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RESEARCH ARTICLE

Understanding genetic diversity and population structure in forage pea (*Pisum sativum* var. *arvense* L.) using inter-primer binding site (iPBS) retrotransposon marker

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Abstract Forage peas are widely utilized as a source of animal feed, and their fresh shoots, fruits, and pods exhibit considerable potential for human consumption. This study aimed to assess the genetic diversity and population structure of 61 Turkish forage pea genotypes using inter-primer binding site (iPBS) retrotransposon markers. A total of 37 highly polymorphic iPBS primers were employed, producing an average of 69 bands per primer and a cumulative total of 2529 bands. The average polymorphism information content (PIC) was determined to be 0.345. The results revealed significant genetic diversity within the germplasm, as indicated by diversity indices such as the average effective number of alleles (0.704) and a Shannon's information index (0.299). Molecular analysis of variance (AMOVA) indicated that 74% of the total genetic variation occurred within populations. Population structure analysis identified four distinct subpopulations among the genotypes, with expected heterozygosity values ranging from 0.2404 in the fourth subpopulation to 0.3094 in the second subpopulation, with an overall average of 0.2702. This study represents the first comprehensive investigation of genetic diversity and population structure in forage pea germplasm using the iPBS

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retrotransposon marker system. The findings highlight the effectiveness of iPBS markers in genetic diversity research and underscore their potential for broader application in the characterization of diverse forage pea collections across different geographic regions.

Keywords AMOVA · Molecular characterization · *Pisum sativum* · Polymorphism · Structure

Introduction

Historical records indicate that forage pea is among the oldest cultivated legumes and fodder plants globally (Ascı et al. 2015; Demirkol and Yilmaz 2019). It can be cultivated either as a sole crop or intercropped with cereals for silage preparation (Tan et al. 2012). In addition to its use in animal feed, the fruits and pods of forage pea have promising potential as vegetables in human diets (Kosev 2014). Due to its high nutritional value and cost-effectiveness, it also serve as an alternative protein source in vegetarian diets (Vennela 2023). The nutritional importance of forage pea lies in its high-quality protein content and its capacity for atmospheric nitrogen fixation, a key trait of legumes (Demirkol and Yılmaz 2023). This attributes to its value a forage crop. Beyond its traditional use as fodder, forage pea is increasingly incorporated into processed food products, including cakes, cookies, bread, pasta, and noodles (Hill 2022). Furthermore, it holds economic significance due to its adaptability to low-rainfall environments, where it often outperforms other legumes such as broad beans and lentils (Mohammed et al. 2016). Botanically, forage pea is a diploid, self-pollinating species with a chromosome number of $2n = 14$ (Azam et al. 2024).

Despite its numerous advantages, both research findings and statistical data reveal that global production levels of forage pea remain low, with a decreasing per capita availability for human and animal consumption, largely driven by rapid population growth. To address the increasing demand and enhance the strategic value of the crop, a substantial improvement in forage pea productivity is essential (Yadav et al. 2023). Consistent with global patterns, the average productivity of forage pea in Turkey also remains notably low. Therefore, the development of high-yielding genotypes and the production of quality seeds are critical for achieving sustainable cultivation (Haliloğlu et al. 2022).

Preservation of genetic diversity is vital for the development of improved cultivars and for maintaining ecological stability (Pinar et al. 2017, 2019; Uzun et al. 2020; Yaman and Uzun 2021). However, the genetic base of many crops species has been eroded due to climate change and anthropogenic pressures, thereby posing risks to ecological resilience. This underscores the urgent need for the conservation of genetic resources to mitigate genetic erosion and enhance food security under adverse environmental conditions (Teixeira and Huber 2021). Efforts have been undertaken globally and within Turkey to harness to local genetic resources for improving the agricultural productivity of key forage crops, including forage pea. In this context, landraces are of particular importance due to their genetic richness, adaptability, and resilience in marginal environments. These dynamic populations—also referred to as primitive, farmers', local, or traditional varieties—are characterized by their historical development, genetic diversity, local adaptation, and associations with traditional farming systems (Alzahib et al. 2021). Landraces represent a critical genetic reservoir for breeding programs, offering access to lost or underutilized genetic variability. They provide novel genes and alleles that can be introgressed into modern cultivars through hybridization. Incorporating landraces into breeding populations can increase genetic variation, after allele frequencies, and enable the discovery of beneficial alleles (Lazaridi et al. 2024; Lopes et al. 2015). Although significant research has been conducted on pea landraces (Baloch et al. 2015), there is limited information regarding forage pea landraces. Understanding the genetic variation for morphological and agronomic traits in forage pea can aid in breeding superior cultivars. Notably, all *Pisum* species and subspecies are cross-compatible, offering valuable opportunities to exploit natural genetic diversity in breeding programs (Rispail et al. 2023).

Morphological markers have traditionally been employed to assess genetic diversity (Karakaya et al. 2023). However, these markers are limited in their ability to account for environmental variability and annual fluctuations in climatic conditions, making it challenging for breeders to achieve their objectives. Consequently, morphological

markers alone are inadequate for comprehensive characterization of genetic diversity. Advancements in molecular biology have led researchers to the adoption of DNA-based markers, which offer reliable and reproducible data independent of environmental influences, plant developmental stages, or organ types. These markers have become essential tools in the evaluation of genetic diversity and are now widely favored in modern breeding programs (Al-Ashkar et al. 2020). In recent years, a range of molecular markers—such as simple sequence repeats (SSR) (Haliloglu et al. 2022), inter-primer binding site (iPBS) markers (Baloch et al. 2015), random amplified polymorphic DNA (RAPD) (Khoiriyah et al. 2018), and amplified fragment length polymorphisms (AFLP) (Dyachenko et al. 2014)—have been utilized to investigate genetic diversity in pea. Despite their broad application in pea research, their use in forage pea research remains limited. Retrotransposons, which represent a substantial fraction of the genome in many eukaryotic organisms, have emerged as particularly valuable molecular markers in plant genetic research. In species with large genomes, retrotransposons may constitute more than 50% of nuclear DNA, highlighting their significant for exploring and promoting genetic diversity (Demirel et al. 2024).

