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Genetic Relationship and Diversity among Pea (*Pisum sativum* L.) Genotypes Assessed using Morphological and Molecular Markers

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ABSTRACT

Background: The study assessed the genetic relationship and diversity among twenty-two pea genotypes using morphological traits and SSR markers.

Methods: 22 pea genotypes were analyzed using twenty-one phenotypic characters and eight SSR markers. The data obtained by morphological and molecular profiling was used to analyze genetic diversity among genotypes.

Result: This study reveals that most genetic variation was due to variation within the population and was clustered into two distinct groups. As revealed by molecular markers, the relation among the diversified genotypes was not significantly correlated with those based on morphological traits, suggesting that the two systems give different estimates of genetic relations among the genotypes. The present study's findings can be used to select diverse genotypes to be used as parents of crosses aimed at breeding improved pea cultivars.

Key words: Genetic diversity, Genetic relationship, Morphological traits, Pea, SSR markers.

INTRODUCTION

Pea (*Pisum sativum* L., 2n=14) is commonly known as matar and garden pea. It is an annual herbaceous legume vegetable belonging to the family Fabaceae and native to Ethiopia, Israel, Syria and Turkey, where it is valued for its many uses as pulses and livestock feed (Choudhury *et al*., 2007). In India, it is mainly grown in Uttar Pradesh, Madhya Pradesh, Punjab, Jharkhand, Himachal Pradesh, West Bengal, Chhattisgarh and Haryana, having a 549,000-hectare area, 5,680,000 metric tonnes of production and productivity of 10.34 metric tonnes per hectare (Anonymous, 2022). In Karnataka, it is grown in districts like Bangalore Urban, Bangalore Rural, Chikka Ballapur, Ramanagar, Kolar, Chitradurga, Davanagere, Belagavi, Dharwad, Bidar and Chikkamagalur with a 1612-hectare area, 25732 metric tonnes of produce and productivity of 15.97 metric tonnes per hectare (Anonymous, 2020). It is cultivated for its tender green pods, dried seeds, canned, frozen, or dehydrated form (Santalla *et al*., 2001).

Widespread crop cultivation, replacing landraces and traditional populations, notably those resistant to biotic and abiotic stress, narrowed down the genetic base and led to the loss of genetic variability. New breeding challenges imposed by global climate change and to meet the global demand, pea breeders have to undertake more efficient methods of selection and better take advantage of the extensive genetic diversity present in the gene pool using advanced methods. Morphological traits represent the action of many genes, but they can be unreliable due to significant environmental influences. In contrast, molecular markers are useful to complement the morphological traits because they are independent of environmental effects and allow identification of the cultivar in the early stages of the crop. Among the various DNA markers available for molecular profiling of genotypes, SSRs or microsatellites are highly

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reliable, accurate, co-dominant, highly polymorphic and cost-effective (Jain *et al.,* 2014).

In peas, various genetic diversity studies were done using phenotypic traits (Priyanka and Lal, 2021; Singh *et al.*, 2021a). Loridon *et al*. (2005) initially developed a set of SSRs for genetic diversity and linkage mapping of a pea. Later, Ram *et al.* (2021) and Singh *et al.* (2021b) reported using some polymorphic SSR markers for genetic diversity studies in peas.

Hence, the present study was undertaken to assess the level of genetic diversity among twenty-two pea genotypes using both morphological traits and SSR markers. This would aid the long-term objective of identifying diverse parental lines to generate segregating populations for tagging essential traits with molecular markers.

