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RESEARCH ARTICLE

Selection of parental forage pea [*Pisum sativum* (L.)] genotypes based on morphological characterization and SSR markers for crossbreeding

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Abstract In crossbreeding, the greater the genetic distance between the parental genotypes, the greater the genetic variation between the hybrids. In this study, genetic diversity among 23 parental forage pea genotypes was assessed by phenotypic and molecular characterization. For morphological characterization, unweighted pair group method with arithmetic mean (UPGMA) similarity dendrogram was generated using the following measurements: plant height (cm), number of pods per plant, number of seeds per pod, and number of seeds per plant. The dendrogram divided the pea genotypes into two main groups, each with two subgroups. For molecular characterization, 16 simple sequence repeat (SSR) markers were tested of which 14 markers were polymorphic and 2 markers were monomorphic among the genotypes. A total of 106 alleles were obtained and the number of alleles per primer was changed between 4 and 10 with an average of 7.57. The observed heterozygosity (Ho) was 0.00 for all SSR primers and expected heterozygosity (He) differed from 0.64 to 0.90 with a mean of 0.84. Polymorphism information content (PIC) values ranged from 0.55 to 0.87 and the average PIC value was calculated to be 0.78. The primers were found to be highly informative for peas genome. The similarity coefficient values between the genotypes ranged from 0.00 to 0.80. Both morphological and molecular research revealed significant variation among pea genotypes. In conclusion, both morphological and molecular research showed that there is significant variation among the genotypes and can be used as parents in further research.

Keywords Crossbreeding · Forage · Genetic diversity · Pea · SSR

Abbreviations

AFLPs	Amplified fragment length polymorphism
PIC	Polymorphism information content
RAPDs	Random amplified polymorphic DNA
RFLPs	Restriction fragment length polymorphism
SSR	Simple sequence repeat
UPGMA	Unweighted pair group method with arithmetic mean

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Introduction

The production of high-yielding and high-quality forage crops is essential for livestock. High-yielding and high-quality crops can be developed through plant breeding (Bradshaw 2017). Forage legume crops such as forage peas are a valuable source of protein (Castro-Montoya and Dickhoefer 2020). They are suitable for use as silage and green manure in animal nutrition. The production of forage crops can be increased by the development of new cultivars. Crossbreeding is one of the methods used in cultivar development. When distantly related parents are crossed, high heterosis and wide genetic diversity occur, which provides effective breeding (Singh and Mishra 2008). Genetic diversity in progeny is generally greater when the parents are more genetically distinct, as this increases the number of loci that segregate (Hung et al. 2012). Understanding the genetic traits and gene effects of the parents is important for choosing the most suitable breeding method (Bisht and Singh 2011).

Previously, morphological characterization was effectively used as the primary stage for developing high-yielding cultivars. However, morphological traits are strongly influenced by environmental conditions (Kumar et al. 2023). Nowadays molecular characterization is used more effectively than morphological characterization to identify genetic differences between genotypes in breeding programs (Arul and Selvakumar 2019).

In molecular research with peas, DNA-based molecular markers such as restriction fragment length polymorphism (RFLPs) (Lu et al. 1996); random amplified polymorphic DNA (RAPDs) (Yadav et al. 2007; Ahmad et al. 2010) and amplified fragment length polymorphism (AFLPs) (Simioniuc et al. 2008) have been successfully used for cultivar identification. In recent years, simple sequence repeat (SSR) markers have been a valuable tool for assessing genetic diversity in pea cultivars, offering high polymorphism and co-dominant characteristics. (Reis and Diogo 2012; Adhikari et al. 2018).

Successful identification of genetic diversity is essential for the development of new cultivars in effective breeding programs. Various investigations conducted by Handerson et al. (2014) and Mohamed et al. (2019) have emphasized the importance of combining morphological traits with molecular characterization to achieve more accurate and reliable genetic diversity analyses in forage peas. This study aimed to examine the morphological traits of 23 forage pea genotypes from different origins, analyze their genetic diversity using morphological and SSR markers, and identify potential parent genotypes for developing new forage pea cultivars in future breeding research.

Materials and methods

Plant material

In present study, 23 forage pea genotypes were analysed, including eighteen genotypes originated from Turkey, two from the USA and the remaining genotypes from Poland, Hungary and Bulgaria (Table 1). The study was arranged in the Randomized Complete Plot Design with three replications containing three

Table 1 Origins, leaf shapes and flower colors of pea genotypes used in the study

Genotype Name	Origin	Leaf Shape	Flower Color
G1	Poland	Semi-Leafless	Pink
G2	Hungary	Leaved	White
G3	Bulgaria	Leaved	White
G4	Turkey	Leaved	White
G5	USA	Leaved	White
G6	USA	Leaved	Pink
G7	Turkey	Leaved	Purple
G8	Turkey	Leaved	White
G9	Turkey	Semi-Leafless	White
G10	Turkey	Leaved	Purple
G11	Turkey	Leaved	Purple
G12	Turkey	Leaved	White
G13	Turkey	Semi-Leafless	Pink
G14	Turkey	Leaved	Purple
G15	Turkey	Leaved	Purple
G16	Turkey	Leaved	Purple
G17	Turkey	Leaved	Purple
G18	Turkey	Leaved	Purple
G19	Turkey	Semi-Leafless	Purple
G20	Turkey	Leaved	White
G21	Turkey	Leaved	Purple
G22	Turkey	Leaved	Purple
G23	Turkey	Leaved	Purple

plants per replicate. The seeds of 23 forage pea genotypes were sown in pods in an unheated greenhouse condition in the Department of Field Crops, Faculty of Agriculture, Bursa Uludag University. A mixture of soil and peat (3:1) was used for the growth medium of potted plants. The experiment was conducted in 2021–2022.