In this study, genetic diversity in forage pea was investigated using inter-primer binding site (iPBS) markers. Although previous reports suggest that forage pea possesses considerable genetic diversity, this variability has not been thoroughly characterized using DNA-based molecular markers. Elucidating the genetic variation among selected forage pea genotypes is crucial for understanding the evolutionary history and populations dynamics of this species. Accordingly, the objective of this study was to characterize and evaluate the genetic diversity of forage pea genotypes collected from various provinces in Türkiye's northeastern Anatolia region using the iPBS markers. The results of this research provide valuable insights for the conservation and sustainable management of forage pea genetic resources in Türkiye. Furthermore, the findings support ongoing and future breeding efforts aimed at improving forage pea through the selection of genotypes with desirable agronomic traits.

Materials and methods

Plant material

This study examined 61 local forage pea landraces collected from five provinces in Türkiye. The Turkish forage pea landraces were collected in cultivated fields from five different provinces (Erzurum, Bayburt, Ardahan, Kars, and Giresun) in the northeastern Anatolia region of Turkey (Fig. 1; Table 1) (Haliloglu et al. 2022).

Determination of genetic diversity

The 61 forage pea landraces were cultivated under controlled greenhouse conditions for the purposes of this study. Genomic DNA was extracted from the young leaves of two-week- old seedlings at the Laboratory of Molecular Biology and Genetics, Department of Field Crops, Faculty of Agriculture, Necmettin Erbakan University. Genomic DNA extraction was performed using the protocol described by Zeinalzadehtabrizi et al. (2015), with tissue samples collected from three individual plants per each genotype. The DNA from these subsequently pooled for further analysis. The quality of the extracted DNA was assessed via electrophoresis on a 0.8% agarose gel. DNA concentrations were quantified using a NanoDrop ND-1000 UV/Vis spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA). Final DNA samples were normalized for use in iPBS analysis. Verified DNA samples were appropriately diluted and stored at –20 °C for subsequent PCR amplification (Haliloğlu et al. 2022).

iPBS-retrotransposon amplification

In this study, an initial screening of 83 iPBS primers was performed, from which 37 primers exhibiting high levels of polymorphism were selected for further analysis. The primers used in this investigation were originally developed by Kalendar et al. (2010), with their sequences detailed in Table 2. PCR amplifications were performed in a 20 µL reaction mixture containing 10X PCR buffer, 2 µM (20 pmol) primer, 0.25 mM of each dNTP, 2 mM $MgCl_2$, 0.5 U Taq polymerase, and 50 ng of DNA template. DNA amplification was conducted using a 96-well thermocycler (SensoQuest Labcycler, Germany) following

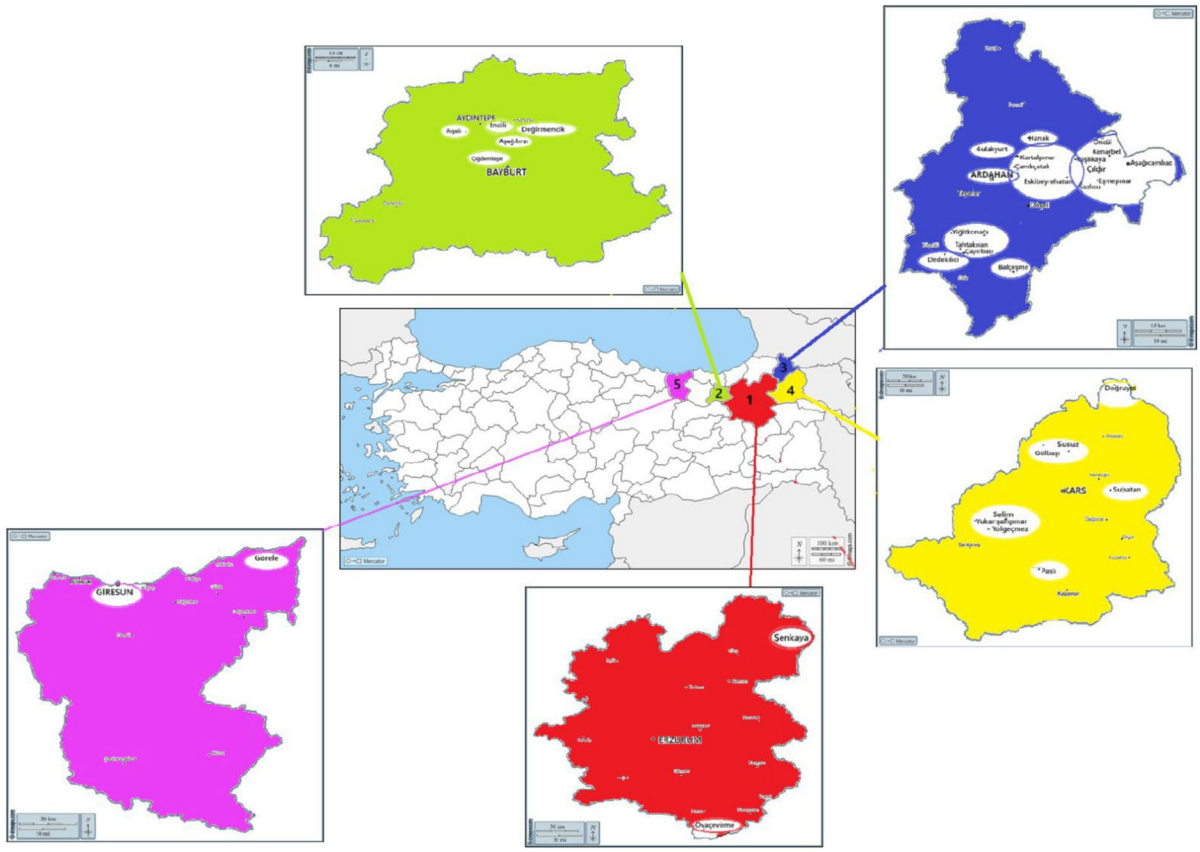


Fig. 1 Geographic distribution of forage pea landraces collected from diverse provinces across the northeastern Anatolia region of Türkiye. The maps **a** and **b** were created using D-Maps (Dalet 2018)

the protocol described by Armağan et al. (2024). The PCR protocol included an initial denaturation step at 95 °C for 5 min, followed by 40 cycles of denaturation at 95 °C for 60 s, annealing at 48–58 °C for 60 s, and extension at 72 °C for 60 s, with a final extension at 72 °C for 10 min. The resulting amplicons were separated on 1.5% agarose gels prepared in 1X SB buffer and electrophoresed at 150 V/cm for 120 min. DNA fragments were visualized using ethidium bromide staining (Sigma-Aldrich GmbH, Darmstadt, Germany). A 50 bp and 1000 bp DNA ladder (Vivantis, product no. NM2421) was utilized as a molecular size reference, as outlined by Haliloğlu et al. (2024).