MATERIALS AND METHODS

The field experiment was conducted during *Rabi* 2021-22 at ICAR-Krishi Vigyan Kendra, Babbur farm, Hiriyur of Keladi Shivappa Nayaka University of Agricultural and Horticultural Sciences, Shivamogga, Karnataka (India) which is situated in the central dry zone (Zone-4) of Karnataka state and lies between 13°57′32″North latitude and 70°37′38″ East longitude with an altitude of 606 meters above mean sea level. In experiment twenty two pea genotypes (Table 1) were evaluated in the randomized block design (RBD) with two replications. The traits *viz.,* PH- Plant height (cm), NBP- number of branches per plant, DF- Days to first flowering, DFF- Days to 50 per cent flowering, LP- Length of the pod (cm), WP- Width of the pod (cm), NSP- Number of seeds per pod, WSTP- Wt. of seeds of ten green pods (g), S- Shelling percentage (%), NPP- Number of pods per plant, GPY- Green pod yield per plant (g), TSS-total soluble solids (°Brix), TC- Total chlorophyll (mg/g), RS- Reducing sugars (%), NRS- Non-reducing sugars (%), F- Firmness (lbs.), DR- Dehydration ratio, DD- Drying duration (min.), PR- Per cent recovery (%), RR- Rehydration ratio and DMC- Dry matter content (%) were recorded for the five randomly selected plants in each replication. The mean values for each trait were analyzed for descriptive statistics and genetic diversity analysis. The genetic divergence among the test garmplasm was evaluated using Mahalanobis (1928) D^2 statistical analysis which was described by Rao (1952).

Molecular studies were carried out in the Department of Studies and Research in Biotechnology, Tumkur

Table 1: List of pea genotypes used in the study along with their source of collection.

Name of the genotype	Source
Magadi Local	Magadi, Ramanagar
Arka Priya	ICAR-IIHR, Bangalore
Arka Karthik	ICAR-IIHR, Bangalore
Arka Pramodh	ICAR-IIHR, Bangalore
Arka Tapas	ICAR-IIHR, Bangalore
Arka Apoorva	ICAR-IIHR, Bangalore
Arka Chaitra	ICAR-IIHR, Bangalore
Arka Ajit	ICAR-IIHR, Bangalore
Arka Uttam	ICAR-IIHR, Bangalore
Arka Sampoorna	ICAR-IIHR, Bangalore
Arkel	ICAR-IARI, New-Delhi
IIHR-570	ICAR-IIHR, Bangalore
IIHR-671	ICAR-IIHR, Bangalore
IIHR-684	ICAR-IIHR, Bangalore
IIHR-688	ICAR-IIHR, Bangalore
IIHR-758	ICAR-IIHR, Bangalore
IIHR-766	ICAR-IIHR, Bangalore
IIHR-774	ICAR-IIHR, Bangalore
IIHR-775	ICAR-IIHR, Bangalore
AFA-10	Ashoka Seeds, Bangalore
PS-101	Nutech Seeds, New-Delhi
GS-10	Golden Seeds, Bangalore

Table 2: List of SSR primers with their sequence and annealing temperature.

Primers	Sequence (“5-3”)	Annealing T_m (°C)
PSMPA5	F 5′-GTAAAGCATAAGGGGTTCTCAT-3′	50
	R 5′-CAGCTTTTAACTCATCTGACA-3′	
PSMPA6	F 5′-CTTAAGAGAGATTAAATGGACAA-3′	50
	R 5′-CCAACTCATAATAAAGATTCAAA-3′	
PSMPA9	F 5′-GTGCAGAAGCATTTGTTCAGAT-3′	50
	R 5′-CCCACATATATTTGGTTGGTCA-3′	
PSMPD23	F 5′-ATGGTTGTCCCAGGATAGATAA-3′	50
	R 5′-GAAAACATTGGAGAGTGGAGTA-3′	
PSMPSAD126	F 5′-TGGCTTTGCAGAGTGTTTGAGTR-3′	50
	R 5′-GGCTTCAACAGCGATCCATAAT-3′	
PSMPSAD134	F 5′-TTTATTTTTCCATATATTACAGACCGR-3′	50
	R 5′-ACACCTTTATCTCCCGAAGACTTAG-3′	
PSMPSAD146	F 5′-TGCTCAAGTCAATATATGAAGAR-3′	50
	R 5′-CAAGCAAATAGTTGTTTTGTTA-3′	
PSMPSAD205	F 5′-TACGCAATCATAGAGTTTGGAA-3′	50
	R 5′-AATCAAGTCAATGAAACAAGCA-3′	

University, Tumkur, Karnataka (India), during 2021-2022. For genotyping, genomic DNA was isolated using the CTAB extraction method (Doyle and Doyle, 1987) and quantified using agarose gel electrophoresis. PCR amplification was performed using 8 SSR primer pairs (Table 2) and evaluated according to Ram *et al*. (2021). A dendrogram was constructed based on Jaccard's dissimilarity matrix using Agglomerative hierarchical cluster analysis. A total number of alleles, major allele frequency, heterozygosity and the polymorphic information content (PIC) were estimated using Power Marker V3.25 (Liu and Muse, 2005) and genetic diversity was estimated based on the Agglomeration method: Ward's method using XLStat Software.