Morphological characterization

Morphological characterization of 23 forage pea plants was performed with 10 randomly selected plants per genotype. Four morphological traits including plant height, number of pod per plants, number of seeds per pod, and number of seeds per plant were observed according to International Union for the Protection of New Varieties of Plants (UPOV) guidelines for pea (UPOV 2010).

Molecular characterization

DNA samples were obtained from 25 mg lyophilized and fine powdered young leaves according to the modified cetyltrimethylammonium bromide (CTAB) method (Fütterer et al. 1995). DNA concentrations carried out by using a Quibit fluorometer (Invitrogen, USA) and diluted to 50–150 ng/µL. Amplification reactions were conducted in a final volume of 20 µL using a template DNA concentration of 50 ng, 1X reaction buffer, 1.0 U Taq DNA polymerase (Thermo Scientific, USA), and 0.10 µM of M13 sequence tailed forward primer. Additionally, 0.20 µM of the reverse primer, 0.20 µM of an M13 primer labeled with infrared dye at 700 nm or 800 nm (Licor, USA) and 0.25 mM of dNTPs were included. (Schuelke 2000). Amplifications were performed on a Veriti 96-well thermal cycler (Applied Biosystems, USA) with the following profile: 2 min at 94 °C; 6 cycles of 40 s at 94 °C, 1 min at 60 °C (annealing temperature was reduced 1 °C after every cycle for touchdown PCR protocol), 1 min at 72 °C; 28 cycles of 40 s at 94 °C, 1 min at 55 °C and 1 min and 30 s at 72 °C; 7 cycles of 45 s at 94 °C, 1 min at 54 °C and 1 min and 30 s at 72 °C and final extension step of 10 min at 72 °C. Polyacrylamide gels (7.5%) were used to separate the PCR products at 30 W for three hours using a Li-COR 4300 DNA analyser (Fig. 1).

Data analysis

JMP 13.0 software was used for statistical analysing morphological data and statistical groups were calculated Fisher's least significant difference (LSD) at a probability level of 0.05 (Steel and Torrie 1960). Genetic diversity based on morphological and molecular characterization was analysed by NTSYSpc v2.21 program (Exeter Software, NY, USA). Cluster analyses were performed by using the unweight pair group method with arithmetic averages (UPGMA) method of SAHN based on the Euclid Coefficient of dissimilarity for morphological characterization and Dice similarity for molecular characterization (Sneath and Sokal 1973).

The polymorphism information content (PIC) values for SSR markers were calculated according to the method of Botstein et al. (1980). Expected heterozygosity (He) and observed heterozygosity (Ho) values were calculated using POPGEN32 software v.1.32 (Yeh et al. 1997) according to the method of Levene (1949) (Table 2). The population structure was revealed using

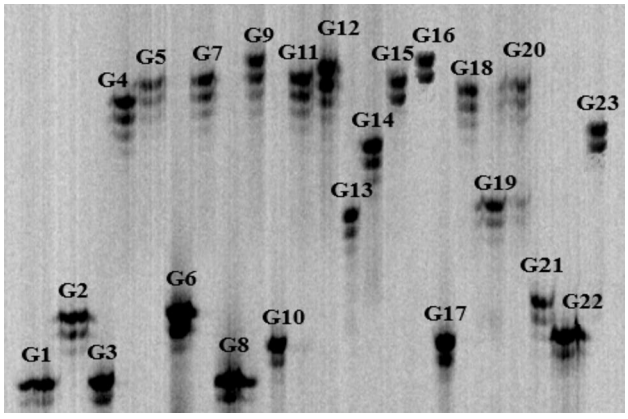


Fig. 1 Polyacrylamide gel picture demonstrating the alleles of AA122 simple sequence repeat (SSR) marker among 23 pea genotypes

Table 2 Sequences of 16 simple sequence repeat (SSR) primers, allele numbers, observed heterozygosity (Ho), expected heterozygosity (He) values and polymorphism information content (PIC)

SSR Markers	Sequence (5′–3′)	Number of allels	Ho	He	PIC
AF004843	F: ccatttctggttatgaaaccg R: ctgttcctcattttcagtggg	5	0.00	0.76	0.71
AA430902	F:ctggaattcttgcggtttaac R:cgttttggttacgatcggcta	4	0.00	0.64	0.55
AA205	F: gctacgcaatcatagagtttggaa R: aatcaagtcaatgaaacaagca	10	0.00	0.89	0.87
A9	F:gtgcagaagcatttgttcagat R: cccacatatatttggttggtca	4	0.00	0.78	0.62
AA355	F:agaaaaattctagcatgatactg R: ggaaatataacctcaataacaca	8	0.00	0.85	0.81
AB53	F:cgtcgttgttgccggtag R: aaacacgtcatctcgacctgc	9	0.00	0.88	0.84
AD61	F:ctcattcaatgatgataatccta R: atgaggtacttgtgtgagataaa	8	0.00	0.88	0.85
AD73	F:cagctggattcaatcattggtg R: atgagtaatccgacgatgcctt	10	0.00	0.89	0.86
PSGAPA1	F:gacattgttgccaataatgg R: ggttctgttctcaatacaag	7	0.00	0.83	0.80
A5	F:gtaaagcataaggggttctcat R: cagcttttaactcatctgaca	8	0.00	0.83	0.77
AB25	F:ttttcactcaaaacactcggct R: gatgccattgctgaaggagatt	7	0.00	0.80	0.76
AA170	F:gtgcacaaggtgaaatgaatga R: ttgatgagttaaatcccgaagg	9	0.00	0.88	0.85
AA122	F:gggtctgcataagtagaagcca R: aaggtgtttcccctagacatca	9	0.00	0.88	0.86
AD146	F:tgctcaagtcaatatatgaaga R: caagcaaatagttgttttgtta	8	0.00	0.90	0.85
AA446	F: ttagcttgcagcccactc R: atccgacccatggattta	Monomorphic	–	–	–
AB72	F: atctcatgttcaacttgcaaccttta R: ttcaaaacacgcaagttttctga	Monomorphic	–	–	–
Total		106			
Mean		7.57	0.00	0.84	0.78

