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RESEARCH ARTICLE

Genetic diversity analysis in peas based on morphological, biochemical and molecular markers

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Abstract

This study aimed to evaluate the genetic diversity of cultivated pea (*Pisum sativum*) varieties using morphological, biochemical and molecular markers. Qualitative trait analysis revealed that 90.01 % of varieties had cream-yellow cotyledons, 72.72 % exhibited rough seed surfaces and 63.63 % displayed irregular seed shapes. Notably, the Algerian variety Séfrou had a pigmented seed coat, anthocyanin coloration and colored flowers. SDS-PAGE analysis detected 175 protein bands, of which 5 were polymorphic. The variety Latcha exhibited the highest number of bands 18, while Guifilo had the lowest (13 bands). Other varieties (Sirma, Aroubia, Dorian, Utrillo, Guifredo, Onward, Sara, Kelvedon) showed intermediate banding patterns (15-17 bands). Molecular analysis using 12 SSR markers revealed substantial allelic diversity, with 43 alleles across 12 loci (3-5 alleles per locus). Major allele frequency (MAF) ranged from 0.199 to 0.757, expected heterozygosity (He) averaged 0.674 (range: 0.578-0.776) and Shannon's index averaged 1.186 (range: 0.934-1.546). The mean polymorphic information content (PIC) was 0.608 (range: 0.486-0.740), confirming high variability. These findings highlight the genetic diversity among pea cultivars, which can inform breeding programs targeting traits like seed color, protein content and stress resistance. The SSR markers and protein profiles provide valuable tools for future conservation and selection efforts.

Keywords: biochemical markers; diversity; molecular markers; *Pisum sativum* L.; pea varieties; qualitative traits

Introduction

Pisum sativum L., a member of the Fabaceae family and the Papilionoideae subfamily, has been cultivated worldwide for centuries as a valuable source of human nutrition and animal feed. As a diploid species with a large genome size of 5000 Mbp, it serves as an excellent model for functional genomics studies (1). Peas are rich in starch (50 %), fiber, minerals, protein (23-25 %) and vitamins (2), making them a nutritionally significant crop. However, despite their global importance, local pea landraces in Algeria are increasingly neglected in favor of imported varieties, leading to a concerning decline in native genetic diversity. This genetic erosion threatens biodiversity and limits breeding opportunities for stress-resilient pea varieties, underscoring the need for conservation efforts.

Accurate variety identification is essential for safeguarding genetic resources under the Farmers' and Plant Breeders' Rights Act. While traditional studies have relied on morphological and biochemical markers (3, 4), these descriptors are often unreliable due to their susceptibility to environmental influences (5, 6). Molecular markers, in contrast, provide a more precise assessment of genetic variation, as they are unaffected by environmental conditions and enable early varietal discrimination (7). Integrating morphological, biochemical and molecular markers offers a comprehensive understanding of genetic diversity, as each method contributes unique insights. Protein storage analysis has proven effective as a genetic diversity indicator in several key applications, including assessing intra- and inter-accession variation, studying domestication patterns, supporting genetic resource conservation and aiding crop improvement programs (8-10). The SDS-PAGE technique, in particular, has been widely and successfully employed for varietal discrimination and genetic diversity assessment (11).

Over the past two decades, genetic diversity research has gained significant importance in breeding strategies and germplasm conservation, especially with the rise of seed banking (12). Various molecular techniques such as restriction fragment length polymorphisms (RFLPs), amplified fragment length polymorphisms (AFLPs) and simple sequence repeats (SSRs)have been utilized in genetic mapping. However, studies applying DNA markers to forage pea (*Pisum sativum* ssp. *arvense* L.) remain limited. Among PCR-based approaches, SSR markers are particularly advantageous for rapidly and cost-effectively characterizing landraces. Their uniform genome distribution, co-dominant inheritance, high polymorphism, ease of PCR detection and reproducibility make them ideal for comprehensive genetic analysis (13). Given these considerations, this study aims to evaluate the genetic diversity of cultivated pea varieties in Algeria using an integrated approach combining morphological, biochemical and molecular markers.

Materials and Methods

Plant material

The pea varieties used were supplied by the Cereals and Pulses Cooperative (CNCC) Constantine. Algeria, the Technical Institute for Market and Industrial Crops (ITCMI) Algiers in Algeria and farmers. The names of the varieties, origins, places of cultivation and suppliers are summarized in Table 1. The selected pea varieties were chosen based on their availability in specific national institutions and their preference among local farmers. No prior scientific studies have been conducted on these varieties, making this research a valuable first step in assessing their genetic diversity and potential for breeding and conservation efforts in Algeria.