RESULTS AND DISCUSSION

Genetic diversity assessment using morphological traits

Based on phenotypic data, analysis of variance showed a significantly higher variation among the genotypes for all the characters studied (Table 3). On the basis of Mahalanobis values, all pea genotypes were classified into five clusters (Priyanka and Lal, 2021). A comparison of cluster mean values revealed that for improving specific characters, genotypes should be chosen from the cluster with the highest mean value for that character. This comparison shows that clusters IV and V had better cluster means for most characters; thus, these clusters may be better for selecting genotypes as divergent parents (Table 4). Similar results are exhibited in the findings of Bijalwan *et al*. (2018).

Genetic diversity assessment using molecular markers SSR marker analysis of pea germplasm

Out of the 8 SSR primer pairs, three primers that generated clear and distinct polymorphic alleles used in this study produced a total of 6 alleles. The average number of bands per locus was 1, with a range of 1 to 2 effective alleles produced per locus. Similar reports were presented by Ram *et al*. (2021) and Haliloglu *et al*. (2022). The PCR amplification profile of SSR markers in twenty-two garden pea genotypes for primers PSMPA5, PSMPA6 and PSMPA9 are shown in Fig 1, Fig 2 and Fig 3, respectively.

Polymorphic information content (PIC) and genetic diversity estimates of SSR primers.

Further, the most informative locus under this study was PSMPA5, with a PIC value of 0.75. A marker with a PIC value greater than 0.5 is considered to be very informative (Prakash *et al*., 2016). Few studies also suggested that a more significant number of genotypes and primer pairs need for improving allelic richness (Negisho *et al*., 2017). The highest (0.50) and lowest (0.34) expected heterozygosity (He) values were obtained with PSMPA9 and PSMPA6, respectively. In contrast, the highest unbiased expected heterozygosity and Shannon's information index were obtained with PSMPA9. It formed the average Shannon information index (I) value of 0.60, which was lower

Table 3: Descriptive statistics of morphological data.

Statistics	PH	NBP	DF	DFF	LP	WP	NSP	WSTP	S%	NPP	GPY	TSS	TC	RS	NRS	F	DR	DD	PR	RR	DMC
Minimum	50.63	23.90	30.50	36.00	5.20	10.30	5.20	8.05	28.85	18.24	36.85	13.56	0.48	2.33	3.74	2.16	2.50	223.78	29.92	1.57	15.04
Maximum	147.50	55.45	48.90	52.00	10.0	18.80	10.00	23.58	54.88	49.66	73.81	21.91	1.72	3.96	8.13	2.70	3.32	299.49	39.48	2.68	19.84
Mean	81.91	33.28	42.70	46.32	7.78	13.25	6.75	15.00	44.56	32.46	60.14	16.08	1.25	3.31	5.19	2.43	2.77	272.33	35.81	2.32	18.15
CV	2.31	1.94	6.35	4.13	5.27	2.64	4.73	2.96	4.41	7.27	4.93	2.00	3.03	1.90	1.68	2.03	1.90	2.45	2.43	2.73	2.61
S.Em ±	1.34	0.46	1.92	1.35	0.29	0.25	0.23	0.31	1.39	1.67	2.10	0.23	0.03	0.04	0.06	0.04	0.04	4.72	0.62	0.04	0.34
CD @ 5%	3.94	1.35	5.64	3.98	0.85	0.73	0.66	0.92	4.08	4.91	6.17	0.67	0.08	0.13	0.18	0.10	0.11	13.83	1.80	0.13	0.98

PH- Plant height (cm), NBP- Number of branches per plant, DF- Days to first flowering, DFF- Days to 50 per cent flowering, LP- Length of the pod (cm), WP- Width of the pod (cm), NSP- Number of seeds per pod, WSTP- Wt. of seeds of ten green pods (g), S- Shelling percentage (%), NPP- Number of pods per plant, GPY- Green pod yield per plant (g), TSS- Total soluble solids (°Brix), TC- Total chlorophyll (mg/g), RS- Reducing sugars (%), NRS- Non-reducing sugars (%), F- Firmness (lbs.), DR- Dehydration ratio, DD- Drying duration (min.), PR- Per cent recovery (%), RR- Rehydration ratio and DMC- Dry matter content (%).

