



Genetic Divergence and Principal Component Analysis for Identification of Superior and Heat Stress-tolerant Genotypes in Field Pea (*Pisum sativum* L.)

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ABSTRACT

Background: Genetic diversity is an important prerequisite for the improvement of field pea through hybridization and selection. The current study was undertaken to assess genetic divergence among 10 parental lines and 45 F₁ hybrids of field pea under TS and LS conditions to identify genetically diverse and superior genotypes for breeding high-yielding and heat stress-tolerant cultivars.

Methods: A total of 55 genotypes, comprising 10 parental lines and 45 F₁ hybrids, were evaluated under TS and LS conditions. Genetic divergence was assessed through Mahalanobis' D² statistics, while PCA was employed to determine the contribution of different traits to overall variability and to identify promising genotypes.

Result: The maximum inter-cluster distances were recorded between clusters V and VI (timely sown) and clusters II and V (late sown), suggesting their potential for generating superior recombinants. PCA demonstrated that the leading four principal components explained 80.51% and 78.18% of the total variation under timely and late sowing, respectively. Seed yield/plant, biological yield/plant, pods/plant and primary branch/plant, secondary branches/plant were the main contributors to genetic divergence. The hybrids IPF 22-22 × IPF 22-24 and RFP 2020-2 × IPF 22-24 were identified as promising across environments, while parental lines IPF 22-22 and IPF 22-24 showed superior performance under late sowing, indicating their potential as heat stress-tolerant parents.

Key words: Cluster analysis, D² analysis, Field pea, Genetic divergence, Heat stress tolerance, Mahalanobis, Principal component analysis.

INTRODUCTION

Field pea (*Pisum sativum* L.) is a cold-season legume belonging to family *Leguminosae* (2n = 2x = 14), is a vital protein source, carbohydrates, vitamins and minerals for human as well as animal nutrition. Beyond its nutritional importance, field pea contributes to sustainable agriculture through biological nitrogen fixation, thereby enhancing soil fertility and reduces the need for nitrogen fertilizers (Smykal *et al.*, 2012). Temperature abiotic stress is among the key environmental factors that significantly affect field pea productivity. Delayed sowing often exposes the crop to elevated temperatures during flowering and pod development, adversely affecting pollen viability, pod set and seed filling, ultimately reducing yield (Devi *et al.*, 2023). Therefore, developing high-yielding and heat-tolerant cultivars is a most important breeding goal. Crop improvement requires genetic diversity, since it enables breeders to identify and exploit desirable traits (Begna, 2024). Multivariate statistical approaches such as Mahalanobis' D² statistics and PCA are widely used to measure genetic divergence and determine the characteristics that lead to variability. D² analysis groups genotypes based on genetic distance, while PCA summarizes complex datasets and reveals the major sources of variation within a population (Mahalanobis, 1936; Rao, 1952; Jolliffe, 2002). Therefore, the current research was undertaken to examine genetic diversity

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among field pea genotypes across TS and LS conditions using Mahalanobis' D^2 statistics and PCA, with the objective of identifying diverse and promising genotypes targeted for future crop improvement programs.

MATERIALS AND METHODS

This study was carried out across two successive Rabi seasons (2023-24 and 2024-25) at the research farm of Genetics and Plant Breeding, Acharya Narendra Deva University of Agriculture and Technology, Kumarganj, Ayodhya, Uttar Pradesh, India. During *Rabi* 2023-24, ten diverse field pea genotypes, namely Pant P 545, Pant P 550, RFP 2020-2, KPMR 907, Pant P 554, IPF 22-22, IPF 22-24, HFP 1960, HFP 1961 and VL 42, were subjected to crossing in a half-diallel fashion excluding reciprocals (Griffing, 1956) to generate 45 F_1 hybrids. In *Rabi* 2024-25, the 45 F_1 hybrids along with their 10 parents were subjected to evaluation under timely-sown and late-sown conditions in three replications using randomized block design. Each genotype was planted in a single row at a spacing of 45 cm $\times$ 10 cm and the recommended agronomic practices were followed throughout the crop season. Data collection was carried out on five randomly chosen plants for eleven traits, namely days to 50 per cent flowering, days to maturity, primary branches/plant, secondary branches/plant, plant height, pods/plant, seeds/pod, biological yield/plant, harvest index, seed index and seed yield/plant. The average values were utilized for multivariate analysis using R software. Genetic divergence was estimated using Mahalanobis D^2 statistics (Mahalanobis, 1936) and Genotypes were classified into clusters using Tocher's method (Rao, 1952). PCA was performed on standardized data to determine the principal sources of variation among genotypes. components with eigenvalues exceeding unity were retained according to Kaiser's rule (Kaiser, 1960) and genotype relationships were visualized using principal component scores.