Bayesian clustering model with STRUCTURE v.2.3.4 software (Pritchard et al. 2000). The range of possible cluster numbers (K) was set between 1 and 12, with five replications for each K value. Each independent run had a burning period of 100.000 steps followed by 100.000 Monte Carlo Markov Chain (MCMC) replications. The most probable number of subpopulations (K) was assessed by the method described by Evanno et al. (2005).

Results and discussion

Morphological characterization

In greenhouse experiments, plant height, number of pods per plant, number of seeds per pod, and number of seeds per plant were measured for all forage pea genotypes. The variance analysis results showed that the differences between genotypes

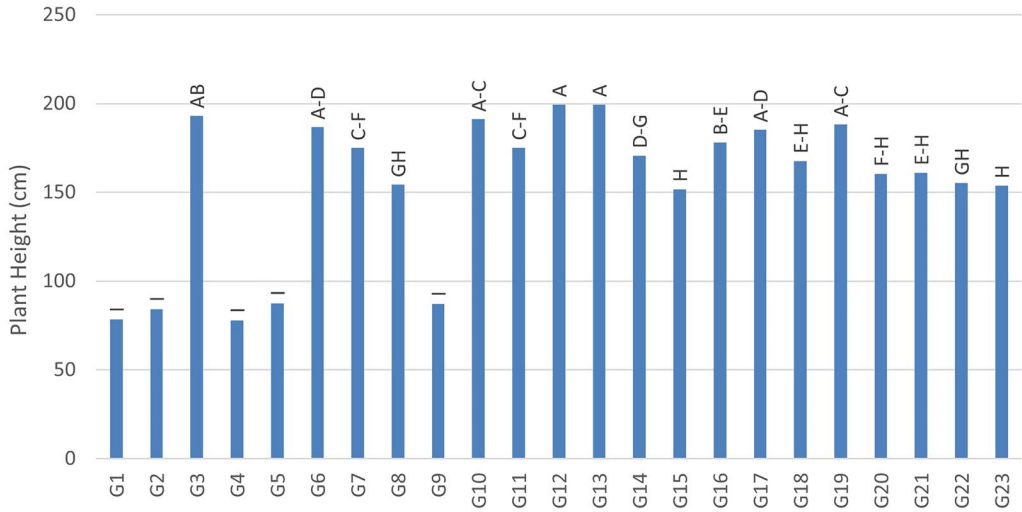


Fig. 2 Average values of plant height in forage pea genotypes

were significant at the 0.05 probability level for all characteristics. The means and statistical groups of the characteristics for the genotypes are given in Fig. 2. The plant height ranged between 77.92 and 199.50 cm and the mean value was 154.86 cm. The lowest plant height values were in G4 (77.92 cm), G1 (78.33 cm), G2 (84.25 cm), G9 (87.17 cm) and G5 (87.56 cm) genotypes. The plant heights were higher in G13 (199.50 cm), G12 (199.47 cm), G3 (193.11 cm), G10 (191.17 cm), G19 (188.21 cm), G6 (186.75 cm) and G17 (185.25 cm) than the other genotypes (Fig. 2). Several investigations on peas have reported that plant height values range from 51.68 to 192.80 cm (Krga et al. 2019; Meena et al. 2022; Singh and Prakash 2022). The plant height range observed in this study aligns with the values reported by these researchers. Plant heights in peas vary depending on genotype characteristics, the location of the experiment, and climatic conditions. Krizmanić et al. (2020) reported that plant height

