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RESEARCH ARTICLE

Assessment of genetic variability at morpho-qualitative levels and molecular characterization of selected promising lines of field pea (*Pisum sativum* L. var. *arvense*)

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Abstract

A field study was conducted during the rabi season of 2023-24 at the Genetics and Plant Breeding Research Farm of Banda University of Agriculture and Technology, Banda, to characterize 30 diverse field pea (*Pisum sativum* L. var. *arvense*) genotypes at the phenotypic and molecular levels. The study utilized two replications in an alpha lattice design with four checks and investigated 16 morpho-qualitative characters. Analysis of variance revealed significant variability among genotypes, with traits such as plant height (PH), number of pods per plant (NPP), number of effective pods per plant (NEPP), biological yield per plant (BYP), seed yield per plant (SYP), total sugars (TS), non-reducing sugars (NRS), reducing sugars (RS) and trypsin inhibitor activity (TIA) showing high values of both phenotypic and genotypic coefficients of variation (PCV and GCV). These traits also exhibited high heritability and genetic advance as a percent of the mean (GAM), indicating additive gene effects and suggesting their suitability for effective selection. Eighteen field pea genotypes were molecularly characterized using 18 polymorphic SSR markers, revealing 36 alleles and indicating moderate genetic diversity. Primer AA-446 was the most informative, while AD-249 was the least informative. Cluster analysis grouped the genotypes into three distinct clusters and Principal Coordinate Analysis (PCoA) captured 43.36 % of the total genetic variation across the first two axes. These findings confirm sufficient diversity among genotypes for effective selection and future breeding programmes.

Keywords: analysis of variance; genetic advance as percent mean; principal coordinate analysis; simple sequence repeat; unweighted neighbour joining

Introduction

Field pea (*Pisum sativum* L.), a winter legume from the Fabaceae family (2n=14) is valued for its high protein content, complex carbohydrates, minerals, antioxidants and cholesterol-free composition, making it important for both human and animal nutrition (1–3). According to ICAR's vision document, India's growing population is projected to demand 32 million tonnes of pulses by 2030, which highlights the urgent need for developing nutritionally rich and genetically improved varieties (4). Traditional breeding based solely on morphological traits shows minimal response to direct selection (5). These limitations are due to low polymorphism and environmental influence, hence creating a gap in accurately identifying superior genotypes (6). Molecular markers, especially simple sequence repeats (SSRs), which are microsatellite-based markers, offer a reliable alternative due to their high polymorphism, reproducibility, co-dominant inheritance and genome-wide distribution (7). This study aims to evaluate morpho-qualitative traits and molecular diversity among selected field pea germplasms using SSR markers, with the objective of identifying promising genotypes for present and future crop improvement programmes.

Materials and Methods

Experimental location and material details

The experiment was performed during the rabi season of 2023-24 at the P.G. Research Farm, College of Agriculture, Banda University of Agriculture and Technology, Banda, Uttar Pradesh, India. It is geographically located in the Bundelkhand region and has a semi-arid climate. The location lies at a latitude of 25.475° N and a longitude of 80.335° E. The morpho-qualitative study was carried out on thirty field pea germplasms with two replications in an alpha lattice design, including four checks viz. IPFD 14-2, Pant P-243, HFP-1961 and Pant P-550 evaluating a total of 16 traits i.e. 11 morphological and 5 qualitative traits. Subsequently, molecular

characterization of eighteen selected promising genotypes was conducted using 20 SSR markers, which included two checks i.e. HFP-1961 and Pant P-243. The promising lines for molecular assessment, out of thirty diverse genotypes were selected on various superior morpho-qualitative characters such as yield and yield-contributing traits, as well as qualitative traits like protein content, etc. This strategic selection ensured that the molecular analysis captured the most genetically diverse and agronomically relevant genotypes. The lists of germplasms for morpho-qualitative assessment, promising genotypes used for molecular characterization and primers utilized for molecular assessment are presented in Table 1, 2 and 3 respectively.

Morphological traits description and methodology used

The observations were recorded by selecting five sampled plants randomly in each plot for eleven morphological characters of field pea, except for the characters viz. days to 50 % flowering (DF) and days to maturity (MD), which were recorded on an individual plot basis. For carrying out various statistical analyses, the mean value of the data was calculated from the sampled plants of each plot for different characters. Observations were recorded on 16 morpho-qualitative characters viz. DF, recorded when 50 % of the plants attained flowering; MD, the stage at which plants attained physiological maturity; plant height (PH) (in cm), regarded as the