RESULTS AND DISCUSSION

Genetic divergence analysis

Genetic diversity among 45 F_1 hybrids and 10 parental lines of field pea was evaluated using Mahalanobis D^2 statistics. The 55 genotypes were organized into six distinct clusters under both timely and late sown conditions (Table 1 and 2; Fig 1 and 2), indicating substantial genetic variability among the studied material. Under timely sowing, Cluster III represented the largest cluster with 15 genotypes, whereas Cluster IV accounted for the smallest with 5 genotypes. Under late sowing, Clusters I and III each contained 11 genotypes, while Cluster II was the smallest with 7 genotypes. The variation in cluster composition across environments suggests that environmental conditions influenced the expression of quantitative traits and altered genetic relationships among genotypes. Similar findings have been reported by Singh *et al.* (2021) and Guruprasad *et al.* (2021).

Inter and intra cluster distance

Intra-cluster and inter-cluster distances among the six clusters are available in Table 3. In timely sown conditions, cluster V exhibited the high intra-cluster distance (26.76), whereas in late sown conditions, cluster II recorded the high intra-cluster distance (30.28), suggesting greater variability under clusters II and V. The largest inter-cluster value was observed between clusters V and VI (51.12) under timely sowing and between clusters II and V (63.30) under late sowing, reflecting the highest genetic divergence among these cluster combinations. In contrast, the lowest inter-cluster value was recorded between clusters III and VI under both environments, indicating closer genetic relationships among the genotypes. Overall, inter-cluster distances were generally greater than intra-cluster distances, suggesting substantial genetic diversity among clusters. Therefore, crosses involving genotypes from clusters V and VI under timely sowing and clusters II and V

Table 1: Clustering pattern of 10 parents along with 45 F_1 crosses of eleven characters under timely sown (TS) condition.

Clusters	No. of genotypes	Genotypes
Cluster_1	10	Pant P 550 $\times$ Pant P 554, IPF 22-24 $\times$ VL 42, IPF 22-22, IPF 22-22 $\times$ IPF 22-24, Pant P 545 $\times$ IPF 22-22, Pant P 545 $\times$ IPF 22-24, Pant P 545 $\times$ Pant P 554, Pant P 545 $\times$ RFP 2020-2, Pant P 550 $\times$ VL 42, Pant P 554
Cluster_2	11	Pant P 554 $\times$ HFP 1960, Pant P 545 $\times$ Pant P 550, HFP 1960 $\times$ HFP 1961, IPF 22-22 $\times$ HFP 1960, IPF 22-22 $\times$ HFP 1961, IPF 22-24, KPMR 907 $\times$ HFP 1960, KPMR 907 $\times$ HFP 1961, KPMR 907 $\times$ Pant P 554, Pant P 545 $\times$ HFP 1961, Pant P 554 $\times$ HFP 1961
Cluster_3	15	Pant P 554 $\times$ VL 42, HFP 1961 $\times$ VL 42, HFP 1960 $\times$ VL 42, IPF 22-22 $\times$ VL 42, KPMR 907 $\times$ IPF 22-22, KPMR 907 $\times$ IPF 22-24, Pant P 545 $\times$ KPMR 907, Pant P 550 $\times$ HFP 1960, Pant P 550 $\times$ HFP 1961, Pant P 550 $\times$ KPMR 907, Pant P 554 $\times$ IPF 22-24, RFP 2020-2 $\times$ HFP 1960, RFP 2020-2 $\times$ HFP 1961, RFP 2020-2 $\times$ KPMR 907, RFP 2020-2 $\times$ VL 42
Cluster_4	5	Pant P 554 $\times$ IPF 22-22, Pant P 550 $\times$ IPF 22-22, Pant P 545, Pant P 550, VL 42
Cluster_5	6	IPF 22-24 $\times$ HFP 1961, IPF 22-24 $\times$ HFP 1960, HFP 1960, HFP 1961, KPMR 907, RFP 2020-2
Cluster_6	8	RFP 2020-2 $\times$ IPF 22-22, Pant P 545 $\times$ VL 42, KPMR 907 $\times$ VL 42, Pant P 545 $\times$ HFP 1960, Pant P 550 $\times$ IPF 22-24, Pant P 550 $\times$ RFP 2020-2, RFP 2020-2 $\times$ IPF 22-24, RFP 2020-2 $\times$ Pant P 554