Data scoring and analysis

The resulting DNA bands were analyzed using TotalLab TL120 software (TotalLab Ltd., Gosforth, Newcastle upon Tyne, UK). For iPBS amplification products, each locus was scored in a binary format—"1" indicating the presence and "0" the absence of a band. Only distinct and well-defined bands were included in the scoring to ensure data accuracy, while faint or ambiguous bands were excluded to maintain data integrity of the dataset (Turkoglu 2023; Türkoğlu et al. 2022, 2023a, 2023b).

Genetic similarities among the forage pea genotypes were calculated using the Numerical Taxonomy and Multivariate Analysis System (NTSYSpc) v.2.0, based on the Dice similarity coefficient (Dice 1945). A dendrogram was constructed using the Unweighted Pair-Group Method with Arithmetic Mean (UPGMA) algorithm implemented in NTSYSpc v.2.0. Additionally, molecular analysis of variance (AMOVA) and Principal Coordinate Analysis (PCoA) were performed using GenAlEx v.6.5 software (Peakall and Smouse 2006) to evaluate the distribution of genetic variation. The polymorphism information content

Table 1 List of information on locations where forage pea genotypes are collected

Code number	Accession name	Location	Latitude	Longitude	Altitude (m)
G1	Ovaçevirme-1	Erzurum	39.19.57 N	41.47.51 E	1586
G2	Ovaçevirme-2	Erzurum	39.19.57 N	41.47.51 E	1586
G3	Ovaçevirme-3	Erzurum	39.19.57 N	41.47.51 E	1586
G4	Ovaçevirme-4	Erzurum	39.19.57 N	41.47.51 E	1586
G5	Ovaçevirme-5	Erzurum	39.19.57 N	41.47.51 E	1586
G6	Şenkaya Merkez	Erzurum	40.33.27 N	42.20.37 E	1826
G7	İncili-1	Bayburt	40.23.24 N	40.12.16 E	1579
G8	İncili-2	Bayburt	40.23.22 N	40.12.14 E	1578
G9	İncili-3	Bayburt	40.23.22 N	40.12.14 E	1575
G10	Çiğdemtepe	Bayburt	40.19.50 N	40.08.16 E	1564
G11	Arpalı	Bayburt	40.21.56 N	40.06.17 E	1538
G12	Aşağıkırzı	Bayburt	40.23.23 N	40.08.48 E	1535
G13	Değirmencik-1	Bayburt	40.21.29 N	40.14.30 E	1532
G14	Değirmencik-2	Bayburt	40.21.29 N	40.14.30 E	1532
G15	Ardahan Merkez-1	Ardahan	41.09.45 N	42.56.15 E	1905
G16	Ardahan Merkez-2	Ardahan	41.09.45 N	42.56.15 E	1905
G17	Ardahan Merkez-3	Ardahan	41.09.45 N	42.56.15 E	1905
G18	Çamlıçatak-1	Ardahan	41.07.42 N	42.49.59 E	1798
G19	Çamlıçatak-2	Ardahan	41.07.42 N	42.49.59 E	1798
G20	Sulakyurt	Ardahan	41.09.50N	42.36.59E	1920
G21	Döşeli-1	Ardahan	41.08.59 N	42.44.45 E	1815
G22	Döşeli-2	Ardahan	41.08.59 N	42.44.45 E	1815
G23	Kartalpınar	Ardahan	41.08.59 N	42.44.45 E	2063
G24	Tepeler	Ardahan	41.03.51 N	42.34.30 E	2044
G25	Oburcak	Ardahan	41.20.59N	42.52.10E	2100
G26	Seyitören	Ardahan	41.23.18 N	42.48.35 E	2224
G27	Serhat	Ardahan	41.20.31 N	42.85.11 E	1950
G28	Cumhuriyet	Ardahan	41.20.57 N	42.52.16 E	1922
G29	Burmadere	Ardahan	41.18.09 N	42.47.05 E	2044
G30	Tepeköy	Ardahan	41.20.50 N	42.48.26 E	2076
G31	Selamverdi	Ardahan	41.14.06 N	42.51.44 E	1810
G32	Ortakent	Ardahan	41.13.45 N	42.56.05 E	1819
G33	Yamçılı	Ardahan	42.20.29 N	42.80.12 E	2034
G34	Koyunpınarı	Ardahan	41.14.20 N	42.46.40 E	1860
G35	Çayağzı	Ardahan	41.11.34 N	42.51.02 E	1813
G36	Avcılar	Ardahan	41.15.02 N	42.49.33 E	1801
G37	Öncül	Ardahan	41.14.18 N	43.09.59 E	1793
G38	Aşağıcambaz	Ardahan	41.14.41 N	43.24.10 E	2044
G39	Eşmepınar	Ardahan	41.06.50 N	43.09.07 E	1889
G40	Eskibeyrehatun	Ardahan	41.07.42 N	42.57.30 E	2040
G41	Kaşlıkaya	Ardahan	41.09.38 N	43.04.12 E	1857
G42	Sazlısu	Ardahan	41.06.16 N	43.06.58 E	1913
G43	Kenarbel	Ardahan	41.11.26 N	43.09.53 E	1788
G44	Tahtakıran	Ardahan	40.30.32 N	42.35.23 E	2042
G45	Dedekılıç	Ardahan	40.51.45 N	42.33.31 E	2040
G46	Yiğitkonağı	Ardahan	40.57.16 N	42.34.04 E	2048
G47	Çayırbaşı-1	Ardahan	40.52.24 N	43.37.56 E	2045