Table 1. List of 30 field pea genotypes utilized in the study

S. No.	Name	Source	S. No.	Name	Source
1	Chahitara local	Chahitara	16	VL-202	CSAUAT, Kanpur
2	Jaspura local	Jaspura	17	HFP-1960	CCSHAU, Hisar
3	Pachulla local	Pachulla	18	HFP-715	CCSHAU, Hisar
4	HFP-1961	CCSHAU, Hisar	19	P-1440-10	IIPR, Kanpur
5	Pant P-243	GBPUAT, Pant Nagar	20	RFP 2020-4	CCSHAU, Hisar
6	IPFD 14-2	CCSHAU, Hisar	21	KPMR-839	IIPR, Kanpur
7	Pant P-550	GBPUAT, Pant Nagar	22	IPF 15-8	CCSHAU, Hisar
8	P-600	IIPR, Kanpur	23	P-1457	IIPR, Kanpur
9	P-1679	IIPR, Kanpur	24	IPFD 9-2	IIPR, Kanpur
10	RFP 2020-2	CCSHAU, Hisar	25	NDP-2	ANDUAT, Ayodhya
11	IPF 16-13	CCSHAU, Hisar	26	IPF 22-18	CCSHAU, Hisar
12	IPFD 10-12	IIPR, Kanpur	27	IPFD 6-3	IIPR, Kanpur
13	Aman	BUAT, Banda	28	SKNP 04-09	ANDUAT, Ayodhya
14	IPFD 11-5	CCSHAU, Hisar	29	Pant P-508	GBPUAT, Pant Nagar
15	Aadarsh	CCSHAU, Hisar	30	NDP-24	ANDUAT, Ayodhya

Table 2. List of 18 promising genotypes of field pea selected for molecular characterization

S. No.	Name	S. No.	Name
1	HFP-1961	10	Aman
2	IPF-16-13	11	P-1679
3	Chahitara local	12	Aadarsh (IPF 99-25)
4	Pachulla local	13	IPFD 11-5
5	Jaspura local	14	Pant P-243
6	RFP 2020-4	15	IPF 22-18
7	HFP-1960	16	KPMR-839
8	IPFD 6-3	17	P-600
9	IPFD 10-12	18	VL-202

Table 3. Sequence details of markers used to assess molecular characterization in promising field pea genotypes

S. No.	Primer	Forward sequence	Reverse sequence	Annealing (critical) temperature (°C)
1	C- 20	GAGTTCTCCGTAATAGAAGGCT	AGCCTTCTATTACGGAGAACTC	58
2	AD-147	AGCCCAAGTTTCTTCTGAATCC	GGATTCAGAAGAAACTTGGGCT	58
3	D-21	TATTCTCCTCCAAAATTTCCTT	AAGGAAATTTTGGAGGAGAATA	54
4	AA-67	CCCATGTGAAATTCTCTTGAAGA	TCTTCAAGAGAATTTCACATGGG	56
5	AD-249	TCTAAACGAATCCTTGCATACT	AGTATGCAAGGATTCGTTTAGA	54
6	AA504	TGAGTGCAGTTGCAATTTCG	CGAAATTGCAACTGCACTCA	56
7	AA-205	TACGAATCATAGAGTTTGGAA	TTCCAAACTCTATGATTCGTA	54
8	AA-175	TTGAAGGAACACAATCAGCGC	GCGCTGATTGTGTTCCTTCAA	58
9	AA-174	GGAGGGATGATTCTAACAAGT	ACTTGTTAGAATCATCCCTCC	58
10	AA-355	AGAAAAATTCTAGCATGATCTG	CAGATCATGCTAGAATTTTTCT	54
11	AD-270	CTCATCTGATGCGTTGGATTAG	CTAATCCAACGCATCAGATGAG	57
12	AA-122	GGGTCTGCATAAGTAGAAGCCA	TGGCTTCTACTTATGCAGACCC	57
13	A-9	GTGCAGAAGCATTTGTTCAGT	ACTGAACAAATGCTTCTGCAC	57
14	AB-23	TCAGCCTTTATCCTCCGAACTA	TAGTTCGGAGGATAAAGGCTGA	58
15	AD-79	ACAAGACTTCCAGAAATTTTGCAT	ATGCAAAATTTCTGGAAGTCTTGT	58
16	AA-399	CCATTGGTATATGAAAGATCGT	ACGATCTTTCATATACCAATGG	57
17	AD-51	ATGAAGTAGGCATAGCGAAGAT	ATCTTCGCTATGCCTACTTCAT	56
18	AD-60	CTGAAGCACTTTTGACAACTAC	GTAGTTGTCAAAAGTGCTTCAG	57
19	AA-416	TTACTGTTACTTTGCGACATCA	TGATGTCGCAAAGTAACAGTAA	54
20	AA-446	TTAGCTTGCAGCCCACTC	GAGTGGGCTGCAAGCTAA	54

stature of the plant from the base or ground level; total number of pods per plant (TPP), defined as the total count of all pods including effective as well as non-effective pods; number of effective pods per plant (NEPP), denoting the number of pods in which seed formation was recorded; number of seeds per pod (NSPP), representing the number of seeds found inside a single pod; pod length (LP), measured from end to end (in cm); 100-seed weight (SW) (g); seed yield per plant (SYPP) (g), defined as the total weight of seeds produced by a single plant; biological yield per plant (BYPP) (g), defined as the total dry weight of the entire plant (whole biomass) and harvest Index (HI), which is the ratio of seed yield to biological yield (in %) (8).