The studied seed traits (cotyledon color, seed surface, seed shape and seed coat color and anthocyanin pigmentation) were evaluated following the description reported (14). Flower color also was used according to (15).

For protein extraction, five seeds were ground into a fine powder and 0.1 g of the seed flour was mixed with 1 mL of Tris-urea buffer (0.05 M Tris-HCl, pH 8.0, 0.4 % SDS, 5 M urea, 2.5 % glycerol, 1.5 % 2-mercaptoethanol, 1 mM EDTA, 0.01 % bromophenol blue). The mixture was vortexed for 5 min, incubated at room temperature for 3 hr and then centrifuged (15000 rpm, 10 min room temperature). The supernatant containing the extracted proteins was collected, while the pellet was discarded (16). Protein extracts were stored at 4 °C until analysis. Electrophoresis was performed using a discontinuous SDS-PAGE system (17) with a 15 % resolving gel and a 4 % stacking gel, run at 180 V for 2 hr. Gels were stained with 0.2 % Coomassie Brilliant Blue R250 for 1 hr and destained overnight in a solution of 10 % acetic acid, 40 % methanol and 50 % distilled water with gentle shaking. Finally, gels were preserved, scanned and photographed for further analysis.

Table 1. List of pea varieties (*Pisum sativum* L.), origins, growing locations and suppliers

Varieties	Origin	Cultivated	Sources
Aroubia	Algeria	Jijel	Farmer
Onward	Greece	Constantine	CNCC
Utrillo	Turkey	Constantine	CNCC
Kelvedon	England	Constantine	CNCC
Latcha	Algeria	Chlef	Farmer
Dorian	Turkey	El oued	Farmer
Guifredo	Italy	Algiers	ITCMI
Guifilo	Italy	Algiers	ITCMI
Séfrou	Algeria	Constantine	CCLS
Sara	France	Mostaganem	Farmer
Sirma	Turkey	Jijel	Farmer

DNA extraction

Plants were grown in the greenhouse of the Genetics, Biochemistry and Plant Biotechnology laboratory. Université mentouri Constantine 1 in Algeria and also in the greenhouse of the Finnish Museum of Natural History. The plants were grown under controlled greenhouse conditions with a constant temperature of 24 °C, a 12 hr light/12 hr dark photoperiod and artificial lighting. The relative humidity was maintained at 60-70 % to ensure optimal growth conditions. DNA was extracted from 5 mg leaf segments using the DNeasy Plant Mini Kit (Qiagen, Germany) following the manufacturer's protocol. NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, Waltham, USA) was used to evaluate the quantity and quality of the DNA extracts.

SSR genotyping

PCR amplification with primers described (18) and summarized in Table 2 In order to amplify the target DNA, a total volume of 20 µL of reaction mixture was utilised. This reaction mixture contained 20 ng of genomic DNA, 0.2 mM dNTPs (obtained from Thermo Fisher Scientific), 2 µL of 1× PCR buffer, 20 pM of each fluorescently labeled primer and 0.25 units of DyNAzyme DNA polymerase (obtained from Thermo Fisher Scientific). The thermal cycling process was conducted using a MasterCycler ep96 (Eppendorf, Hamburg, Germany) under the following thermal profile: an initial denaturation at 94 °C for 2 min; 35 cycles of denaturation at 94 °C for 30 sec, primer annealing at 55 °C for 1 min and strand extension at 72 °C for 2 min; followed by a final elongation at 72 °C for 5 min. Subsequent examination of the amplified PCR products was undertaken using an ABI 3730XL DNA analyzer (Applied Biosystems, Foster City, California, USA) with a GeneScan 500 LIZ Size Standard (also from Applied Biosystems).

Data analysis

XLSTAT were used to construct an agglomerative hierarchical clustering (AHC) using morphological traits, the data obtained with SDS-PAGE analysis were scored in a binary form as the presence or absence (1/0) of bands for each sample. XLSTAT was used to calculate Jaccard coefficient. A hierarchical cluster analysis was performed using the average linkage method and Jaccard coefficient. The dendrogram, based on total seed protein patterns of pea cultivars. iMEC (19) was used to analyze microsatellite patterns and determine polymorphic information content (PIC), expected heterozygosity (He), marker index (MI) and discriminating power (D). PopGene 1.32 (20) was employed to compute further diversity parameters, including mean allele