Table 1 (continued)

Code number	Accession name	Location	Latitude	Longitude	Altitude (m)
G48	Çayırbaşı-2	Ardahan	40.52.24 N	43.37.56 E	2045
G49	Balçeşme	Ardahan	40.49.54 N	42.49.29 E	2165
G50	Subatan	Kars	40.36.12 N	43.26.07 E	1788
G51	Doğruyol	Kars	41.03.00 N	43.20.10 E	2031
G52	Paslı	Kars	40.17.05 N	42.57.32 E	1838
G53	Yolgeçmez	Kars	40.25.07 N	42.45.11 E	1898
G54	Iğdır	Kars	40.21.12 N	42.45.11 E	1815
G55	Gölbaşı	Kars	40.76.57 N	42.98.47 E	2015
G56	Yukarışallıpınar-1	Kars	40.43.63 N	42.61.42 E	2081
G57	Yukarışallıpınar-2	Kars	40.43.63 N	42.61.42 E	2081
G58	Sarvan	Giresun	40.53.03 N	38.27.19 E	266
G59	Görele-1	Giresun	41.01.54 N	39.00.04 E	24
G60	Görele-2	Giresun	41.01.54 N	39.00.04 E	24
G61	Giresun Merkez	Giresun	40.52.07 N	38.23.38 E	18

Table 2 List of 37 inter primer binding site (iPBS)-retrotransposon primers with their sequence used to elucidate genetic diversity among 61 forage pea landraces

Marker	Primers Sequences ($5^1 \rightarrow 3^1$)	Marker	Primers Sequences ($5^1 \rightarrow 3^1$)
2074	GCTCTGATACCA	2279	AATGAAAGCACCA
2077	CTCACGATGCCA	2298	AGAAGAGCTCTGATACCA
2078	GCGGAGTCGCCA	2375	TCGCATCAACCA
2079	AGGTGGGCGCCA	2377	ACGAAGGGACCA
2080	CAGACGGCGCCA	2378	GGTCCTCATCCA
2083	CTTCTAGCGCCA	2380	CAACCTGATCCA
2085	ATGCCGATACCA	2381	GTCCATCTTCCA
2095	GCTCGGATACCA	2382	TGTTGGCTTCCA
2217	ACTTGGATGTCGATACCA	2383	GCATGGCCTCCA
2219	GAACTTATGCCGATACCA	2384	GTAATGGGTCCA
2221	ACCTAGCTCACGATGCCA	2385	CCATTGGGTCCA
2225	AGCATAGCTTTGATACCA	2386	CTGATCAACCCA
2228	CATTGGCTCTTGATACCA	2389	ACATCCTTCCCA
2242	GCCCCATGGTGGGCGCCA	2390	GCAACAACCCCA
2270	ACCTGGCGTGCCA	2391	ATCTGTCAGCCA
2271	GGCTCGGATGCCA	2392	TAGATGGTGCCA
2274	ATGGTGGGCGCCA	2401	AGTTAAGCTTTGATACCA
2276	ACCTCTGATACCA	2402	TCTAAGCTCTTGATACCA
2278	GCTCATGATACCA		

(PIC) value was calculated to assess the diversity of each iPBS marker (Botstein et al. 1980). The POPGEN v.1.32 software was employed to estimate key diversity parameters, including the effective number of alleles (Ne), Nei's genetic diversity index (h), and Shannon's information index (I) (Yeh et al. 1997). The genetic structure of the genotypes was analyzed using Structure v.2.3.4 software (Pritchard et al. 2000a, b), with the optimal value of K determined using Evanno's ΔK method (Evanno et al. 2005) and the Structure Harvester approach (Earl and VonHoldt 2012). This analysis incorporated Markov chain Monte Carlo (MCMC) simulations, executed with a burn-in period of 10,000 iterations followed by 100,000 iterations under a model permitting admixture and correlated allele frequencies. The most likely

K value was visualized through a bar plot generated using the Clumpak software package (Kopelman et al. 2015).

Results

Genetic diversity of forage pea genotypes using iPBS markers

All primers utilized in this study produced clear, reproducible, and scorable bands. A total of 2529 bands were amplified and analyzed using the 37 primers employed. The number of alleles detected per primer ranged from 38 (iPBS 2385) to 155 (iPBS 2077), with an average of 68 alleles per primer. Major allele frequencies ranged from 0.597 (iPBS 2375) and 0.814 (iPBS 2391), with a mean value of 0.704. The polymorphism information content (PIC), which reflects the discriminatory power and informativeness of each marker, was calculated based on the iPBS marker data. PIC values ranged from 0.226 to 0.464, with a mean PIC value of 0.345 (Table 3).

Table 4 presents a summary of the effective number of allele (ne), Nei's genetic diversity (h), and Shannon's information index (I) for the forage pea genotypes. The highest values of ne (1.563), h (0.351), and I (0.516) were observed for the iPBS-2242 marker. In contrast, the lowest values were observed for the iPBS-2389 marker, with ne, h, and I amounting to 0.660, 0.071, and 0.111, respectively. The mean values across all markers were 1.264 for ne, 0.200 for h, and 0.299 for I.

Cluster principal coordinate analysis (PCoA) and dendrogram generated from iPBS markers

The unweighted pair-group method with arithmetic mean (UPGMA) dendrogram classified the 61 forage pea landraces into three distinct clusters. The distribution of genotypes among the clusters was as follows: Cluster I contained 27 genotypes (44.26%), Cluster II included 23 genotypes (37.7%), and Cluster III comprised 11 genotypes (18.03%). Specifically, Cluster I encompassed the following genotypes: G1, G2, G3, G4, G5, G6, G7, G8, G9, G10, G11, G12, G13, G14, G15, G16, G17, G18, G19, G20, G21, G22, G23, G24, G25, G26, and G27. Cluster II consisted of genotypes G28, G29, G30, G31, G32, G33, G34, G35, G36, G37, G38, G39, G40, G41, G42, G43, G44, G45, G46, G47, G48, G49, and G50. Finally, Cluster

Table 3 Thirty-seven inter primer binding site (iPBS) primers used in the detection of polymorphism among landraces of forage pea