Biochemical traits description and methodology used

Protein content (PC) (in %) refers to the proportion of protein present in a sample, expressed as a percentage of its total weight. It is based on the reaction involving peptide nitrogen with copper ions under alkaline conditions, followed by colour development using the Folin-Ciocalteu reagent and is calculated using a method of total proteins determination (9). Total sugars refer to the combined amount of all types of sugars present in a sample, including both reducing and non -reducing sugars. It is typically estimated by a standard method using the phenol-sulphuric acid method, where sugars react with phenol and concentrated sulphuric acid to produce a coloured compound (10). The intensity of this colour is measured using a spectrophotometer and the sugar concentration is calculated using a glucose standard curve (10-100 µg). The result is expressed as a percentage of the sample's weight. Reducing sugars are types of sugars that have free aldehyde or ketone groups, allowing them to act as reducing agents in chemical reactions. They are typically estimated using a standard method of sugar estimation which involves the reaction of sugars with an alkaline copper reagent, followed by colour development with an arseno-molybdate reagent (11). The calculation is done using the formula (g) × sample weight. The intensity of the resulting colour is measured spectrophotometrically and the sugar concentration is calculated using a standard curve, usually based on glucose. Non-reducing sugar (%) was deduced by subtracting reducing sugar values from the total sugars obtained earlier in the experiment and then, multiplying the value by 0.95 (12). Trypsin inhibitor activity (TIA) is an anti-nutritional compound that inhibits the activity of trypsin and its calculation was performed using a standard procedure, wherein one TIU is defined as a decrease of 0.01 absorbance units at a wavelength of 280 nm (13).

Methodology and materials used for molecular characterization

Total DNA was extracted from the leaves of eighteen selected promising genotypes using the CTAB (Cetyl Trimethyl Ammonium Bromide) method with some modifications (14). To assess the purity, quality and concentration of the extracted DNA, a NanoDrop spectrophotometer, model ND-ONE-W, was used and the observations were recorded at 280 nm. Molecular characterization of the test genotypes was carried out using 20 SSR markers (Table 3) derived from public domain. PCR amplification was performed using a PCR thermal cycler, model Agilent Sure 8800. PCR products were then separated on a 3 % agarose gel and later assessed using a gel documentation system under UV illumination.

Gel electrophoresis and band scoring

Gel electrophoresis was carried out following standard procedures and the banding patterns were carefully observed. A 1 kb DNA ladder was used in every run to accurately determine the size of the DNA fragments. Band scoring was done manually and verified by two separate individuals to ensure consistency. Bands that appeared faint or were missing, possibly due to weak amplification, poor DNA quality, or the presence of null alleles, were excluded from the final analysis to maintain overall accuracy. Only distinct and repeatable bands were used for scoring and any unclear results were rechecked through repeated amplification. This careful approach helped ensure that the molecular data collected were dependable and suitable for assessing genetic diversity.

Statistical analysis

Statistical analysis was performed using the mean values of various quantitative traits. The data were subjected to analysis of variance (ANOVA) to determine differences among the test genotypes (15). Different variability parameters such as genotypic and phenotypic coefficients of variation, heritability and genetic advance (GA) were estimated using R software version 4.0 and the packages viz. Variability and Metan. Genetic similarity among genotypes was evaluated using the Unweighted Neighbour Joining (UNJ) methodology. Analysis based on clustering of genotypes was administered using DARWin software, version 6.0.21, which is used to construct phylogenetic trees (dendrograms) based on dissimilarity matrices derived from SSR marker data. For analysing marker data related to molecular diversity parameters such as total number of alleles, major allele frequency, gene diversity and polymorphism information content (PIC) values, PowerMarker software, version 3.25 was used.