under late sowing may be promising for generating broad variability and superior segregants in breeding programmes.

Cluster means

Substantial variability in cluster means across different traits under timely and late-sown conditions provided further evidence of diversity. Cluster mean values for all eleven characters in TS and LS environments are given in Table 4.

As revealed by Table 4, under timely sown conditions, cluster I showed the largest mean value for PH (113.41 cm) and BYPP (80.62 g) and smallest mean values for DTF (67.50 days). Cluster II exhibited the largest mean values for DTM (112.27 days), harvest index (39.83%) and SYPP (31.65 g). Cluster III showed high mean values for PB (2.87), SB (4.86), PPP (29.51), SPP (6.18), SI (19.41), HI (41.70%) and SYPP (35.32 g). Cluster IV recorded the

Table 2: Clustering pattern of 10 parents along with 45 F_1 crosses of eleven characters under late sown (LS) condition.

Clusters number	No. of genotypes	Genotypes
I	11	Pant P 554 × VL 42, Pant P 545 × IPF 22-24, KPMR 907 × IPF 22-24, KPMR 907 × Pant P 554, Pant P 545, Pant P 545 × HFP 1961, Pant P 550 × KPMR 907, Pant P 550 × Pant P 554, Pant P 554 × HFP 1960, RFP 2020-2 × Pant P 554, RFP 2020-2 × VL 42
II	7	IPF 22-22 × HFP 1961, IPF 22-22 × HFP 1960, HFP 1960, HFP 1961, KPMR 907, Pant P 550, RFP 2020-2
III	11	Pant P 554 × IPF 22-22, Pant P 550 × VL 42, IPF 22-24 × HFP 1961, KPMR 907 × IPF 22-22, Pant P 550 × HFP 1960, Pant P 550 × HFP 1961, Pant P 550 × IPF 22-22, Pant P 550 × IPF 22-24, Pant P 554 × IPF 22-24, RFP 2020-2 × IPF 22-22, RFP 2020-2 × KPMR 907
IV	9	HFP 1961 × VL 42, HFP 1960 × VL 42, HFP 1960 × HFP 1961, KPMR 907 × HFP 1960, KPMR 907 × HFP 1961, Pant P 545 × KPMR 907, Pant P 545 × RFP 2020-2, RFP 2020-2 × HFP 1960, RFP 2020-2 × HFP 1961
V	8	Pant P 545 × Pant P 550, IPF 22-24 × VL 42, IPF 22-22, IPF 22-22 × IPF 22-24, IPF 22-24, Pant P 545 × IPF 22-22, Pant P 545 × VL 42, RFP 2020-2 × IPF 22-24
VI	9	Pant P 550 × RFP 2020-2, KPMR 907 × VL 42, IPF 22-22 × VL 42, IPF 22-24 × HFP 1960, Pant P 545 × HFP 1960, Pant P 545 × Pant P 554, Pant P 554, Pant P 554 × HFP 1961, VL 42