Marker	An	Maf	PIC	Marker	An	Maf	PIC
2074	76	0.753	0.294	2279	111	0.720	0.312
2077	155	0.676	0.374	2298	48	0.725	0.382
2078	96	0.781	0.299	2375	63	0.597	0.374
2079	66	0.759	0.274	2377	96	0.674	0.362
2080	48	0.637	0.370	2378	66	0.727	0.371
2083	78	0.663	0.397	2380	75	0.629	0.452
2085	57	0.631	0.425	2381	66	0.636	0.425
2095	93	0.699	0.331	2382	69	0.706	0.342
2217	96	0.670	0.402	2383	63	0.606	0.464
2219	52	0.808	0.226	2384	63	0.646	0.359
2221	47	0.657	0.331	2385	38	0.693	0.310
2225	58	0.795	0.265	2386	90	0.627	0.451
2228	49	0.783	0.256	2389	54	0.652	0.377
2242	39	0.769	0.271	2390	64	0.717	0.294
2270	50	0.658	0.346	2391	75	0.814	0.242
2271	72	0.727	0.328	2392	41	0.684	0.332
2274	63	0.748	0.342	2401	42	0.752	0.345
2276	63	0.740	0.314	2402	78	0.772	0.323
2278	69	0.702	0.383	**Mean**	68	0.704	0.345

Maf, major allele frequency; An, Alelle number; PIC, polymorphism information content

Table 4 Effective number of alleles (Ne), Nei's gene diversity (h) and Shannon information index (I) based on thirty-seven iPBS loci forage pea landraces

Marker	ne	h	I	Marker	ne	h	I
2074	1.376	0.229	0.346	2279	1.196	0.254	0.374
2077	1.348	0.216	0.328	2298	1.391	0.247	0.380
2078	1.342	0.220	0.342	2375	1.338	0.209	0.317
2079	1.006	0.114	0.171	2377	1.264	0.231	0.345
2080	1.295	0.177	0.267	2378	1.414	0.242	0.359
2083	1.036	0.177	0.262	2380	1.353	0.205	0.302
2085	1.513	0.295	0.432	2381	1.396	0.233	0.346
2095	1.378	0.233	0.341	2382	1.403	0.239	0.358
2217	1.421	0.245	0.361	2383	1.038	0.143	0.214
2219	1.411	0.244	0.363	2384	1.474	0.262	0.378
2221	1.032	0.172	0.265	2385	1.449	0.254	0.372
2225	1.188	0.116	0.176	2386	1.404	0.240	0.357
2228	1.305	0.176	0.260	2389	0.660	0.071	0.111
2242	1.563	0.351	0.516	2390	0.936	0.086	0.130
2270	1.043	0.182	0.267	2391	1.212	0.133	0.205
2271	1.263	0.225	0.332	2392	1.416	0.240	0.355
2274	1.291	0.172	0.258	2401	1.351	0.218	0.334
2276	1.290	0.183	0.279	2402	1.173	0.109	0.167
2278	0.791	0.076	0.112	**Mean**	1.264	0.200	0.299

III included genotypes G51, G52, G53, G54, G55, G56, G57, G58, G59, G60, and G61 (Fig. 2).

Principal coordinate analysis (PCoA) was employed to visualize the genetic relationships among the forage pea genotypes, revealing three clearly defined clusters. Genotypes originating from the Erzurum and Bayburt regions predominantly clustered in the lower-right quadrant of the two-dimensional coordinate plot. Most genotypes from the Ardahan region were concentrated in the upper-left quadrant, with additional genotypes positioned in the lower-right quadrant, and two genotypes located in the upper-right quadrant. Of the eight genotypes collected from the Kars region, seven were clustered in the lower-left quadrant, while one genotype was situated in the upper-left quadrant. Genotypes collected from Giresun in the Eastern Black Sea region were all clustered in the lower-left quadrant of the coordinate axis (Fig. 3). The first three principal coordinates of the analysis explained 18.26%, 9.58%, and 3.87% of the total genetic variation, respectively, accounting for a cumulative 31.71% of the total diversity (Table 5). Cluster I included genotypes G1, G2, G3, G4, G5, G6, G7, G8, G9, G10, G11, G12, G13, G14, G15, G16, G17, G18, G19, G20, G21, G22, G23, G24, G25, G26 and G27, all of which were collected from the Erzurum and Bayburt regions. Cluster II consisted of genotypes G28, G29, G30, G31, G32, G33, G34, G35, G36, G37, G38, G39, G40, G41, G42, G43, G44, G45, G46, G47, G48, G49 from Ardahan and G50 from Giresun. The total genetic variation was predominantly observed within populations (74.00%), while 26.00% of the variation occurred between populations (Table 6).

Population genetic structure analysis for iPBS markers

To investigate the population structure of the 61 forage pea genotypes, a model-based approach was employed using the STRUCTURE software, which assigned each genotype to its specific subpopulations. The optimal value of K was determined by calculating the ΔK statistic. The genetic structure analysis indicated that the optimal value of K was 4, corresponding to four subgroups represented by the colors red (A), green (B), blue (C), and yellow (D) (Fig. 4a). At K = 4, Group I comprised 16 genotypes of forage pea landraces: G6, G7, G4, G10, G5, G11, G9, G8, G13, G2, G12, G3, G14, G1, G16, and G15. Group II included 11 genotypes: G55, G57, G59, G58, G60, G56, G54, G53, G61, G52, and G51. Group III