Results and Discussion

Variability parameters analysis for morpho-qualitative traits

The estimates of mean, range, phenotypic coefficient of variance (PCV), genotypic coefficient of variance (GCV), heritability in the broad sense (h^2b) and expected genetic advances as a percent of mean (GAM) for each trait under consideration are represented in Table 4. Across all traits, PCV values were consistently higher than GCV values, indicating the influence of the environment on trait expression. PCV and GCV values ranged from 4.05 % and 2.98 % for MD to 48.51 % and 48.16 % for TIA respectively. High variability was observed in traits such as PH, NPP, NEPP, NSPP, BYPP, SYPP, total sugar content (TSC), reducing sugar content (RSC), non-reducing sugar (NRS) and TIA. Moderate estimates of variability were noted for HI, SW and PC, while traits viz., DF, MD and LP showed low variability. Broad sense heritability was grouped into low (below 30 %), moderate (30-60 %) and high (above 60 %) categories. In accordance with the above criteria, high heritability was reported for the traits viz., PH, NPP, NEPP, LP, NSPP, SW, BYPP, SYPP, HI, RSC, TSC and TIA, suggesting a high degree of genetic control and minimal environmental effect. Moderate estimates of heritability were observed for traits viz., DF, MD and NRS. GA ranged from 0.80 (LP) to 47.76 (PH), while, GAM values varied from 4.53 % (MD) to 98.52 % (TIA). Traits such as PH, NPP, NEPP, LP, NSPP, SW, BYPP, SYPP, HI, RSC, TSC and TIA exhibited both high heritability and high GAM, indicating that these traits are likely governed by additive gene action and are effective for selection. The representation of genetic traits is shown in Fig. 1. ANOVA revealed significant differences among all the traits studied, confirming the presence of substantial genetic variability among the field pea genotypes indicating significant variation across genotypes in similar studies (16-18).

Table 4. Estimates of genetic parameters viz. heritability, GA, GAM, GCV, PCV and mean values

Traits	Mean	Heritability (%)	GA	GAM	GCV (%)	PCV (%)
DF (in days)	71.78	64.29	9.23	12.96	7.85	9.79
MD (in days)	121.38	54.34	5.51	4.53	2.98	4.05
PH (cm)	71.30	96.22	47.76	66.99	33.15	33.80
NPP	8.60	89.39	5.81	68.42	35.13	37.15
NEPP	7.25	81.60	4.09	57.06	30.66	33.94
LP (cm)	5.32	91.92	0.80	15.14	7.66	7.99
NSPP	4.58	81.48	1.62	35.60	19.14	21.20
SW (g)	14.03	87.55	3.31	23.64	12.26	13.11
BYPP (g)	11.15	92.38	6.32	56.79	28.68	29.84
SYPP (g)	4.54	93.36	2.90	63.96	32.13	33.26
HI (%)	40.69	97.85	11.21	27.56	13.52	13.67
PC (%)	27.99	92.10	6.08	21.82	11.04	11.50
TSC (mg/100 DW)	10.81	96.80	7.41	68.70	33.89	34.45
RSC (mg/100 DW)	7.24	97.20	5.27	72.76	35.82	36.33
NRS (mg/100 DW)	3.56	63.45	1.98	55.86	34.04	42.74
TIA (mg/100 DW)	5.97	98.58	5.88	98.52	48.16	48.51

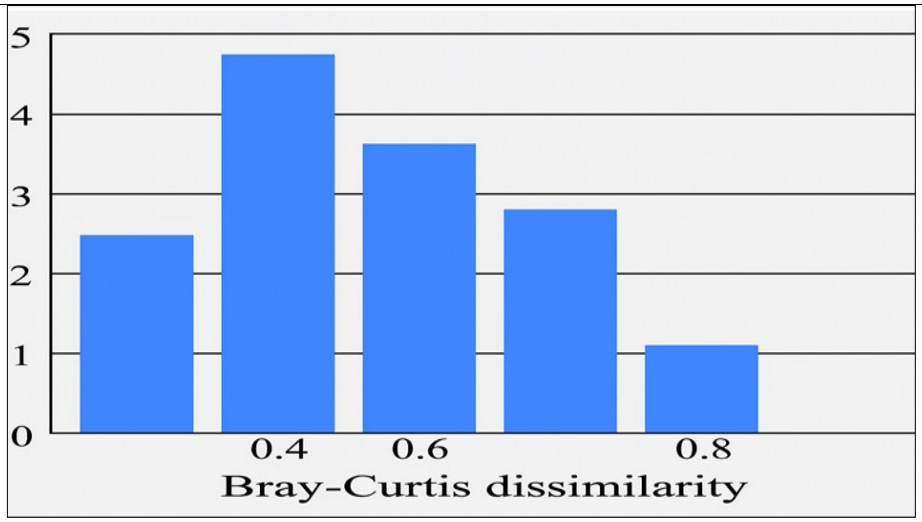


Fig. 1. Bray-Curtis dissimilarity matrix representing extent of dissimilarity in 18 test genotypes.

Across all traits, the PCV was higher than the GCV, indicating that environmental factors had a noticeable influence on trait expression (19-22). This indicates that while genetic factors play a role, the environment also contributes significantly to the observed variability. High PCV and GCV values were recorded for traits such as PH, NPP, NEPP, BYPP, SYPP, TSC, RSC, NRS and TIA depicting high variability in yield-related and biochemical traits (23, 24). On the other hand, traits such as DF, MD, LP and PC showed low PCV and GCV values, indicating limited genetic variability (25). Broad-sense heritability was found to be high for PH, NPP, NEPP, NSPP, LP, SW, BYPP, SYPP, HI, PC, RSC, TSC and TIA, suggesting that these traits are largely controlled by genetic factors. Previous studies have also reported high heritability for these traits, reinforcing their potential for effective selection in breeding programmes (26, 27). GAM was highest for traits such as NPP, NEPP, PH, NSPP, BYPP, SYPP, TSC, RSC, NRS and TIA, while MD showed the lowest genetic gain. Traits that exhibited both high heritability and high GAM such as PH, NPP, NEPP, BYPP, SYPP, TSC, RSC and TIA are likely governed by additive gene action. This indicates that selection based on these traits can lead to significant genetic improvement (28, 29). These studies emphasized the role of additive genes in determining trait inheritance.