Table 2. Microsatellite primers used for genetic diversity analysis

M N	Forward (5'-3')	Reverse (5'-3')	A t (°C)
PB14	GAGTGAGCTTTTTAGCTTGCAGCCT	TGCTTGAGAACAGTGACTCGCA	67
PSAA18	CTGTAGACCAAGCCCAAAAGAT	TGAGACACTTTTGACAAGGAGG	61
PSAA175	TTGAAGGAACACAATCAGCGAC	TGCGCACCAAACTACCATAATC	62
PSAC58	TCCGCAATTTGGTAACACTG	CGTCCATTTCTTTTATGCTGAG	60
PSAC75	CGCTCACCAAATGTAGATGATAA	TCATGCATCAATGAAAGTGATAAA	62
PSAA219	ATTTGTGCAATTGCAATTTCATT	CGAAAACGCTTTGCATCCTA	61
PSAD83	CACATGAGCGTGTGTATGGTAA	GGGATAAGAAGAGGGAGCAAAT	62
PSAD270	CTCATCTGATGCGTTGGATTAG	AGGTTGGATTTGTTGTTTGTTG	61
PSAA456	TGTAGAAGCATAAGAGCGGGTG	TGCAACGCTCTTGTTGATGATT	63
PSAB23	TCAGCCTTTATCCTCCGAACTA	GAACCCTTGTGCAGAAGCATTA	61
PSAB47	TCCACAATACCATCTAAATGCCA	AATTTGTTCAGTTGAAATTTCGTTTC	60
PSAA497	TTGTGACTGATTTAGAAGTTTCCCAC	TTGATGAGTTGCAATTTCGTTTC	67

MN: marker name**, A t:** annealing temperature

frequency (MAF), effective number of alleles (Ne) and Shannon's information index (I). Additionally, Microsoft Excel was utilized to calculate standard deviations.

Results

Morphological traits

Fig. 1. and Fig. 2. show the seeds morphological and the flower color polymorphism of the studied varieties and these traits are summarized in the Table 3. Two cotyledon colors were observed: cream yellow (90.01 %) and green (9.09 %), with only Kelvedon exhibiting the latter. Regarding the seed surface, Aroubia, Sirma, Dorian, Onward, Latcha, Utrillo, Sara and Kelvedon have a rough surface (72.72 %), while Séfrou, Guifredo and Guifilo have a smooth surface (27.27 %). The seed shape is irregular for Aroubia, Sirma, Dorian, Onward, Latcha, Utrillo and Sara (63.63 %), elliptical for Séfrou, Guifredo and Guifilo (27.27 %) and cylindrical for Kelvedon (9.09 %). The coloration of the seed coat is absent in all varieties except for the variety Séfrou (9.09 %). Anthocyanin pigmentation is only observed in Séfrou (9.09 %). All the studied varieties have a white flower except for Séfrou characterized by purple flower (9.09 %).

Based on the observed qualitative traits an agglomerative hierarchical clustering (AHC) was conducted using XLSTAT, the clustering analysis grouped the varieties into three distinct clusters (Fig. 3), with Séfrou forming a separate group due to its unique pigmentation.

SDS-PAGE analysis

SDS page analysis revealed a total of 175 bands of which 5 were polymorphic Fig. 4. Latcha had the highest number of bands (18), while Guifilo had the lowest (13). Sirma, Aroubia, Dorian, Utrillo, Guifredo and Onward had 16 bands, while Sara and Kelvedon had 17 and 15 bands respectively. Polymorphic bands were observed between 14-43 kDa, corresponding to vicilin, Legumin α and Legumin β, characterized by the presence of polymorphic bands, Difference in bands intensity of some of the monomorphic bands were observed between the studied varieties profiles, this difference is clearly visible in 3 sites, the first one in ≈ 38 kDa region corresponding to (Legumin α), the second one were observed in ≈ 24 kDa corresponding to (Legumin β) while the last one were under the region of ≈ 14 kDa.