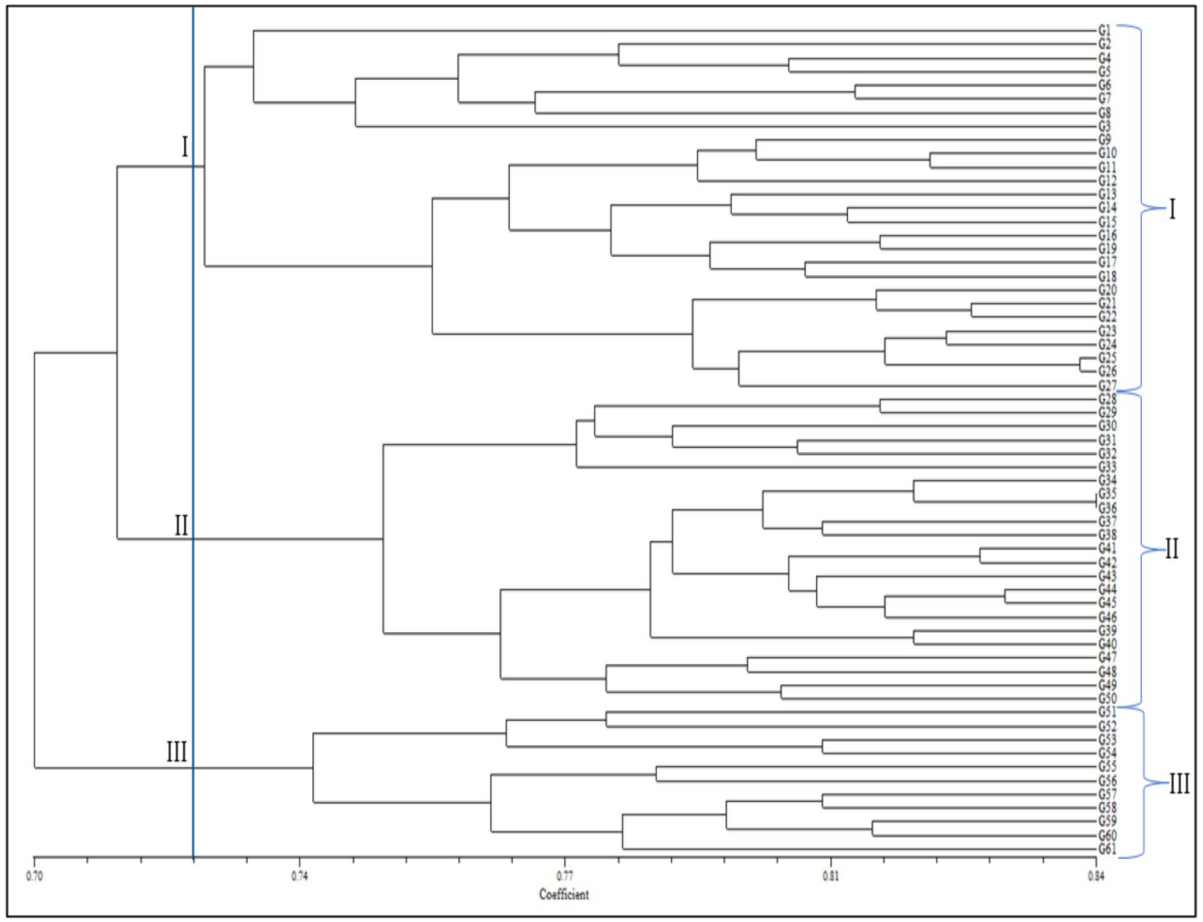


Fig. 2 Dendrogram of 61 forage pea landraces generated with data from 37 inter primer binding site (iPBS) primers

contained 22 genotypes: G44, G36, G41, G45, G38, G42, G43, G34, G35, G39, G40, G46, G37, G32, G33, G48, G47, G50, G30, G31, G49, and G29. Finally, Group IV consisted of 12 genotypes: G22, G23, G21, G25, G26, G20, G24, G19, G27, G18, G17, and G28 (Fig. 4b). The membership coefficients for each genotype in the four inferred subpopulations are presented in Supplementary Table ST1. Although STRUCTURE was initially designed to detect allelic fixation as a result of evolutionary processes, the subpopulations of forage pea displayed varying levels of genetic differentiation, as quantified by in F_{ST} values. This analysis aimed to investigate the correlation between alleles at various hierarchical levels within the structured population. The F_{ST} values for the first through fourth subpopulations were calculated as 0.2117, 0.2173, 0.2849, and 0.3280, respectively, indicating varying degrees of genetic differentiation. Additionally, the expected heterozygosity (He) values for these subpopulations were 0.2882, 0.3094, 0.2428, and 0.2404, respectively (Table 7).

Discussion

Assessing genetic diversity within plant germplasm is essential for the efficient development and management of plant breeding programs (Salgotra and Chauhan 2023). Molecular markers, such as iPBS, serve as powerful tools for evaluating genetic diversity and analyzing relationships among genotypes. These markers offer a significant advantage for parental selection, as they are not influenced by environmental factors. In addition to their role in genetic

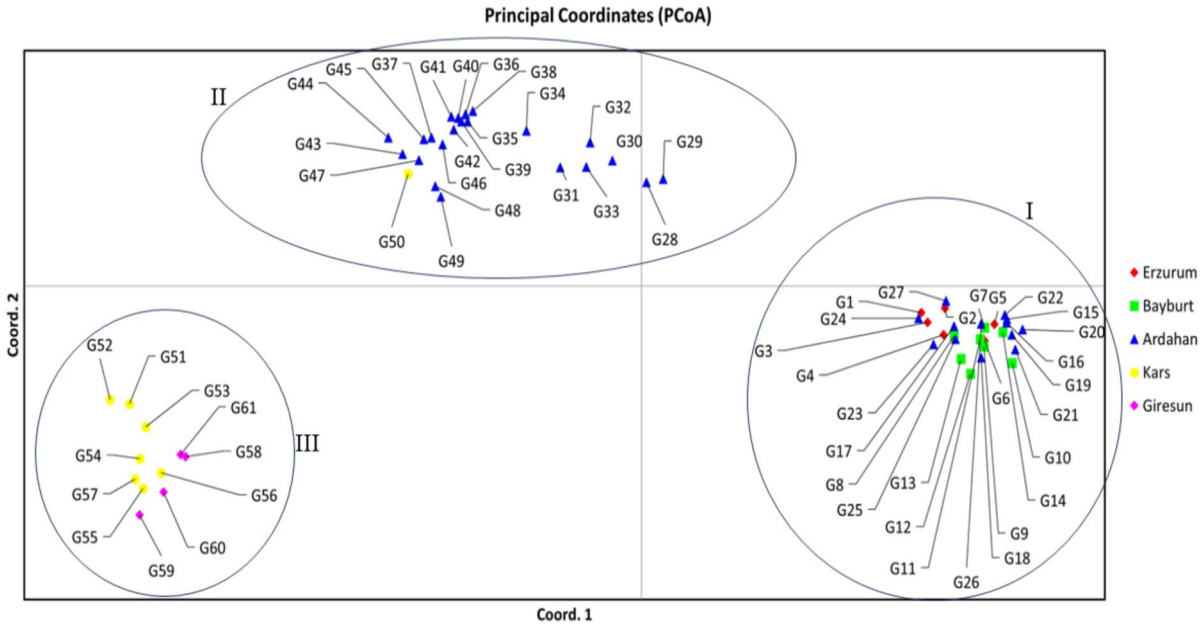


Fig. 3 Principal coordinates analysis (PCoA) calculated from the pooled data of 37 inter primer binding site (iPBS) primers in 61 forage pea genotypes