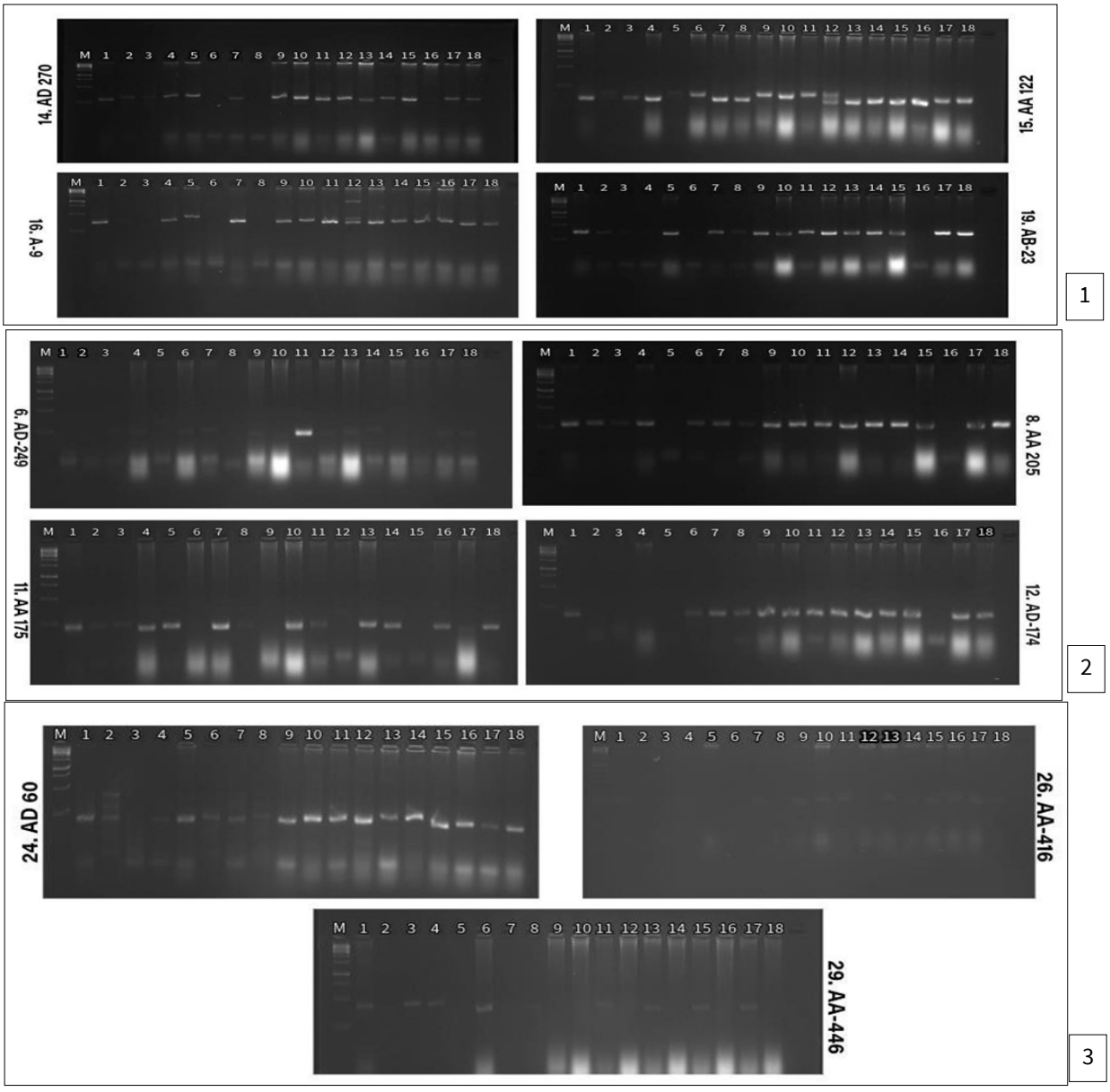
Molecular characterization

Molecular characterization was carried out after thorough screening of 18 outstanding accessions out of a total of 30 field pea genotypes using 20 SSR markers, of which 18 markers were found to be polymorphic, revealing substantial genetic variability. The level of variability present among different microsatellite loci was determined by estimating the total number of alleles present (TA), major allele frequency (MAF), gene diversity (GD) and polymorphism information content (PIC). Based on observations from 18 polymorphic markers, a total of 36 alleles were detected, with a mean of two alleles concerning single locus, indicating a moderate level of allelic richness (30, 31). This level of polymorphism suggests that the SSR markers utilized in the study were successful in capturing GD among the selected genotypes. The presence of polymorphisms in 90 % of the markers (18 out of 20) signifies that SSR markers can be effectively utilized for diversity analysis, genotype differentiation and marker-assisted selection. Polymorphic markers (scored as 1 or present) play a major role in portraying GD (32). The two markers that were monomorphic (scored as 0 or absent) did not show any variation among the test genotypes. This typically arises due to a number of reasons such as DNA regions targeted by those primers being highly conserved, depicting similarity among different genotypes, limited GD in the sampled population, or low mutation rates at those specific loci. Although these monomorphic markers did not represent genetic differences, they do not undermine the overall quality of the molecular analysis. The remaining polymorphic markers were sufficient to detect meaningful genetic variation, allowing for reliable genotype classification and diversity assessment. This kind of outcome is common in SSR-based studies and still supports the use of these markers in selection and hybridization programmes. With respect to the allelic data, MAF ranged from 0.50 (AA-246) to 0.94 (AD-249), with a mean value of 0.73, indicating that some loci were highly variable while others were dominated by a single allele. This variation marks the presence of both conserved as well as variable regions within the genome. Concerning the data regarding diversity of gene, the range was recorded between 0.10 (AD-249) to 0.50 (AA-446 and AD-249) with an