The dendrogram (Fig. 5) classified varieties into three major clusters, with Séfrou forming a separate group due to its

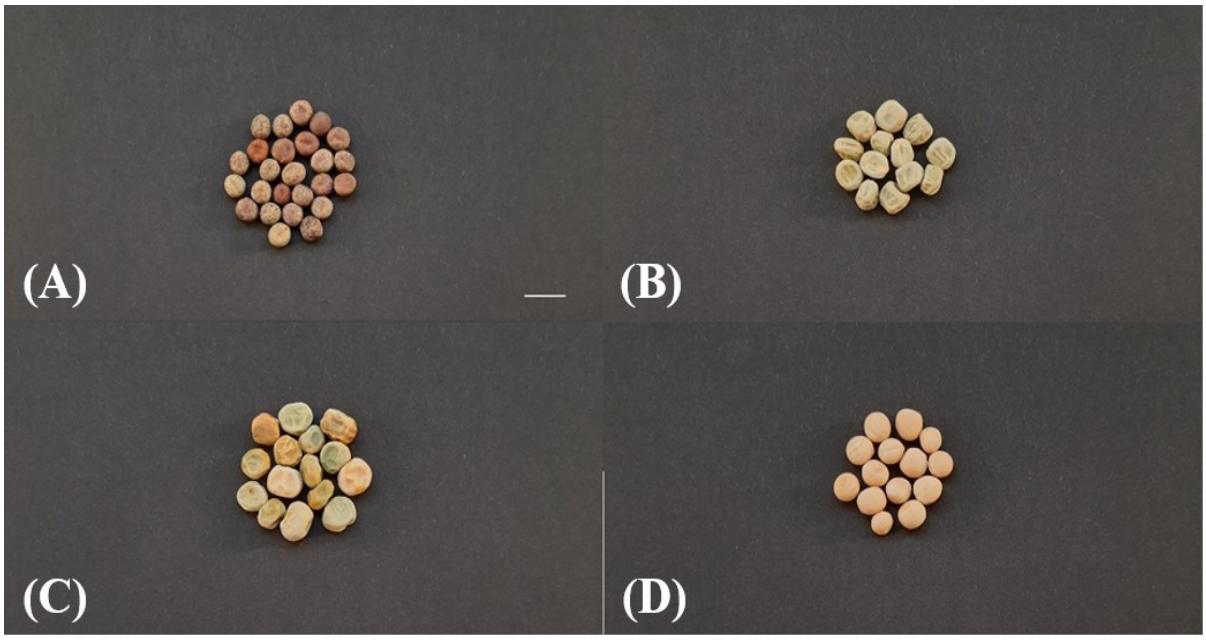

Fig. 1. Seeds polymorphism of studied pea varieties; (A): colored eleptical smooth seed, (B): translucent cylindrical rough seed, (C): translucent irregular rough seed, (D): translucent eleptical smooth seed. Scale bar = 1 cm.

Fig. 2. Flowers polymorphism of studied pea varieties; (A): purple flower, (B): white flower.

Table 3. Morphological seed traits of pea studied varieties

Varieties	Cc	Ss	Ssh	Scc	Ap	Fc
Aroubia	cream yellow	rough	irregular	translucent	absent	white
Séfrou	cream yellow	smooth	eleptical	colored	present	purple
Sirma	cream yellow	rough	irregular	translucent	absent	white
Dorian	cream yellow	rough	irregular	translucent	absent	white
Kelvedon	green	rough	cylindrical	translucent	absent	white
Onward	cream yellow	rough	irregular	translucent	absent	white
Latcha	cream yellow	rough	irregular	translucent	absent	white
Utrillo	cream yellow	rough	irregular	translucent	absent	white
Sara	cream yellow	rough	irregular	translucent	absent	white
Guifredo	cream yellow	smooth	eleptical	translucent	absent	white
Guifilo	cream yellow	smooth	eleptical	translucent	absent	white

Cc: cotyledon color, **Ss**: seed surface, **Ssh**: seed shape, **Scc**: seed coltyledon color, **Ap**: anthocyanin pigmentation, **Fc**: flower color