compared to the studies of Ram *et al.* (2021). Expected heterozygosity (He) ranged from 0.34 to 0.50 in primers PSMPA6 and PSMPA9, respectively. Unbiased expected heterozygosity (uHe) ranged from 0.34 to 0.51 in primers PSMPA6 and PSMPA9 (Table 5). Low allelic richness in the analyzed germplasm is indicated by lower estimates of all diversity parameters, suggesting that more germplasm must be added to enhance the allelic richness.

Levels of genetic dissimilarity

Jaccard's dissimilarity index ranged from 9.32 between dwarf type genotypes 'Arka Pramodh, Arka Uttam, Arkel' and tall type genotypes 'IIHR-688' (distant) to 1.78 between the pairs of tall type genotypes 'Magadi local, IIHR-758, IIHR-775, GS-10, Arka Priya, Arka Apoorva, Arka Chaitra and Arka Ajit.' and 'IIHR-688' (closest).

Dissimilarity and cluster analysis using Agglomeration method: Ward's method

Cluster analysis based on Agglomeration method: Ward's method, dendrogram (Fig 4) outlined by SSR primer pairs classified the 22 pea genotypes into two major groups based on tall and dwarf cultivars, with the major groups further subdivided into five subgroups. The highest dissimilarity was found between subgroups II and V, indicating that the genotypes in these subgroups had high genetic divergence

Table 4: Mean values of 21 characters for five clusters in garden pea genotypes.

Characters	Clusters				
	I	II	III	IV	V
PH	73.77	80.27	94.43	64.00*	147.50**
NBP	31.62	29.89	38.33	26.25*	55.45**
DF	43.14	42.22	42.90	37.70*	44.50**
DFF	46.86	45.45	46.63	40.25*	49.50**
LP	7.27	9.09	7.51	9.91**	5.82*
WP	13.26	14.71**	12.55	11.65	10.30*
NSP	6.40	7.34	6.28*	10.00**	6.40
WSTP	14.84	16.39**	15.29	15.60	8.05*
S	44.50	43.81*	43.90	45.95	50.22**
NPP	33.04	34.46	28.22	21.44*	44.02**
GPY	56.66*	66.08**	61.13	58.88	65.96
TSS	15.37*	16.53	16.04	21.60**	16.35
TC	1.34**	1.15	1.23	1.10*	1.14
RS	3.43**	3.37	3.06	3.17	2.87*
NRS	4.29	6.45	5.70	3.73*	8.19**
F	2.47**	2.45	2.33	2.29*	2.39
DR	2.77	2.74	2.89**	2.59*	2.69
DD	272.00	275.85	261.32*	290.07**	279.68
PR	36.27	36.78	34.84*	38.68**	37.29
RR	2.36	2.38**	2.19	2.16*	2.30
DMC	18.13	18.39	17.42*	19.34**	18.65

PH- Plant height (cm), NBP- Number of branches per plant, DF- Days to first flowering, DFF- Days to 50 per cent flowering, LP- Length of the pod (cm), WP- Width of the pod (cm), NSP- Number of seeds per pod, WSTP- Wt. of seeds of ten green pods (g), S- Shelling percentage (%), NPP- Number of pods per plant, GPY- Green pod yield per plant (g), TSS- Total soluble solids (°Brix), TC- Total chlorophyll (mg/g), RS- Reducing sugars (%), NRS- Non-reducing sugars (%), F- Firmness (lbs.), DR- Dehydration ratio, DD- Drying duration (min.), PR- Per cent recovery (%), RR- Rehydration ratio and DMC- Dry matter content (%).

Table 5: Primer/locus name, PIC, polymorphism % and genetic diversity estimates of SSR primers.