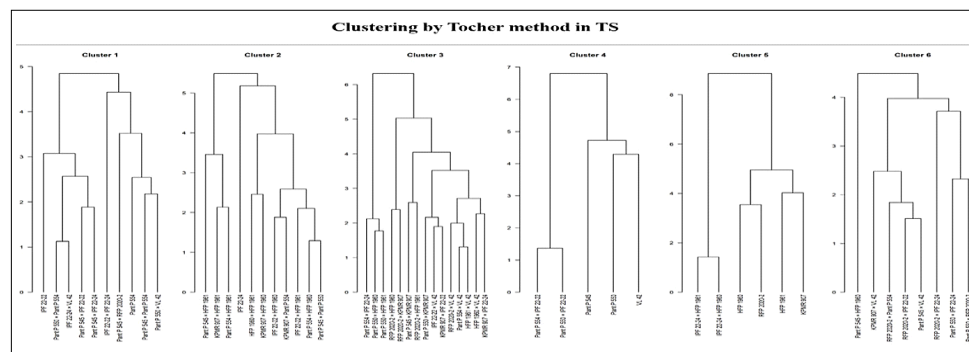


Fig 1a: Clustering pattern of 10 parents and their F_1 hybrids under timely sown condition.

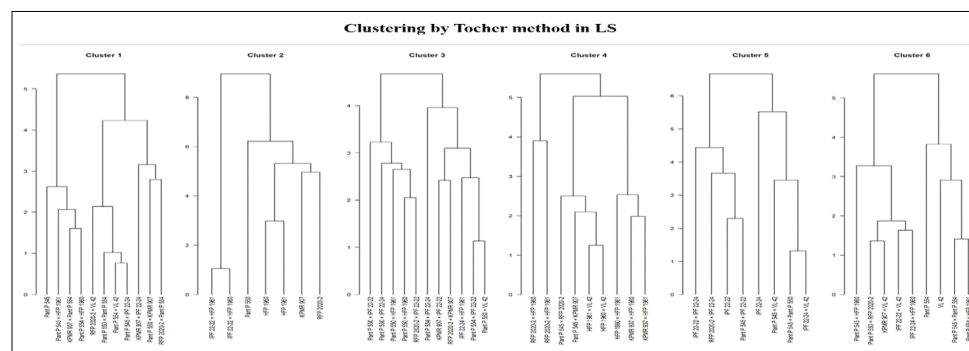


Fig 1b: Clustering pattern of 10 parents and their F_1 hybrids under late (LS) sown condition.

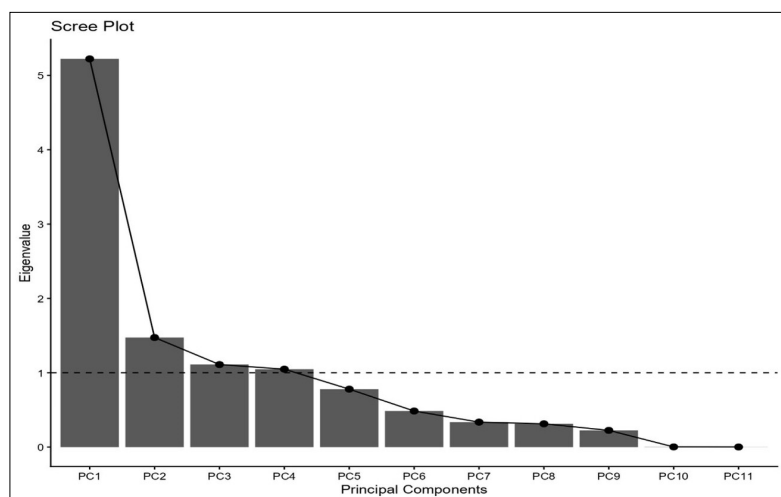


Fig 2: Scree plot showing the eigenvalues of eleven principal components under timely sown (TS) condition.