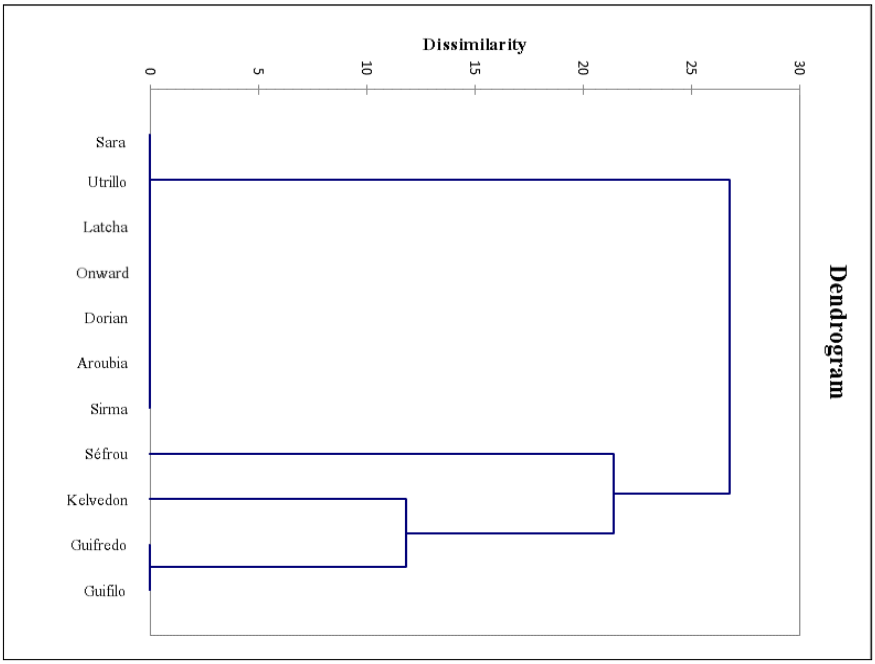

Fig. 3. Dendrogram of studied varieties based on qualitative traits.

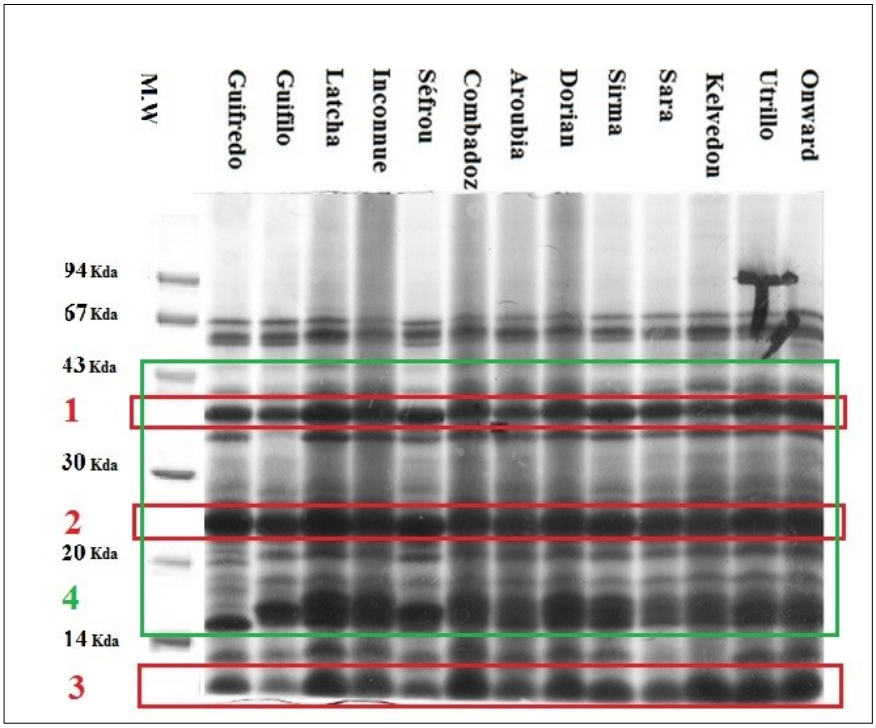

Fig. 4. SDS-PAGE profile of seed storage proteins of studied pea varieties.