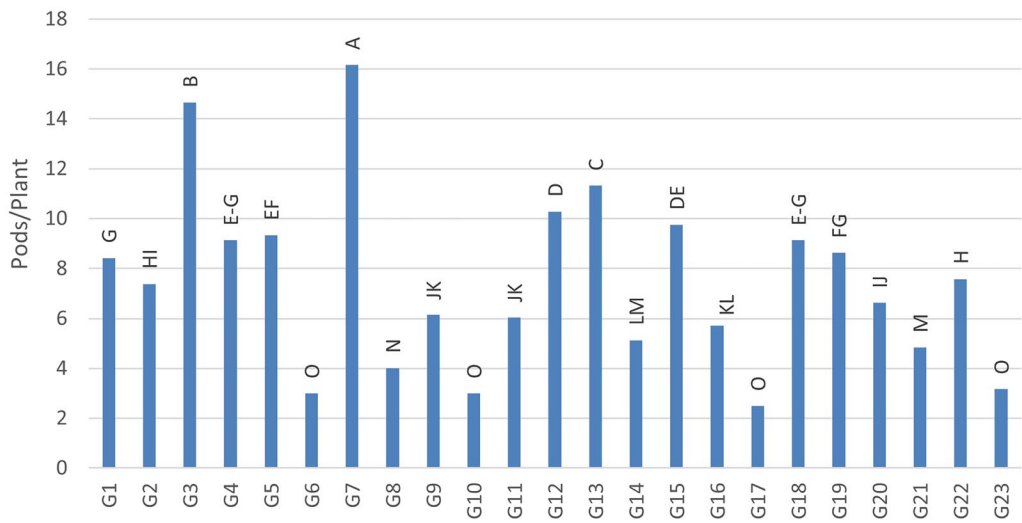


Fig. 3 Average values of the number of pods per plant in forage pea genotypes

is important in developing a productive and high-quality forage pea cultivar. The number of pods per plant ranged between 2.50 and 16.17 (average 7.47). The lowest values were in G17 (2.5), G6 (3.00), G10 (3.00), G23 (3.17) and the highest value was in G7 (16.17) (Fig. 3). In previous research conducted with different pea genotypes across different years and locations, the number of pods per plant was reported to range from 2.60 to 40.80 (Jiang et al. 2020; Kadıoğlu et al. 2020; Sayed and Ouis 2022; Sharma et al. 2022a; Ton et al. 2022). Kosev (2014) stated that the number of pods per plant is important in increasing yield. The number of seeds per pod ranged 1.33–4.67 (average 3.03). The lowest and highest values were in G10 (1.33) and G11 (4.67) genotypes, respectively (Fig. 4). Some researchers have reported that the number of seeds per pod ranges from 2.50 to 8.54 (Prasad et al. 2019; Haq and Ahmed 2021; Meena et al. 2022; Singh and Prakash 2022).

The number of seeds per plant ranged between 5.33 and 44.50 (average 19.44). The lowest values were in G23 (5.33), G21 (6.21) and G17 (7.00). G7 genotype (44.50) was higher than the other genotypes (Fig. 5). Uzun et al. (2005) and Mart (2022) stated in their research that the number of seeds per plant ranged from 1.70 to 118.00. Many researchers in forage peas reported that the number of seeds per pod, the number of pods per plant and important in developing a productive and high-quality cultivar (Kosev 2014; Georgieva et al. 2016; Krizmanić et al. 2020).

In the study, based on the observations, the genotypes were grouped based on early, mid, and late flowering characteristics, and they were also evaluated in terms of leaf types and petal colors (Table 1). Early flowering genotypes were G1, G2, G3, G4, G5, G6, G7, G9, G11, G12, G13, G15, G16, G18, G19, G20 (about 120 days) and late flowering genoypes were G8, G10, G21, G2 (about 210 days). The other genotypes (G17, G23, G14) were investigated to be mid-flowering genotypes. By crossing early and late flowering genotypes, breeders can develop new cultivars that increase genetic diversity and have a broad adaptive capacity. Genetic diversity research emphasizes that flowering time plays a critical role in the management of genetic resources (Smith and Whitelam 2004; Buckler et al. 2009). According to leaf and petal color of the plants, four genotypes were semi-leafless (G1, G9, G13 and G19) whereas the remaining nineteen genotypes were normal-leaved. Besides, eight genotypes had white petals (G2, G3, G4, G5, G8, G9, G12 and G20) while pink petals were observed only in three genotypes (G1, G6 and G13). The remaining twelve genotypes had purple petals.

The relationship among the genotypes was estimated using morphological characters (plant height,