average of 0.33. PIC estimates ranged from 0.10 (AD-249) to 0.38 (AA-446), with a mean value of 0.27, further supporting the moderate informativeness of the SSR markers utilized in the study as presented in Table 5 (33, 34). Markers with high PIC values (AA-446 and AA-246) can be utilized for genotypic differentiation and marker-assisted selection programmes. The gel images depicting banding patterns clearly demonstrate the genetic differences among the field pea genotypes as revealed by the SSR markers (Plates 1-3). Each genotype displayed unique banding patterns, confirming that the markers used were indeed polymorphic. The inclusion of a 1 kb DNA ladder helped in precisely estimating the size of the amplified

Table 5. Details of amplification products viz. total alleles (TA), MAF, GD and PIC generated from 18 polymorphic SSR markers

S. No.	Marker name	TA	MAF	GD	PIC
1	C-20	2	0.71	0.27	0.23
2	AD-147	2	0.83	0.39	0.31
3	D-21	2	0.74	0.37	0.30
4	AA-67	2	0.83	0.27	0.23
5	AD-249	2	0.94	0.10	0.10
6	AA-205	2	0.72	0.40	0.32
7	AA-175	2	0.88	0.19	0.17
8	AD-174	2	0.72	0.40	0.32
9	AD-270	2	0.52	0.50	0.37
10	AA-122	2	0.69	0.42	0.33
11	AA-9	2	0.83	0.15	0.20
12	AB-23	2	0.77	0.34	0.28
13	AD-79	2	0.55	0.49	0.37
14	AA-399	2	0.84	0.25	0.21
15	AD-51	2	0.88	0.19	0.16
16	AD-60	2	0.52	0.49	0.37
17	AA-416	2	0.72	0.34	0.27
18	AA-446	2	0.50	0.50	0.38
Mean		**2.00**	**0.73**	**0.33**	**0.27**



Plates 1-3. Representative banding patterns generated by selected primers across different genotypes. Lane M represents a 1 kb DNA ladder.

fragments, making it easier to compare allele profiles across samples and ensuring the accuracy of the molecular analysis.

UNJ clustering

The cluster analysis showed that the 18 field pea genotypes could be grouped into three distinct genetic clusters. These groups were formed based on genetic dissimilarity among the genotypes, which ranged from 20 % to 80 % (Fig. 1). The genotype pairs IPFD6-3 vs. SKNP04-09 (0.78), RFP-2020-4 vs. Pant P-243 (0.74) and IPFD6-3 vs. Pant P-508 (0.72) exhibited high genetic divergence and are ideal candidates for hybridization to exploit heterosis and broaden the genetic base. This suggests that the SSR markers utilized in the study successfully depicted polymorphic variation. The PCoA also showed the same three groups and explaining about 43 % of the total genetic variation through the first two axes (Fig. 2, Table 6), representing moderate variation (35). Although this pattern is moderate, it is sufficient to reveal genetic differentiation among the test genotypes. The genetic tree and dendrogram (Fig. 3) provided a clear picture of how these genotypes are related with each other, confirming the

Table 6. PCoA showing the percentage of variation explained by the first five axes (1st, 2nd, 3rd, 4th and 5th) in 18 genotypes

Percent variation	1st	2nd	3rd	4th	5th
Variation (%)	23.19	20.17	17.88	8.43	7.62
Cumulative variation (%)	23.19	43.36	61.24	69.67	77.29