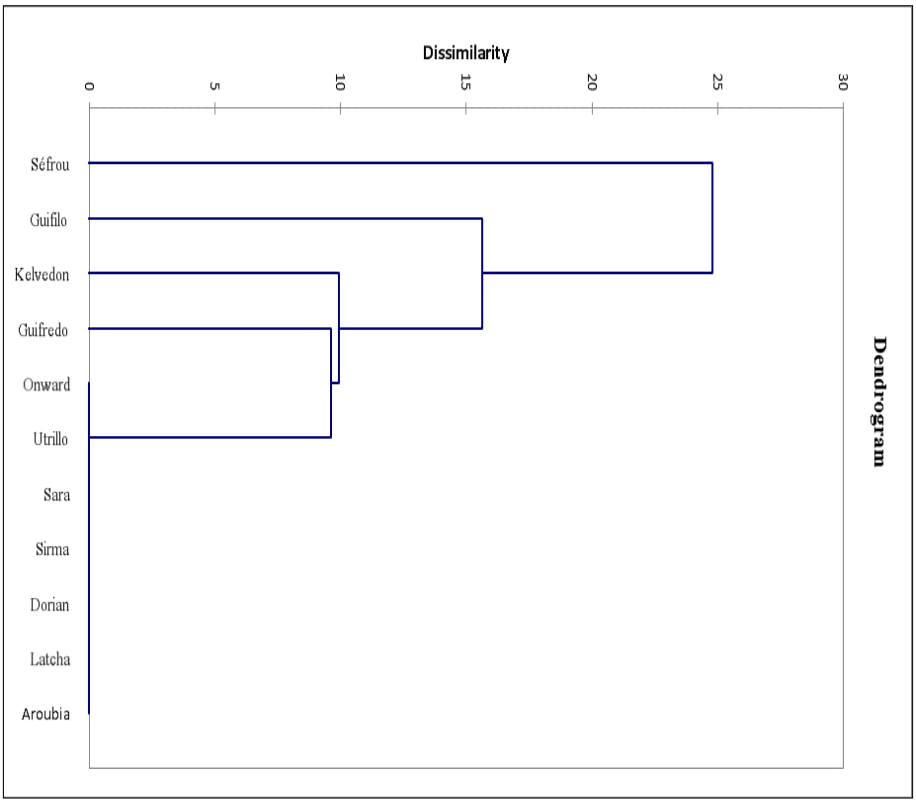

Fig. 5. Dendrogram based on markers of SDS-PAGE of seed storage proteins illustrating the relationships of studied pea varieties.

unique protein profile The first group is divided into two sub-groups: the first sub-group comprises the varieties Aroubia, Latcha, Dorian, Sirma, Sara, Utrillo, Onward and Guifredo, the second sub-group includes only the Kelvedon. The second group consists of the Guifilo and the third group includes the Séfrou.

SSR analysis

the obtained results with the twelve chosen simple sequence repeat markers are resumed in Table 4. In the germplasm inventory from Algeria, a total of 43 alleles were observed. Allelic diversity ranged from 3 alleles (PB14, PSAC75, PSAD83, PSAD270, PSAA456, PSAB47, PSAA497) to 5 alleles (PSAA175, PSAC58), On average, the observed number of alleles across all loci was 3.58.

Our study revealed an average major allele frequency (MAF) of 0.355, spanning from 0.199 (PSAA175 and PSAC58) to 0.757 (PSAD270). The mean value of the expected heterozygosity (He) across all the utilized SSR markers was determined to be 0.674, with the lowest value of 0.578 (PB14 and PSAD83) and the highest value of 0.776 (PSAA175). In this study, the Shannon's Information index was observed to vary between 0.934 (PB34 and PSAD83) and 1.546 (PSAA175 and PSAC58), with the mean value of 1.186. The effective number of alleles (Ne) exhibited a

Table 4. Genetic diversity indices of pea varieties

Marker name	PIC	MI	D	MAF	Na	Ne	He	I
PB14	0.486	0.578	0.636	0.333	3	2.372	0.578	0.934
PSAA18	0.636	0.694	0.763	0.249	4	3.270	0.694	1.263
PSAA175	0.740	0.776	0.854	0.199	5	4.481	0.776	1.546
PSAC58	0.737	0.765	0.852	0.199	5	4.481	0.771	1.546
PSAC75	0.572	0.644	0.709	0.696	3	2.814	0.644	1.067
PSAA219	0.678	0.727	0.800	0.249	4	3.666	0.727	1.342
PSAD83	0.486	0.578	0.636	0.333	3	2.372	0.578	0.934
PSAD270	0.586	0.661	0.727	0.757	3	2.951	0.661	1.091
PSAA456	0.551	0.828	0.690	0.333	3	2.688	0.628	1.036
PSAB23	0.655	0.710	0.781	0.249	4	3.456	0.710	1.294
PSAB47	0.586	0.661	0.727	0.333	3	2.951	0.661	1.091
PSAA497	0.584	0.660	0.721	0.333	3	2.951	0.660	1.091
Mean	0.608	0.696	0.741	0.355	3.583	2.372	0.674	1.186
St. Dev.	0.084	0.068	0.072	0.173	0.792	0.679	0.064	0.202

PIC: polymorphism information content, **MI:** marker index, **D:** discriminating power, **MAF:** mean allele frequency, **Na:** number of alleles, **Ne:** effective alleles, **He:** Expected heterozygosity, **I:** Shannon Index, **St. Dev**: Standard deviation

range from 2.372 (PB14 and PSAD83) to 4.481 (PSAA175 and PSAC58), with an average value of 2.372. The PIC value serves as an indicator of the marker's discriminatory power. In our study, the mean value of the polymorphic information content was found to be 0.608, in a range of 0.486 (PB14 and PSAD83) to 0.740 (PSAA175).