Table 3: Estimates of average intra and inter-cluster distance for six clusters of field pea genotypes under timely (TS) and late sown (LS) conditions.

Cluster number	Conditions	Intra-cluster distance	II	III	IV	V	VI
I	TS	9.74	15.18	14.37	23.51	32.32	15.83
	LS	8.44	35.54	13.49	13.71	20.64	13.85
II	TS	11.16		16.73	29.82	28.29	21.58
	LS	30.28		43.63	35.02	63.3	32.57
III	TS	10.04			38.55	43.18	12.46
	LS	8.97			16.07	19.05	13.84
IV	TS	22.21				29.6	42.15
	LS	11.8				27.96	14.75
V	TS	26.76					51.12
	LS	18.15					26.13
VI	TS	9.56					
	LS	9.42					

Table 4: Cluster mean for different characters in field pea under timely (TS) and late (LS) sown conditions.

Clusters number	Conditions	DTF	DTM	PH	PB	SB	PPP	SPP	SI	BYPP	HI	SYPP
I	TS	67.5	107.63	113.41**	2.67	4.50	26.19	6.03	19.26	80.62	37.67	30.29
	LS	65.33	102.87	103.5	2.51	3.62	25.5	5.85	15.62	61.5	37.98	23.32
II	TS	71.3	112.27**	103.6	2.66	4.57	27.27	6.07	19.12	79.63	39.83**	31.65
	LS	67.76	104.85	92.14	2.18	2.97	18.63	5.35	15.5	45.57	34.5	15.48
III	TS	67.82	106.4	105.5	2.87**	4.86**	29.51**	6.18**	19.41**	84.53**	41.7**	35.32**
	LS	62.87	99.69	103.03	2.69	3.53	25.85	5.56	16.65	69.67	34.52	23.95
IV	TS	70.13**	107.2	114.04**	2.35	4.21	23.92	5.66	17.22	66.32	35.21	23.2
	LS	66.62	101.07	93.55	2.41	3.14	24.56	5.95	15.96	68.52	34.29	23.31
V	TS	71.94**	111.11**	93.46	2.52	3.63	22.8	5.58	18.48	64.25	37.1	23.69
	LS	63.75	103.41	107.76	2.93	4.1	26.2	5.9	17.69	74.64	36.67	27.32
VI	TS	67.54	106.04	110.14	3.13**	5.35**	29.96**	6.08**	19.56**	93.69**	37.98	35.53**
	LS	64.51	103.48	103.27	2.49	3.4	24.13	5.64	15.53	68.76	30.77	21.12

DTF: Day to 50% flowering, DTM: days to maturity, PH: Plant height, PB: Primary branches/plant, SB: Secondary branches/plant, PPP: Pods/plant, SPP: Seeds/pod, SI: Seed index, BYPP: Biological yield/plant, HI: Harvest index, SYPP: Seed yield/plant.

highest value for PH (114.04 cm) but lowest for PB (2.35), SI (17.22), BYPP (66.32 g), HI (35.21%) and SYPP (23.20 g). Cluster V showed highest values for DTF (71.94 days) and DTM (111.11 days), while exhibiting lower values for PH (93.46 cm), SB (3.63), PPP (22.80), BYPP (64.25 g) and SYPP (23.69 g). Cluster VI exhibited the high values for PB (3.13), SB (5.35), PPP (29.96), SI (19.56), BYPP (93.69 g) and SYPP (35.53 g), while lowest values for DTM (106.04 days). Under late sown conditions, cluster I exhibited one of the highest mean values for HI (37.98%). Cluster II showed the highest mean values for DTF (67.76 days) and DTM (104.85 days), but recorded the lowest values for PH (92.14 cm), PB (2.18), SB (2.97), PPP (18.63), SPP (5.35), SI (15.50), BYPP (45.57 g) and SYPP (15.48 g). Cluster III showed the lowest values for DTF (62.87 days) and DTM (99.69 days), while maintaining comparatively higher values for PPP (25.85), SI (16.65), BYPP (69.67 g) and SYPP (23.95 g). Cluster IV was among the superior clusters for SPP (5.95) and BYPP (68.52 g). Cluster V emerged as the most promising cluster under late sowing, exhibiting

the largest values for PH (107.76 cm), PB (2.93), SB (4.10), PPP (26.20), SI (17.69), BYPP (74.64 g) and SYPP (27.32 g). Cluster VI showed moderate performance for most traits but recorded the lowest harvest index (30.77%).

