
	Pod bearing length				
	No. of pod Plant^{-1}				
	No. of effective pods Plant^{-1}				
	No. of seeds Plant^{-1}				
	Biological yield Plant^{-1}				
	Seed yield Plant^{-1}				

variability explained by each principal component. A high PC score for a particular genotype in a particular component denotes high values for the variables in that particular genotype/trait.

The PC1 was loaded with yield traits*, i.e.*, plant height, number of nodes Plant^{-1}, pod-bearing length, number of pods Plant^{-1}, number of seeds Plant^{-1}, number of effective pods Plant^{-1}, number of seeds Plant^{-1}, biological yield Plant^{-1}, and seed yield Plant^{-1} as shown in Table 7. The second principal component (PC2) dominated with phenological traits viz., days to first flower opening, days to 50% flowering and days to maturity, while PC3 consisted of harvest index. The fourth principal component was also dominated by 100 seed weight. In contrast, the fifth principal component was loaded with yield-related traits, i.e. pod lengthand number of seeds pod^{-1}.

In PC1, genotype FP 14–21 (5.824) had the high PC score, followed by HVP 2 (4.517), FP 14–17 (4.500), PP 115 (4.461) and FP 14–27 (4.481), respectively. In PC2, FP 14–56 (2.408), NDVP 4 (2.408) and FP 95 39 (2.373) showed high

Table 7: PC scores of Pea Genotypes showed positive value >1.0 in each PCs

Sl. No.	PC1	PC2	PC3	PC 4	PC 5
1.	FP 14-21 (5.824)	FP 14-56 (2.408)	JP 180 (3.796)	DDR 52 (2.336)	AMAN (2.878)
2.	HVP 2 (4.517)	NDVP 4 (2.387)	JP 885 (2.864)	FP 14-21 (2.149)	FP 14-33 (1.612)
3.	FP 14-17 (4.50)	FP 95-39 (2.373)	DDR 52 (2.077)	DDR 55 (2.125)	P 3 (1.567)
4.	PP 155 (4.461)	P 3 (2.345)	PUSA PRA-GATI (2.009)	DDR 27 (1.743)	HVP 2 (1.496)
5.	FP 14-27 (4.431)	VRP 5 (2.258)	VRP 5 (1.982)	GOL BA-TRA TEN-DUA (1.74)	VRP 5 (1.396)
6.	JP 180 (4.38)	FP 14-82 (1.978)	FP 14-15 (1.963)	FP 14-27 (1.639)	FP 14-21 (1.347)
7.	JM 6 (3.842)	RACHNA (1.971)	ARKEL (1.925)	ARKA SAMPUR-NA (1.589)	JP 180 (1.032)
8.	B 22 (3.522)	PUSA PRAGATI (1.722)	RP 3 (1.683)	FP 7562 (1.444)	JAYANTI (1.020)
9.	JAYANTI (2.435)	AMAN (1.502)	PP 155 (1.402)	FP 14-15 (1.435)	
10.	DDR 54 (2.351)	KPMR 30 (1.430)	SAFED BATRA GUDDA (1.232)	FP 14-13 (1.199)	
11.	IPF 99-25 (2.257)	FP 14-46 (1.240)	DDR 27 (1.133)	KPMR 585 (1.189)	
12.	DDR 55 (2.173)	FP 94-12 (1.155)	PSM 3 (1.025)	AMAN (1.146)	
13.	FP 94-12 (1.851)	KPMR 327 (1.126)			
14.	KPMR 405 (1.766)	FP 14-8 (1.008)			
15.	KPMR 30 (1.122)	KPMR 502 (1.075)			
16.	FP 95-39 (1.121)				

PC scores, while in PC3, a high PC score was obtained by JP 180 (3.796), JP 885 (2.864) and DDR 52(2.07). Similarly, in PC4 genotypes, DDR 52 (2.336), FP 14–21(2.149), and DDR 55 (2.125) obtained high PC scores, while PC5 Aman (2.878) and FP 14–33 (1.612) recorded high PC scores presented in Table VIII. Similar findings have been reported by Parihar et al. (20I4), Bhuvaneswari et al. (2016), Singh et al. (2017), and Kumari et al. (2019). From the above results it was evident that yield contributing traits have the highest variation in PC1, followed by PC3, PC4 and PC5. Genotype HVP–2 and FP 14–17 had high PC scores, and Genotypes, namely FP 14–21 (PC1, 4 and 5), JP 180 (PC1, 3 and 5), VRP 5 (PC 2, 3 and 5) and Aman (PC2, 4 and 5) plunge in three PCs (Table IX). The hybridization programme must include these genotypes to develop superior varieties dominated by yield-attributing traits.

4. Conclusion

Based on the current study, 52 genotypes obtained from various sources were categorized into 8 clusters using Tocher's method. Cluster I (32 genotypes), cluster II (12 genotypes), and cluster VI (3 genotypes) exhibited polymorphism. The genotypes in cluster II displayed the greatest inter-cluster distance with those in cluster V, suggesting their potential use in widening the genetic diversity of pea. FP 14-21, JP 180, VRP 5, AMAN, HVP-2, and FP 14-17 were identified as potential genotypes based on PCA.

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