Discussion

Morphological characteristics are indispensable for distinctness, uniformity and stability evaluation (21, 22). Morphological traits are not only essential for varietal identification but also hold significant implications for physiological and biochemical characteristics. For instance, seed coat color and texture can influence water imbibition rates, thereby affecting germination efficiency and seedling vigor. Additionally, cotyledon color and texture may be associated with variations in biochemical composition, such as protein and starch content, which are crucial determinants of seed nutritional value and processing quality. Understanding these associations can provide valuable insights for breeding programs aimed at improving both agronomic performance and nutritional quality of pea varieties.

Compared to (23), our results revealed a higher prevalence of cream-colored cotyledons (90.01 % vs. 45.3 %), whereas the distribution of green cotyledons was considerably lower (9.09 % vs. 14 varieties in their study). Similarly, our study observed a dominance of rough seed texture (72.72 %), aligning with the dimpled surface predominance reported (23), although the distribution of smooth and wrinkled textures differed. These differences could be attributed to genetic variations and environmental conditions specific to our studied varieties. In the study (23), cream was the most common seed coat color (45.3 %), while purple-dark was rare. Cotyledon pigmentation varied, with cream being the most frequent. Seed texture was evenly distributed, with dimpled as the most prevalent and seed shapes were mainly oval-elongated (40.0 %), followed by spherical and square. It was investigating an Algerian pea collection, reported polymorphism among the genotypes (24). In comparison, our findings revealed similarities in the occurrence of cylindrical seed shapes, as observed in Kelvedon, aligning with the five genotypes identified (24). However, differences were noted in the frequency and distribution of seed shapes, with irregular and elliptical shapes being predominant in our study. Regarding anthocyanin pigmentation, while Séfrou exhibited pigmentation in our analysis, the same author reported a higher occurrence (58.33 %) across various genotypes, highlighting the potential influence of genetic background and environmental conditions. Morphological features are often subject to environmental influences, yet they remain essential for assessing crop diversity as they constitute the fundamental elements of overall variability (21, 22).

It was classified pea genotypes into three groups based on six qualitative traits (25), with slight differences from our clustering results, likely due to genetic and environmental factors. The first group included four cultivars and two *Pisum fulvum* L. accessions, while the second grouped two cultivars with accessions from Cyprus and Algeria. The third group contained wild taxa, a regional cultivar and ICARDA accessions. Complete similarity was found between two *Pisum sativum* L. cultivars and *Pisum fulvum* L. accessions, indicating genetic identity (26). Also, it was identified five to seven groups in 164 *Pisum sativum* L. accessions using qualitative and quantitative traits (27).

A study on 32 electrophoretic protein bands revealed that only 15 exhibited polymorphism (25). Similar results were obtained by another group, who examined seed storage proteins in 13 *Pisum sativum* L. cultivars and found that just 15 out of 41 bands were polymorphic (28). In contrast, a separate investigation identified 121 polymorphic protein bands across 148 *Pisum* accessions (29). The electrophoretic examination revealed a high level of variation among the analyzed genotypes, with the exception of the two *Pisum fulvum* L. accessions, which exhibited identical banding profiles. A similar investigation also verified that *Pisum fulvum* L. is distinctly differentiable from other *Pisum* species (30). Variations in electrophoretic protein banding patterns can be attributed to the genetic diversity inherent to the origin of the studied varieties and the environmental conditions under which they were grown. The geographic origin of accessions influences their genetic makeup, potentially leading to differences in seed storage protein profiles due to long-term adaptation to specific climates and soil types. Additionally, growth conditions such as soil nutrient availability, temperature and water stress can modulate gene expression related to protein synthesis, further contributing to the observed polymorphism. Understanding these factors is crucial for selecting suitable varieties for breeding programs and optimizing cultivation practices.

Based on the dendrogram classification, the Algerian *Pisum sativum* variety Aroubia clustered within the same sub-group as the European *Pisum sativum* varieties Onward (Greece) and Utrillo (Turkey), whereas the Séfrou variety formed an independent group. Ashraf et al. (25) developed a dendrogram from protein patterns, which delineated two principal clusters. The first cluster included the Algerian *Pisum sativum* L. accession and a subgroup with four cultivars and two *Pisum fulvum* L. accessions, which showed complete similarity. The second cluster contained two cultivars and a wild taxon from El-Dabba. The dendrogram clearly separated *Pisum fulvum* L. accessions from the four cultivars.