The results showed that each cluster exhibited strengths for specific characteristics under both TS and LS conditions. However, clusters III and VI under timely sowing and cluster V under late sowing showed better overall performance for seed yield/plant and most of its contributing traits. Therefore, genotypes belonging to these clusters can be considered promising candidates for use as parents in breeding programme. Crossing these genotypes with those from genetically diverse clusters having high inter-cluster distances may help to generate greater variability and increase the chances of obtaining superior recombinants in subsequent generations.

Principal component analysis (PCA)

Principal component analysis (PCA) was conducted to determine the principal sources of variation and traits

Table 5: Factor loadings of 11 quantitative traits on the first four principal components in 10 parental lines and 45 F_1 hybrids of field pea under timely sown (TS) and late sown (LS) condition.

Traits	Conditions	PC1	PC2	PC3	PC4
DTF	TS	-0.553	0.591	-0.185	0.326
	LS	-0.614	0.433	-0.174	-0.394
DTM	TS	-0.535	0.573	-0.376	0.207
	LS	-0.405	0.689	-0.450	-0.052
PH	TS	0.474	-0.590	-0.255	0.279
	LS	0.522	0.046	-0.499	0.296
PB	TS	0.786	0.170	-0.206	-0.240
	LS	0.791	0.157	0.112	0.190
SB	TS	0.819	-0.020	-0.292	0.187
	LS	0.733	0.427	-0.036	0.247
PPP	TS	0.848	0.091	0.217	-0.008
	LS	0.823	-0.220	0.032	-0.143
SPP	TS	0.477	0.217	0.343	0.661
	LS	0.469	0.100	-0.052	-0.735
SI	TS	0.559	0.298	-0.515	-0.372
	LS	0.619	0.393	-0.036	0.236
BYPP	TS	0.876	0.029	-0.177	0.217
	LS	0.824	-0.237	-0.394	-0.211
HI	TS	0.430	0.469	0.523	-0.321
	LS	0.324	0.445	0.761	-0.082
SYPP	TS	0.952	0.231	0.094	0.044
	LS	0.957	0.027	0.008	-0.242
Components eigen value	TS	5.223	1.474	1.111	1.048
	LS	4.956	1.340	1.235	1.069
% variance explained	TS	47.480	13.402	10.098	9.530
	LS	45.057	12.178	11.227	9.721
Cum. variance explained	TS	47.480	60.882	70.980	80.510
	LS	45.057	57.235	68.463	78.184

DTF: Day to 50% flowering, DTM: days to maturity, PH: Plant height, PB: Primary branches/plant, SB: Secondary branches/plant, PPP: Pods/plant, SPP: Seeds/pod, SI: Seed index, BYPP: Biological yield/plant, HI: Harvest index, SYPP: Seed yield/plant.

The PCA biplot under timely sown (TS) conditions showed that PC1 and PC2 together explained 60.88% of total variability. Traits such as seed yield/plant (SYPP), biological yield/plant (BYPP), pods/plant (PPP), primary branches/plant (PB), secondary branches/plant (SB), seeds/pod (SPP) and seed index (SI) were closely grouped and oriented in the same direction, indicating strong positive

Scree Plot

Eigenvalue

Principal Components

Principal Component	Eigenvalue
PC1	4.9
PC2	1.3
PC3	1.2
PC4	1.0
PC5	0.9
PC6	0.7
PC7	0.4
PC8	0.3
PC9	0.2
PC10	0.05
PC11	0.05