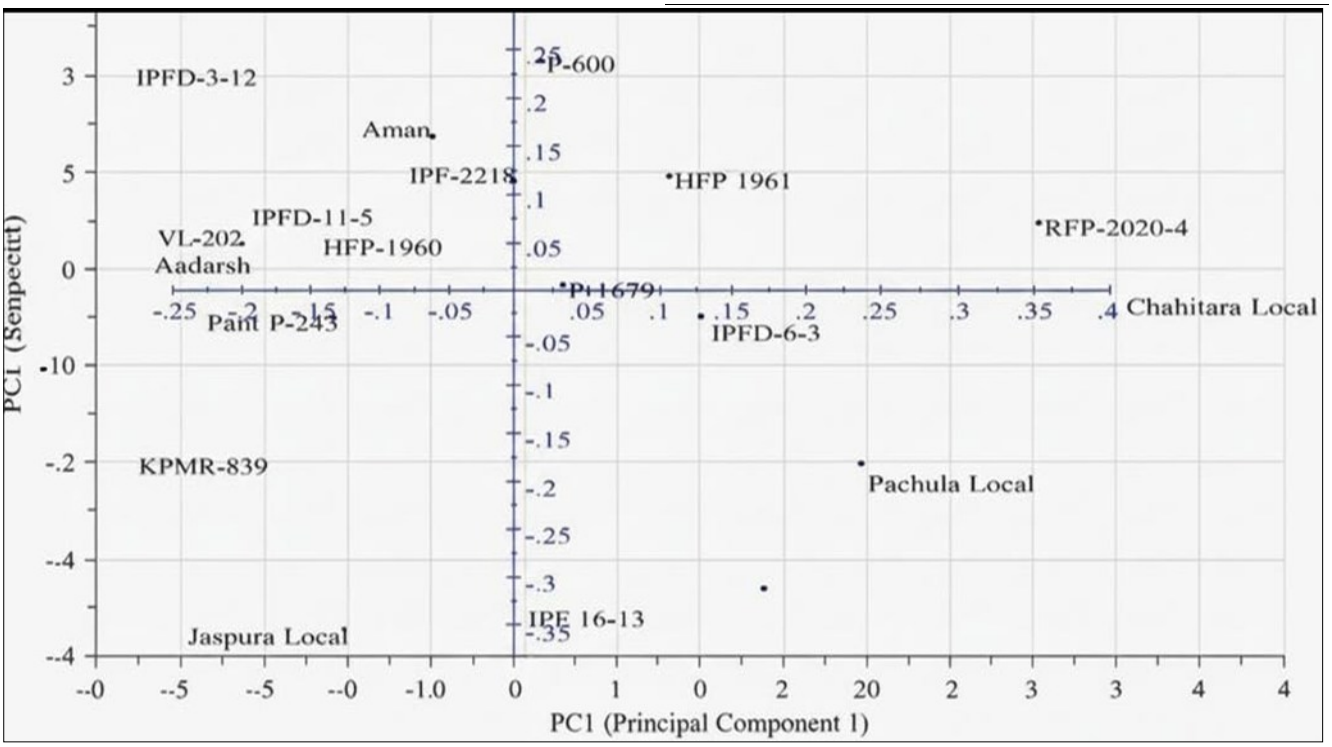


Fig. 2. Principal Coordinate Analysis (PCoA) of 18 field pea test genotypes showing trait-based variation along PC1 and PC2 axes genotypes clustered together share similar profiles, where distant points indicate greater dissimilarity among the test genotypes.