Table 5 Principal coordinate analysis (PCoA) of forage pea landraces

Axis	1	2	3
%	18.26	9.58	3.87
Cum %	18.26	27.84	31.71

diversification, the activation of retrotransposons has been recognized as a critical mechanism in facilitating host genomes adaptation to environmental changes (Baloch et al. 2023).

In this study, polymorphic iPBS markers were employed to discriminate among forage pea genotypes at the molecular level. These markers provided valuable insights into the genetic relationships among forage pea genotypes. The data derived from iPBS markers demonstrated their efficiency and reliability for studying genetic diversity and conducting analyses in forage pea genotypes. Although no previous studies have employed iPBS markers specifically in forage pea (*Pisum sativum* var. *arvense* L.), these markers have been successfully used in related species within the Fabaceae family, including beans (Haliloğlu et al. 2022), *Cicer* species (Andeden et al. 2013), cowpea (Baloch et al. 2023), lentil (Rey-Banos et al. 2017), pea (Baloch et al. 2015), and soybean (Kumlay et al. 2021). For example, a study investigating genetic diversity among 138 pea genotypes from different regions in Turkey employed 12 iPBS primers, yielding 106 scorable bands (Baloch et al. 2015). Similarly, Andeden et al. (2013) used 10 iPBS primers in *Cicer* species, resulting in 130 polymorphic bands. In another study, 6 iPBS markers were used to analyze 12 soybean varieties, producing 49 bands (Kumlay et al. 2021). In the present study, the

Table 6 Analysis of molecular variance (AMOVA) of forage pea landraces using inter primer binding site (iPBS) marker

Source	Degree of Freedom (DF)	Sum of Squares (SS)	Variance Component	%	*p*-Value
Among Pops	4	2669.181	53.928	26.00%	0.258
Within Pops	56	8703.442	155.419	74.00%	0.001
Total	60	11,372.623	209.347	100.00%	

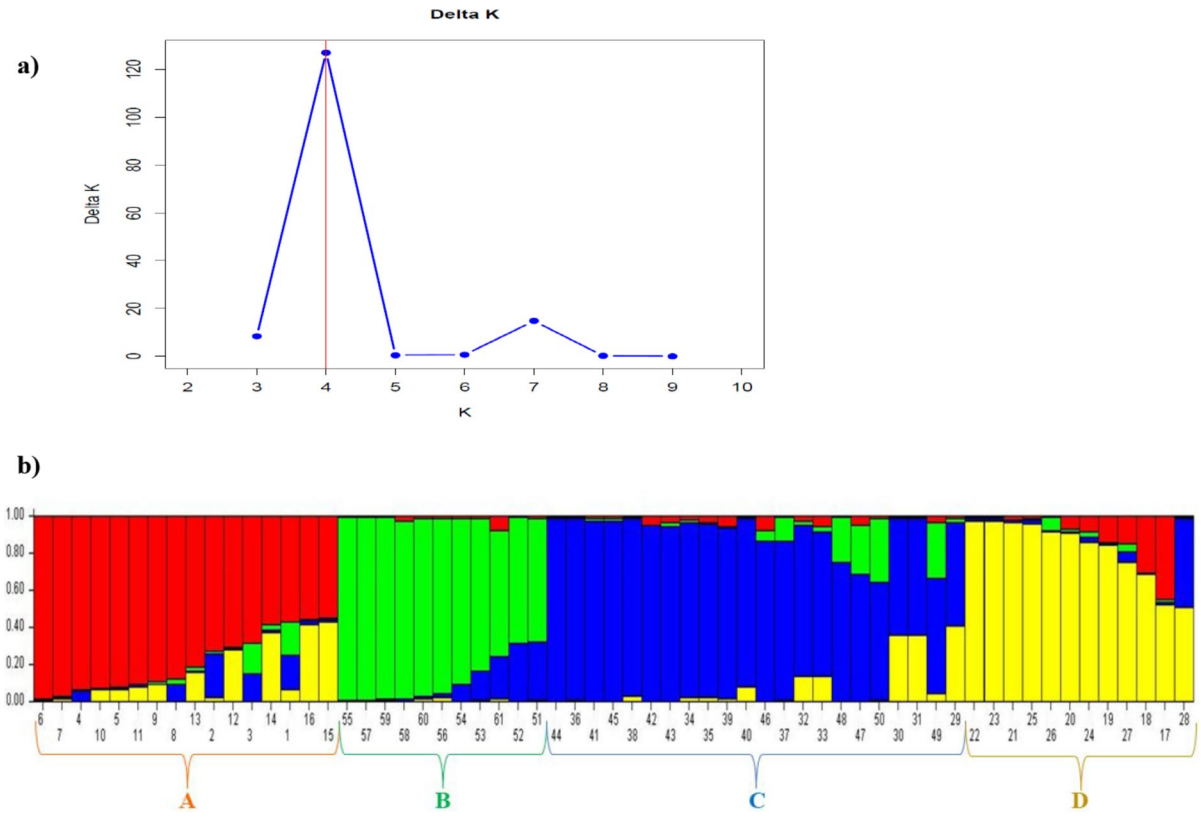


Fig. 4 **a** The Delta K values and **b** the population structure pattern suggests the existence of four distinct subpopulations among forage pea landraces based on the inter primer binding site (iPBS)-retrotransposon marker system