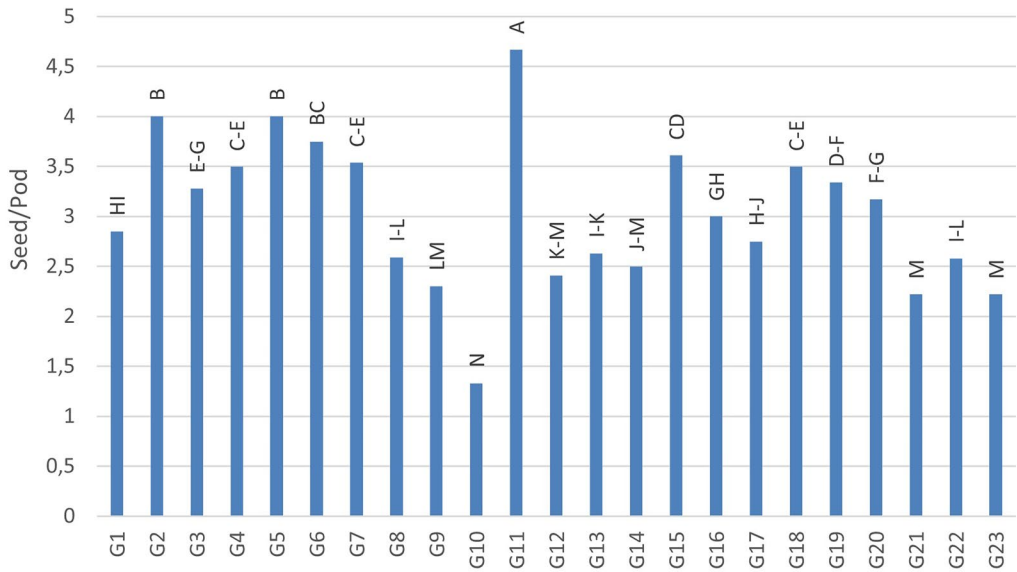


Fig. 4 Average values of the number of seeds per pod in forage pea genotypes

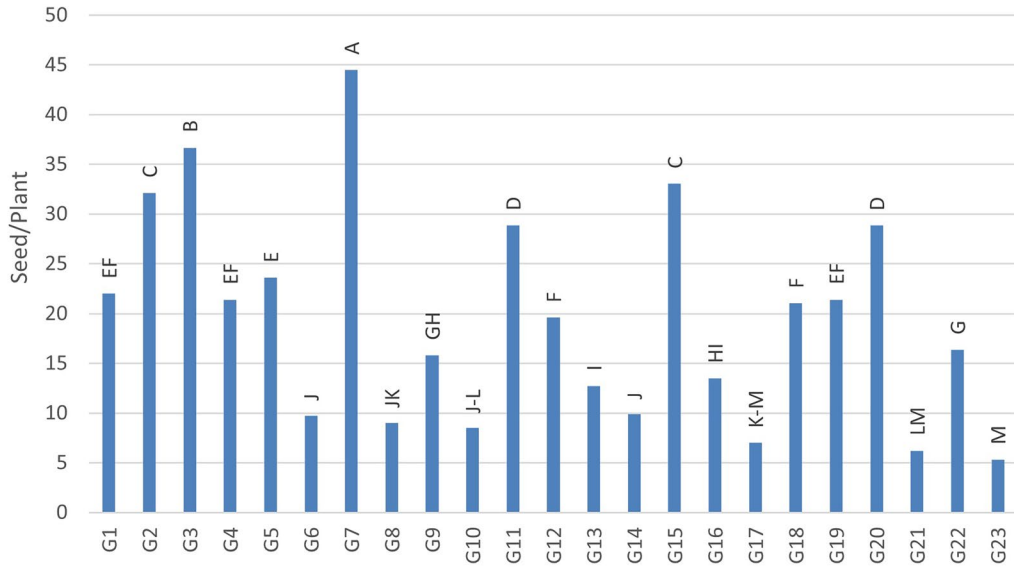


Fig. 5 Average values of seed number per plant in forage pea genotypes

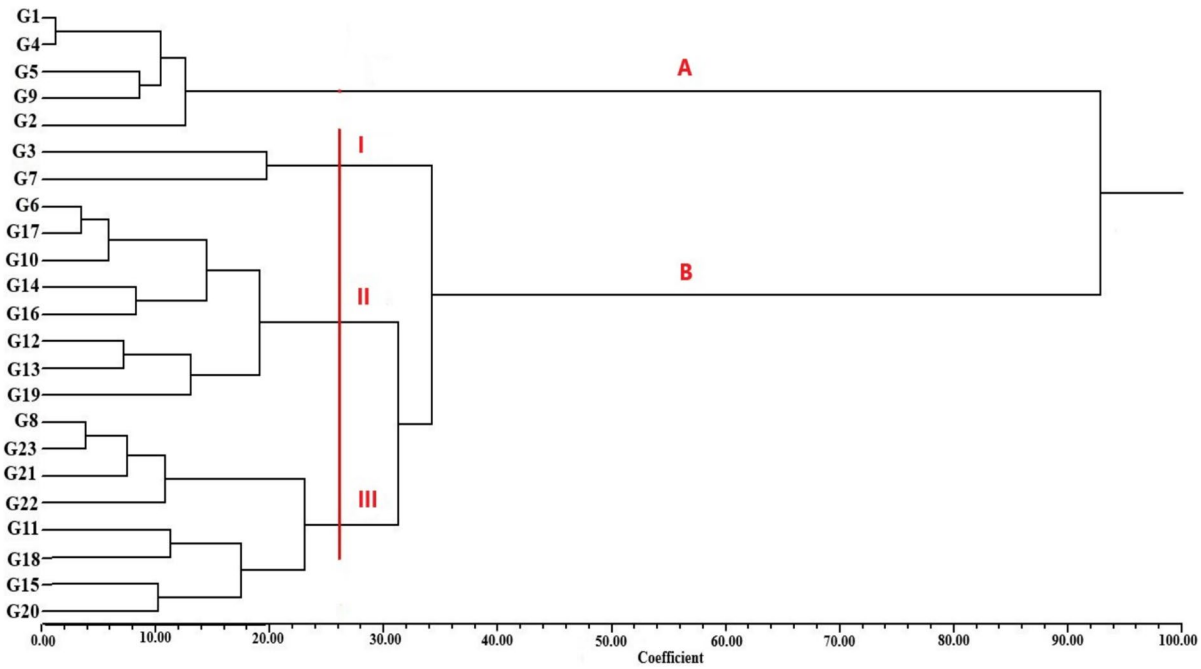


Fig. 6 Dendrogram showing genetic dissimilarities of 23 forage pea genotypes constructed using the unweighted pair group method with arithmetic mean (UPGMA) based on morphological data

number of pods per plant, number of seeds per pod, and number of seeds per plant) and a UPGMA dendrogram was constructed. The dendrogram was also interpreted by considering the genotypes' flowering time, leaf shape, and petal color. Based on Euclidean dissimilarity matrix, two main clusters were observed

in the dendrogram (A and B) (Fig. 6). Cluster A consisted of only five genotypes and Cluster B included three further subgroups; Group I, Group II and Group III which included two, eight and eight genotypes, respectively. Dissimilarity index was calculated using morphological data based varied from 1,28 to 121,93 with an average of 63,60 demonstrating high variation in this study. Among forage pea genotypes, the lowest dissimilarity was observed between the genotypes G1 and G4; the highest dissimilarity was found between G13 and G4 (Table 3). Singh et al. (2021) reported that genetic Euclidean distance (ED) based on morphological data of 47 pea genotypes differed from 25.68 to 195.17.