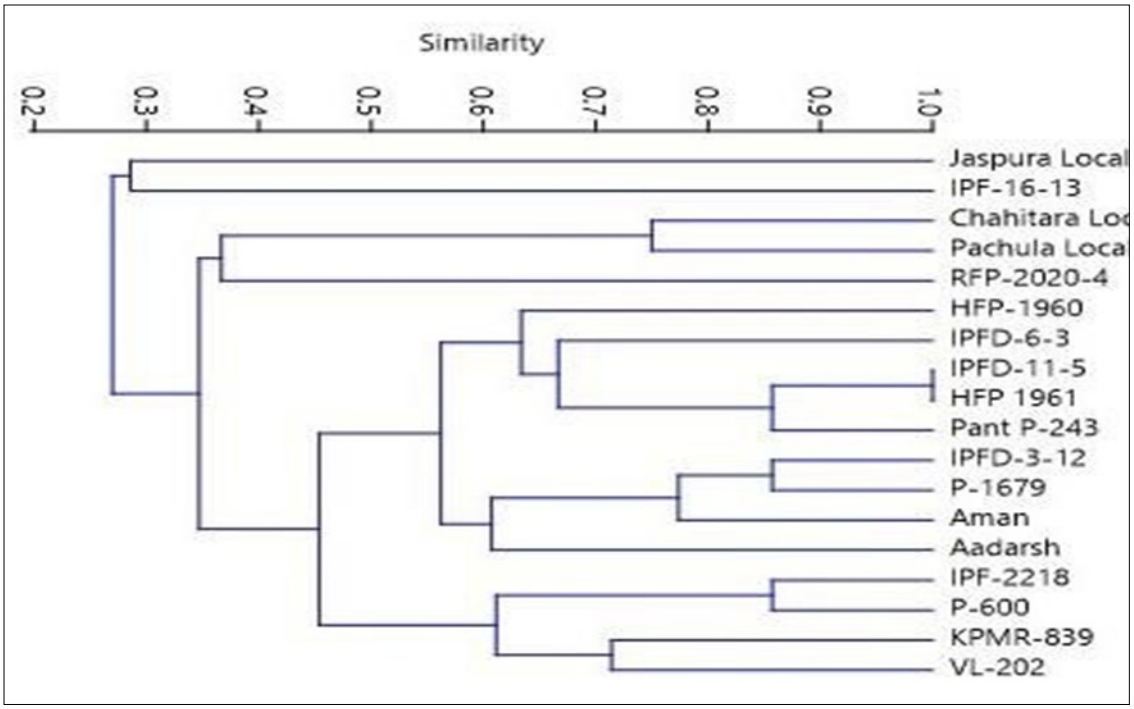


Fig. 3. Dendrogram representation of 18 field pea genotypes.

results obtained from both cluster analysis and PCoA. This indicates that the SSR markers used were effective in detecting genetic differences. From a breeding point of view, these results are useful for identifying genetically distinct genotypes. Crossing genotypes from different clusters can increase diversity and improve traits in future field pea varieties. The clustering pattern appears to be influenced by both pedigree background and trait performance. For instance, genotypes with higher seed yield and pod number (e.g. IPFD6-3, RFP-2020-4) were grouped separately from those with moderate biochemical traits (e.g. Pant P-243, IPF-16-13), suggesting that both molecular and phenotypic diversity contributed to cluster formation (36, 37). These studies highlighted the effectiveness of SSR markers in capturing genetic variation through clustering patterns. From a breeding perspective, selecting parents from different clusters can enhance genetic recombination and improve trait diversity in future field pea lines. As per the breeding recommendations, IPFD6-3, SKNP04-09 and RFP-2020-4 belong to separate genetic groups; therefore, crossing them could result in offspring with better yields, stronger traits and greater adaptability. On the other hand, if a plant such as Pant P-243 shows excellent sugar content even though it is genetically similar to others, it can still be used to improve that specific trait by crossing it back with other lines. This approach will help breeders combine strengths from different plants to create better-performing varieties in future breeding programmes.

Conclusion

This study aimed to assess genetic variability among field pea genotypes using both agro-morphological and biochemical traits, supported by molecular fingerprinting with SSR markers. Significant variation was observed across all traits, confirming the presence of substantial genetic diversity. Genotypes such as P-600 (early flowering), P-1679 (early maturity), IPFD 14-2 (dwarf stature) and IPFD 6-3 (high seed yield and pod length) demonstrated superior performance for specific traits, making them valuable candidates for targeted improvement.

Biochemical profiling highlighted Pant P-243 for high protein content and low trypsin inhibitor activity, while IPFD 10-12 excelled in total and non-reducing sugar content. These trait-specific genotypes offer potential for nutritional enhancement and quality improvement in breeding programmes.

Molecular analysis using 18 polymorphic SSR markers revealed 36 alleles, with AA-446 identified as the most informative locus. Cluster analysis grouped the genotypes into three distinct clusters, supported by PCoA, which explained 43.36 % of the total genetic variation. Genotypes such as IPFD6-3, SKNP04-09 and RFP-2020-4, belonging to separate clusters, showed high genetic divergence and are recommended for hybridization to exploit heterosis and broaden the genetic base.

Recommendations include enhancing genetic mixing and broadening the diversity pool, it is advisable to cross genotypes that fall into separate genetic groups such as pairing IPFD6-3 with SKNP04-09.

Genotypes that show excellence in specific traits, such as Pant P-243 for protein content or IPFD 10-12 for sugar levels, can be used in targeted breeding efforts to improve nutritional value through repeated crossing with other lines.

Genetic markers that proved highly informative such as AA-446, should be prioritized in future studies for tracking diversity and assisting in trait selection.

The patterns revealed through clustering and coordinate analysis offer a reliable roadmap for selecting parent lines and preserving genetically unique varieties for long-term breeding and conservation goals.

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Authors' contributions

SS performed the whole study and collected the germplasms from different sources. MK helped in gathering the data. SS and VS wrote the manuscript. SS and DS conducted the molecular characterization. DKS, CMS, VC and SP provided the technical help relating to different protocols involving morpho-qualitative and molecular data. VS helped in data interpretation. VC, AB, N and HJ reviewed the final draft. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: The authors declare that they have no conflict of interest.

Ethical issues: None

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