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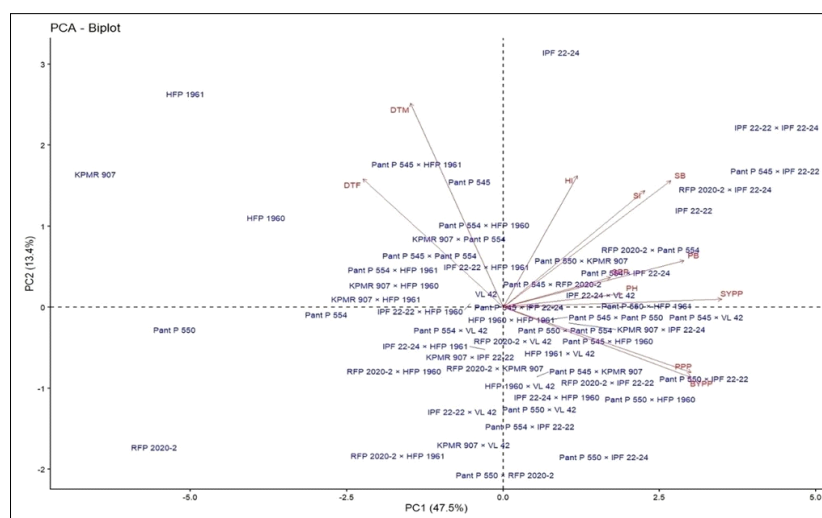


Fig 5: PCA biplot of genotypes and traits under late sown (LS) condition.

associations among these traits. In contrast, DTF and DTM were positioned opposite to the yield-related traits, suggesting a negative association between crop duration and productivity. Parental lines HFP 1961, KPMR 907, Pant P 550, VL 42 and RFP 2020-2 were located away from the origin, indicating greater genetic divergence. Hybrids Pant P 545 × IPF 22-22, Pant P 545 × VL 42, RFP 2020-2 × IPF 22-24 and IPF 22-22 × IPF 22-24 were associated with yield-related vectors, suggesting their potential for yield improvement. Under LS conditions, PC1 and PC2 accounted for 57.24% of the overall variation. A similar clustering of SYPP, BYPP, PPP, PB and SB was observed, indicating strong positive relationships among these traits. The separation between yield-related and phenological traits was more pronounced under LS conditions, highlighting the importance of earliness under terminal heat stress. Parents HFP 1961, KPMR 907, Pant P 550 and RFP 2020-2 occupied extreme positions, while hybrids IPF 22-22 × IPF 22-24, Pant P 545 × IPF 22-22 and RFP 2020-2 × IPF 22-24 were closely associated with yield-enhancing traits. These results revealed that SYPP, BYPP, PPP, PB and SB were the major contributors to variability under both environments. Similar observations have been noted by Patel *et al.* (2023), Pratap *et al.* (2024) and Anand *et al.* (2025), demonstrating the effectiveness of PCA in identifying genetically diverse and high-yielding breeding materials.

CONCLUSION

This study demonstrated considerable genetic divergence within the evaluated material of field pea under TS and LS conditions. Genotypes were grouped into six clusters based on the D² analysis, while PCA revealed that the first four PC explained greater than 78% of the overall variability under both environments. Clusters III and VI under timely sowing and cluster V under late sowing exhibited superiority for seed yield and related traits. Among the parental lines,

IPF 22-22 and IPF 22-24 performed well under late sowing, indicating their potential as heat stress-tolerant parents. The hybrids IPF 22-22 × IPF 22-24 and RFP 2020-2 × IPF 22-24 were identified as promising genotypes due to their strong association with yield and yield-contributing traits. These genotypes may be strategically exploited in breeding programmes focused on developing high-yielding and heat stress-tolerant field pea cultivars.

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Conflict of interest

The authors confirm that no conflicts of interest are associated with the publication of this article. The study was conducted independently, without any external funding or sponsorship influencing its design, data collection, analysis, decision to publish, or manuscript preparation.

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