It was employed the same primers used in our study, along with nine others, to evaluate 731 pea accessions from various origins (18). They detected a total of 109 alleles, with an average of 5.19 alleles for each pair of the used primer. Among these, effective alleles (Ne) made up 57.1 %, with an average of 2.96 alleles. Four primers PSAD270, PSAC58, PSAA18 and PSAC75 were found to be the strongly efficient, showing high values for Shannon's information index (over 1.5), observed alleles (above 6.0) and effective alleles (over 4.0), surpassing the results of our study using the same primers. It was applied three of these primers PSAD270, PSAC58 and PSAC75 to analyze 600 pea accessions, each revealing an allele count (Na) greater than 10 (31).

In a study of 25 pea varieties using SSR markers, (27) identified between 3 to 6 alleles per primer, detecting a total of 38 alleles across all varieties. It was examined 20 pea varieties and 57 wild pea accessions (32), using 10 out of 20 microsatellite primers and found 59 alleles. The PEACPLHPP, AF004843 and AA43090 loci had the highest allele count of 8, while allele numbers per locus

ranged from 2 to 8, with an average of 5.9. A study successfully differentiated 51 genotypes with 32 SSR markers, identifying a total of 127 alleles, with 2 to 7 alleles per primer and an average of 3.97 (33). This average is higher than the results of our study. It was found 11 polymorphic SSR markers in a group of 23 individuals, including the parents of recombinant inbred lines, with allele numbers ranging from 2 to 4 (34).

Comparing genetic diversity across studies is challenging due to variations in genotype sample sizes, the number of alleles detected per marker and the genetic diversity of the markers used (35). It was examined 46 field pea accessions and identified 37 alleles across 12 EST-SSR loci (13). Other studies on pea reported an average of 2.3 (36), 4.5 (22), 3.6 (37), 5 (29), 4 (38), 5 (39), 3.8 (40) and 4 (41, 42) alleles per polymorphic marker.

The Information index varied from 0.08 (A-9) to 1.47 (PSAC58), with an average of 0.81 (43). The diversity index (I) ranged from 0.11 to 1.13, with an average of 0.53 across all loci (13). A study reported Shannon index values from 0.2739 (AD100) to 1.8089 (PSAD270), with an average of 1.1597 (18). Haliloglu et al. (43) found that the effective number of alleles ranged from 1.03 (A-9) to 4.07 (PSAC58), with an average of 2.15. Additionally, the observed number of alleles (Na) per locus exhibited a range from 2 to 6, while the effective number of alleles (Ne) varied from 1.05 to 2.83 (13).

The average PIC value reported by (43) was 0.41, starting from 0.03 to 0.70, while Wu et al. (31) registered an average of 0.647, with a range of 0.408 to 0.827. Smýkal et al. (44) found a range of 0.10 to 0.75 with an average of 0.52 and Nasiri et al. (33) observed values between 0.556 and 0.839, averaging 0.72. Other studies reported PIC values ranging from 0.17 to 0.89, with averages of 0.63, (34), 0.62, (39), 0.53, (38), 0.89, (44), 0.51, (22) and 0.41 (36). These findings highlight the genetic variability among varieties, influenced by factors such as sample size, mode of reproduction and domestication history.

Variations in allele counts, diversity indices and polymorphism information content (PIC) values observed across different studies can be largely attributed to the genetic background of the pea varieties, their geographic origins and the environmental conditions in which they were cultivated. The origin of the accessions plays a crucial role in shaping their genetic diversity, as varieties adapted to distinct agro ecological zones may exhibit different levels of allelic richness and heterozygosity. Furthermore, growth conditions such as soil composition, climate and agronomic practices can influence genetic expression and variability, potentially leading to differences in marker performance across studies. Understanding these factors is essential for effectively utilizing molecular markers in breeding programs and germplasm conservation efforts.

Conclusion

The combination of qualitative traits, protein profiling and molecular markers such as SSRs provided a comprehensive evaluation of diversity levels. The distinct traits of the Algerian variety Séfrou, alongside the variation in SDS-PAGE band patterns and molecular marker diversity indices, emphasize the potential for these varieties to contribute to breeding programs. The findings underline the importance of integrating multiple analytical approaches for effective characterization and conservation of pea genetic resources. This integrated approach can enhance breeding efficiency, improve varietal identification and support conservation efforts for genetic diversity preservation.

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Authors' contributions

OB conceptualised the research work and analysed and interpret the whole data. OB and PP were involved in designing the research work, data collection, data validation and resource collection. OB and DH did the literature review. PP was involved in writing the research article, critical review and providing project administration and funding acquisition. All authors have read and agreed to the published version of the manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interests to declare.

Ethical issues: None

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