All forage pea genotypes which were shorter than 100 cm and early flowering (G1, G4, G5, G9 and G2) placed in Cluster A (Figs. 2 and 6). All genotypes in Group I were normal leaved and longer than 170 cm (Table 1, Fig. 6). Besides, they had the average values of the number of per pod and the seed number per plant higher than 14,0 and 35,0 respectively (Figs. 2, 4 and 5). Group II mostly consisted of normal leaved and purple petal genotypes as well as they were also longer than 170 cm (Table 1, Figs. 2 and 6) All late flowering genotypes in this study were clustered in Group II (G10 and G17). Majority of genotypes in Group III had purple petals and they were normal leaved (Table 1 and Fig. 6). In previous research, Hanci (2019) clustered 132 pea accessions into two distinct clusters based on morphological analyses such as flowering time and plant height. According to the results, late-flowering accessions clustered in the first cluster (63 days) and the second cluster included earliest flowering accession (45 days). Rana et al. (2017) reported that 151 pea accessions were clustered into two majour groups based on morphological traits using Euclidean distance method. The genetic groups and subgroups obtained from the dendrogram provided important data for parent selection and evaluation of genetic material in breeding research. These data are in line with the principles of genetic diversity emphasized by Bisht and Singh (2011), Singh and Mishra (2008).

Molecular characterization

In this research, a total of 16 SSR markers were tested to determine genetic relationship among 23 pea genotypes. While 14 of SSR markers were polymorphic, AA446 and AB472 were monomorphic with single alleles. All genotypes had only one homozygous SSR allele. Among 23 genotypes, 106 polymorphic alleles were amplified and the number of polymorphic alleles per SSR marker varied from 4 (A9 and AA43092) to 10 (AA205 and AD73) with an average of 7.57 (Table 2). Similar results obtained from Nasiri et al. (2009) that AA430902 had the lowest number of alleles per locus (3). In contrast, Hanci (2019) reported that AA205 had the lowest number of allele (2). In other genetic diversity study conducted with 23 pea genotypes, the number of polymorphic alleles ranged from 2 (PEA063) to 5 (Psat8487) with a mean of 4.69 (Nisar et. al. 2017). Besides, Mekonen et al (2022) reported that 8 SSR markers detected a total of 41 alleles varying from 2 to 21 with an average of 5.13 alleles per locus in 25 pea genotypes. These results demonstrated that genotypes used in this study were genetically diverse.

Ho values of each SSR marker was 0.00 and He values ranged from 0.64 (AA43092) to 0.90 (AD146) with an average of 0.84. Considering all SSR markers used in this study, a significant heterozygote deficiency ($Ho < He$) in accordance with that reported in the previous researches (Haliloglu et al. 2022; Sharma et al. 2022b).

PIC values differed from 0.55 (AA43092) to 0.87 (AA205) with an average of 0.73 with a mean of 0.78 in this study (Table 2). Guo and Elston (1999) reported that PIC values indicates the discrimination power of SSR primers and A marker with a PIC value higher than 0,50 is considered to be very informative. Therefore, The PIC values of the SSR markers used in this study exceeded 0.50, indicating high informativeness. The number of average polymorphic alleles per locus was higher than that reported by Handerson et al. (2014), Singh et al. (2021), and Kumar et al. (2023). It was comparable to that reported by Nasiri et al. (2009) and Rana et al. (2017), and lower than that reported by Cupic et al. (2009).

The number of gene pools indicated by the STRUCTURE analysis was estimated to be $K = 2$ with the highest ΔK (14.65) (Fig. 7). As shown in Fig. 7; 23 pea genotypes were divided into two gene pools. A total of 14 genotypes were identified in gene pool I, while gene pool II contained 9 genotypes. In previous researches, Mekonen et al. (2022) assigned grass pea populations into two groups. Ahmad et al. (2015) found four separated groups among 35 diverse pea

Table 3 Eucladian dissimilarity coefficient of the 23 pea genotypes based on morphological analyses

Genotypes	G1	G2	G3	G4	G5	G6	G7	G8	G9	G10	G11	G12	G13	G14	G15	G16	G17	G18	G19	G20	G21	G22	G23
G1	0.00																						
G2	11.92	0.00																					
G3	115.91	109.20	0.00																				
G4	1.28	12.62	116.33	0.00																			
G5	9.56	9.38	106.50	9.91	0.00																		
G6	109.33	105.02	30.12	109.66	100.38	0.00																	
G7	99.59	92.01	19.79	100.04	90.17	39.06	0.00																
G8	77.38	73.95	48.94	77.68	68.65	32.48	43.00	0.00															
G9	11.08	16.71	108.31	11.27	8.57	99.86	92.95	67.64	0.00														
G10	113.91	109.67	30.88	114.25	105.00	5.42	41.87	36.88	104.39	0.00													
G11	97.14	90.98	21.46	97.57	87.82	22.82	18.71	29.24	88.98	26.75	0.00												
G12	121.21	115.94	18.72	121.57	112.00	17.92	35.40	46.93	112.44	16.27	26.45	0.00											
G13	121.61	116.95	25.06	121.93	112.50	15.57	40.46	45.94	112.51	12.80	29.85	7.21	0.00										
G14	93.29	89.31	36.35	93.61	84.40	16.28	36.74	16.41	83.77	20.72	20.01	30.99	29.65	0.00									
G15	74.25	67.51	41.86	74.70	64.85	42.72	26.75	25.19	66.90	47.23	24.13	49.63	52.01	30.50	0.00								
G16	100.16	95.63	29.15	100.50	91.12	9.92	32.94	24.21	90.91	14.55	16.01	22.90	22.22	8.26	33.14	0.00							
G17	108.25	104.24	33.21	108.57	99.39	3.49	41.37	31.02	98.61	6.31	24.81	20.88	17.81	15.11	43.28	10.33	0.00						
G18	89.42	84.20	30.34	89.79	80.19	23.09	25.56	19.02	80.77	27.66	11.33	31.84	32.99	12.57	20.02	13.39	23.68	0.00					
G19	109.91	104.53	17.12	110.29	100.68	13.39	27.65	36.61	101.23	15.25	15.30	11.56	14.68	21.58	38.33	13.51	16.46	20.53	0.00				
G20	82.42	76.26	34.58	82.88	73.12	32.93	23.46	21.42	74.41	37.45	14.82	40.31	42.63	21.99	10.20	23.60	33.65	11.04	28.84	0.00			
G21	84.46	81.20	45.42	84.75	75.78	26.04	42.46	7.36	74.64	30.24	27.15	41.19	39.54	10.28	29.15	18.52	24.30	17.17	31.61	23.10	0.00		
G22	77.40	72.93	43.46	77.73	68.32	32.38	35.43	8.33	68.32	36.95	23.73	44.33	44.40	16.77	17.49	22.87	31.70	13.46	33.34	13.95	12.01	0.00	
G23	77.47	74.59	51.84	77.75	68.94	33.47	46.64	3.85	67.46	37.68	32.42	48.69	47.21	17.76	28.88	25.90	31.67	22.23	38.77	25.19	7.69	12.10	0.00