use of 37 iPBS primers resulted in the detection of 2529 bands. This difference can likely be attributed to the higher number of markers employed and the presence of greater genotypic variation in the current study. The mean major allele frequency (Maf) was 0.704, with values ranging from 0.597 (iPBS 2375) to 0.814 (iPBS 2391). In a study on beans using iPBS markers, the Maf values ranged from 0.528 to 0.888, with an average of 0.706 based on 26 iPBS markers (Haliloğlu et al. 2022). These results are consistent with the findings of the current study. The polymorphism information content (PIC), a key parameter for evaluating the informativeness of polymorphic loci and the discriminatory capacity of primers (Kadri et al. 2024), ranged from 0.226 (iPBS 2219) to 0.464 (iPBS 2383), with a mean of 0.34. This mean PIC value is lower than the 0.460 mean PIC value reported for 35 pea samples analyzed with 15 SSR markers (Ahmad et al. 2012), but comparable to the mean PIC value of 0.32 observed in pea samples from the Czech Republic using RBIP retrotransposon markers (Cieslarová et al. 2012). Another study on pea varieties reported PIC values ranging from 0.10 to 0.75, with a mean of 0.52 for 25 pea varieties analyzed with SSR markers (Smýkal et al. 2008), which was higher than the mean PIC value obtained in the present study.

Table 7 Expected heterozygosity (He) and Fst values in four forage pea landraces subpopulations

Subpopulation (K)	Expected heterozygosity (He)	Fst
1	0.2882	0.2117
2	0.3094	0.2173
3	0.2428	0.2849
4	0.2404	0.3280
Mean	0.2702	0.2605

The average values for the effective number of alleles (Ne), Nei's genetic diversity (h), and

Shannon's information index (I) among forage pea genotypes were 1.264, 0.200, and 0.299, respectively. In their study using iPBS markers in pea (*Pisum sativum* L.), Baloch et al. (2015) reported h and I value of 0.26 and 0.39, respectively. Similarly, in an investigation utilizing 24 iPBS markers on fenugreek, another member of the Fabaceae family, the values were reported as 1.679 (Ne), 0.403 (h), and 0.592 (I) (Haliloğlu et al. 2024). The discrepancies in diversity indices across studies may be attributed to variations in genotypes, species, and the molecular markers employed.

Clustering analysis based on the Dice similarity coefficient and the unweight pair group method with arithmetic means (UPGMA) grouped the forage pea genotypes into three distinct groups. The results highlight the influence of geographical distribution on genetic diversity, underscoring the importance of geographical location in shaping genetic variation. For example, Handerson et al. (2014) classified 34 pea genotypes into five groups using SSR markers, while Sharma et al. (2022) grouped 56 garden pea (*Pisum sativum* L.) genotypes into six clusters using SSR markers. These findings differ from the current study, where 138 pea genotypes were divided into three clusters using iPBS markers, consistent with the clustering pattern observed in the present study (Baloch et al. 2015).

Principal coordinate analysis (PCoA) was conducted to further assess genetic diversity based on both quantitative and qualitative traits. PCoA scales distance data onto multidimensional planes to effectively characterize diversity patterns (Haliloğlu et al. 2022), and offers a spatial visualization of the relative genetic distances among populations (Klaedtke et al. 2016). In the present study, the first three axes of the PCoA accounted for 31.71% of the total variation, a proportion considered reliable given that the cumulative variance explained exceeds the commonly accepted threshold of 25%. Analysis of molecular variance (AMOVA) indicated that 74% of the total genetic variation was attributable to differences among genotypes, while the remaining variation was observed within populations. Such genetic differentiation may be driven by evolutionary forces including selection, gene flow, genetic drift, ecotypic differentiation, and pollination mechanisms (Karagoz et al. 2020).

Based on genetic structure, the forage pea genotypes were grouped into four distinct subpopulations. Over evolutionary time, factors as mutation, recombination, genetic drift, and natural selection have contributed to the observed genetic diversity. The study of population structure has garnered increasing attention for its ability to identify suitable parent lines and marker-trait associations. Population structure analysis also elucidates genetic similarities among genotypes and subpopulations. For instance, Sharma et al. (2022) divided garden pea genotypes into four subpopulations ($K=4$). Similarly, a previous study classified pea genotypes into three subpopulations (Rana et al. 2017).

The inbreeding coefficient (F_{ST}), which measures genetic differentiation among subpopulations relative to the total population, is a key indicator of genetic diversity (Verma et al. 2019). In the present study, an F_{ST} value of 0.2605 was observed, indicating a high level of genetic differentiation among the subpopulations. This degree of differentiation is likely a result of the genotypes being sampled from ecologically diverse environments. According to Wright (1984), F_{ST} values of ≥ 0.25 indicate significant differentiation, values between 0.15 and 0.25 indicate moderate differentiation, and values below 0.05 suggest minimal differentiation.

Conclusions

This study represents the first comprehensive molecular characterization of local forage pea genotypes from the northeastern Anatolia region of Turkey using the iPBS-retrotransposon marker system. The findings revealed a high degree of genetic diversity and well-defined population structure among the accessions, highlighting the potential of these native genotypes as valuable genetic resources for breeding and conservation efforts. The iPBS markers demonstrated high informativeness, marked polymorphic capacity, and strong discriminatory power, affirming their suitability for germplasm evaluation and management. Moreover, the study underscores the critical role of local landraces in breeding programs, given their considerable intra- and inter-population variability. Such genetic diversity is essential for addressing future challenges in crop improvement, particularly in the context of climatic change and environmental

conditions. The integrating of these molecular insights into marker-assisted selection and breeding strategies is expected to facilitate the development of improved forage pea cultivars with enhanced adaptability and resilience. Future research should expand the scope of germplasm sampling across broader geographical regions and explore associations with key agronomic traits to further enhance the utility of native forage pea resources in sustainable breeding programs.

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Data availability All the data has been given in supplementary information.

Declarations

Conflict of interest The authors declare no competing interests.

Ethical approval Not applicable.

Consent to participate All authors agree on their participation in this paper.

Consent for publication All authors agree to be coauthors in the order submitted.

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