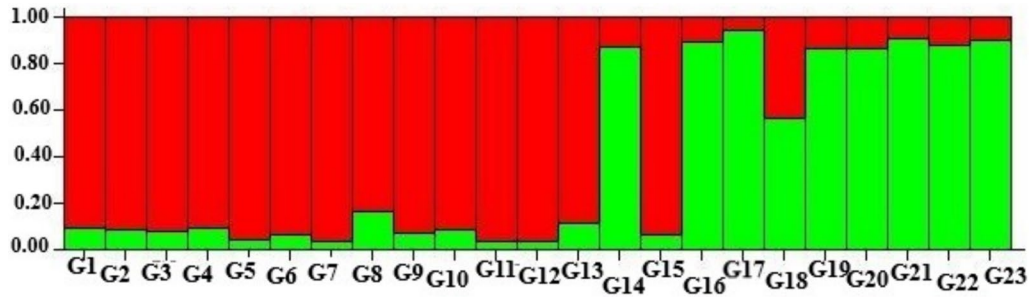


Fig. 7 The STRUCTURE bar graph based on 14 simple sequence repeat (SSR) markers. Red and green colours indicates Gene Pool I and II, respectively (k = 2; ΔK = 14.65)

genotypes and 56 garden pea genotypes were divided into four sub-populations by Sharma et al. (2022b). Haliloglu et al. (2022) found three major populations at K = 3 between 61 forage pea landrace accessions and Yang et al. (2015) clustered 24 pea accession and its wild genotypes into distincSt 3 sub-goups (Mohamed et. al. 2019).

According to the UPGMA dendrogram based on Dice similarity matrix, 23 pea genotypes mainly divided two distinct clusters (A and B) assigned similarly with the identified by STRUCTURE analysis (Fig. 8). The two clusters (A and B) were subdivided into four subgroups (I, II, III and IV). Similarly, Nisar et al. (2017) clustered 35 pea accessions into two major groups which were divided into seven further subgroups and Singh et al (2021) classified 47 pea genotypes into two distinct groups. However, Ahmad et al. (2015) identified five main clusters between 35 pea genotypes and Nasiri et al. (2009) clustered 77 pea accessions into three groups with two-subgroups. Dice similarity coefficient varied from 0.01 to 0.80 with an average of 0.40 indicating high genetic variation among genotypes in this study. G21 and G16 revealed the highest

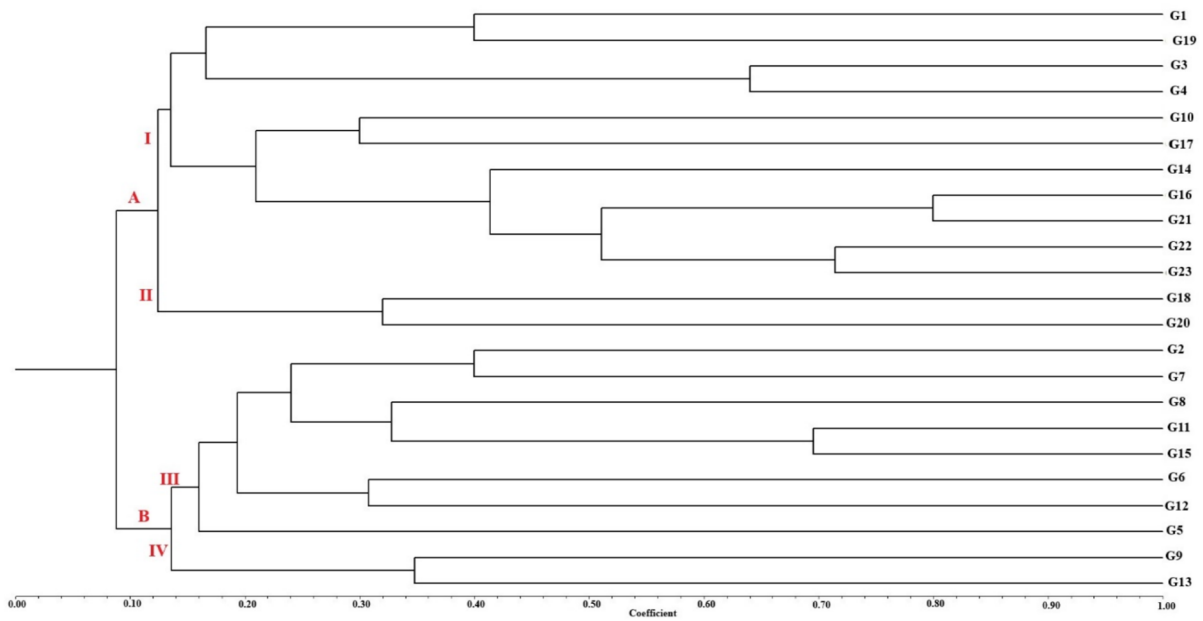


Fig. 8 Unweighted pair group method with arithmetic mean (UPGMA) dendrogram based on the genetic relationships among 23 pea genotypes

Table 4 Dice's similarity coefficient of the 23 pea genotypes based on 14 polymorphic simple sequence repeat (SSR) markers

Genotypes	G1	G2	G3	G4	G5	G6	G7	G8	G9	G10	G11	G12	G13	G14	G15	G16	G17	G18	G19	G20	G21	G22	G23
G1	1.00																						
G2	0.33	1.00																					
G3	0.25	0.17	1.00																				
G4	0.21	0.08	0.64	1.00																			
G5	0.08	0.19	0.17	0.08	1.00																		
G6	0.14	0.16	0.08	0.07	0.01	1.00																	
G7	0.25	0.40	0.17	0.16	0.27	0.16	1.00																
G8	0.14	0.25	0.24	0.21	0.08	0.14	0.25	1.00															
G9	0.17	0.10	0.01	0.08	0.19	0.08	0.31	0.08	1.00														
G10	0.10	0.11	0.10	0.09	0.11	0.20	0.33	0.10	0.23	1.00													
G11	0.09	0.23	0.10	0.01	0.21	0.19	0.33	0.34	0.02	0.01	1.00												
G12	0.16	0.19	0.09	0.08	0.27	0.30	0.27	0.30	0.26	0.20	0.19	1.00											
G13	0.28	0.26	0.08	0.01	0.08	0.21	0.08	0.01	0.34	0.20	0.09	0.15	1.00										
G14	0.01	0.02	0.28	0.20	0.01	0.09	0.10	0.09	0.22	0.30	0.01	0.00	0.09	1.00									
G15	0.15	0.19	0.17	0.07	0.09	0.23	0.18	0.30	0.01	0.01	0.69	0.07	0.15	0.10	1.00								
G16	0.02	0.03	0.11	0.11	0.01	0.30	0.01	0.20	0.14	0.16	0.12	0.31	0.19	0.47	0.22	1.00							
G17	0.14	0.02	0.08	0.07	0.08	0.14	0.01	0.07	0.08	0.30	0.17	0.07	0.07	0.38	0.23	0.40	1.00						
G18	0.08	0.01	0.09	0.16	0.08	0.08	0.02	0.08	0.01	0.02	0.21	0.09	0.06	0.19	0.27	0.10	0.32	1.00					
G19	0.40	0.04	0.20	0.01	0.01	0.01	0.03	0.01	0.01	0.18	0.01	0.00	0.18	0.28	0.00	0.40	0.20	0.00	1.00				
G20	0.23	0.08	0.16	0.07	0.01	0.01	0.02	0.07	0.09	0.03	0.02	0.00	0.14	0.09	0.16	0.21	0.22	0.32	0.18	1.00			
G21	0.03	0.01	0.11	0.12	0.01	0.25	0.01	0.12	0.03	0.01	0.03	0.00	0.05	0.40	0.14	0.80	0.25	0.13	0.25	0.23	1.00		
G22	0.12	0.02	0.01	0.11	0.02	0.12	0.01	0.11	0.02	0.20	0.01	0.00	0.12	0.28	0.02	0.60	0.25	0.02	0.01	0.23	0.40	1.00	
G23	0.14	0.01	0.16	0.26	0.03	0.14	0.04	0.13	0.02	0.02	0.01	0.00	0.01	0.50	0.05	0.44	0.14	0.03	0.02	0.01	0.60	0.71	1.00

similarity of 0.80, followed by G23 and G22 (0.71), and G15 and G11 (0.69) (Table 4). Cupic et al. (2009) found genetic distance varied from 0.24 to 0.84 and Kumari et. al. (2013) estimated a genetic diversity differed from 0.11 to 0.73 among peas based on SSR markers.

Hung et al. (2012) emphasized that genetic diversity depends on the genetic distance between parents. Overall, both morphological and molecular research showed that there is significant variation among the parental genotypes.

Conclusion

In the present study, the genetic diversity among 23 forage pea genotypes was analyzed using morphological traits and SSR (Simple Sequence Repeat) markers. Morphological analyzes were supported by molecular analyzes. Thus, more reliable results were obtained in a shorter time and the differences between the genotypes were more clearly revealed. The results obtained at both morphological and molecular levels showed that the variation among genotypes was high and that there was significant genetic diversity. This enables more efficient and precise decision-making in parent selection for breeding research.

As a result, it is thought that this research can be an important and reliable source of data for parent selection in future breeding programs for the development of new forage pea cultivars.

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Data availability No datasets were generated or analysed during the current study.

Declarations

Conflict of interest The authors declare no conflict of interest.

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