Pop	Locus	PIC	polymorphism %	Band Freq.	p	q	N	Na	Ne	I	He	uHe
Pop1	PSMPA5	0.75	100	0.50	0.29	0.71	22.00	2.00	1.71	0.60	0.41	0.42
	PSMPA6	0.33	100	0.95	0.79	0.21	22.00	2.00	1.50	0.52	0.34	0.34
	PSMPA9	0.09	100	0.77	0.52	0.48	22.00	2.00	2.00	0.69	0.50	0.51
	Average	0.39	100	0.74	-	22.00	2.00	1.74	0.60	0.42	0.43	

Pop= Population; Band frequency; Estimated allele frequency (p and q); N= Samples size; Na= Observed number of alleles; Ne= Effective number of alleles; I: Shannon's information index; He= Expected heterozygosity and uHe= Unbiased expected heterozygosity.

and could be used as parents in a hybridization program to produce superior segregants. These results are in agreement with Choudhury *et al*. (2007); Suman *et al*. (2019) and Singh *et al*. (2021) and also showed that there is no correspondence of genotypic matrix with the geographical location. A similar type of finding where reported by Jain *et al*. (2014); Rana *et al*. (2017); Tahir *et al*. (2018) and Singh *et al*. (2021b). Thus, hybridization between genotypes across clusters may yield combinations with a high heterotic response and superior recombinants.

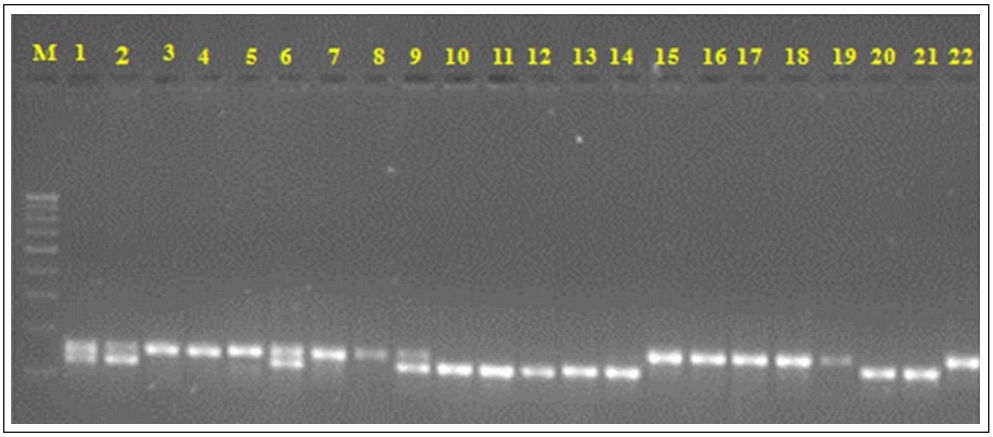

Fig 1: Electrophoretic profile of the 22 pea genotypes amplified with PSMPA5 marker (M= 1 kb molecular size marker).

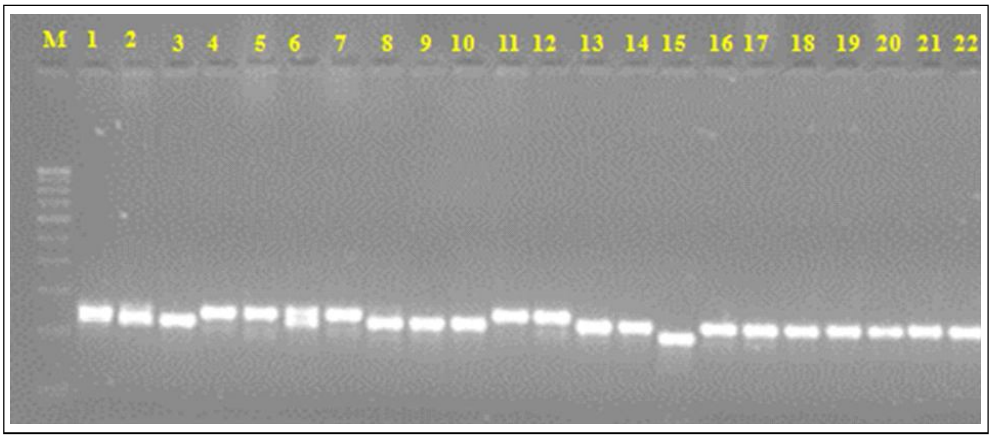

Fig 2: Electrophoretic profile of the 22 pea genotypes amplified with PSMPA6 marker (M= 1 kb molecular marker).

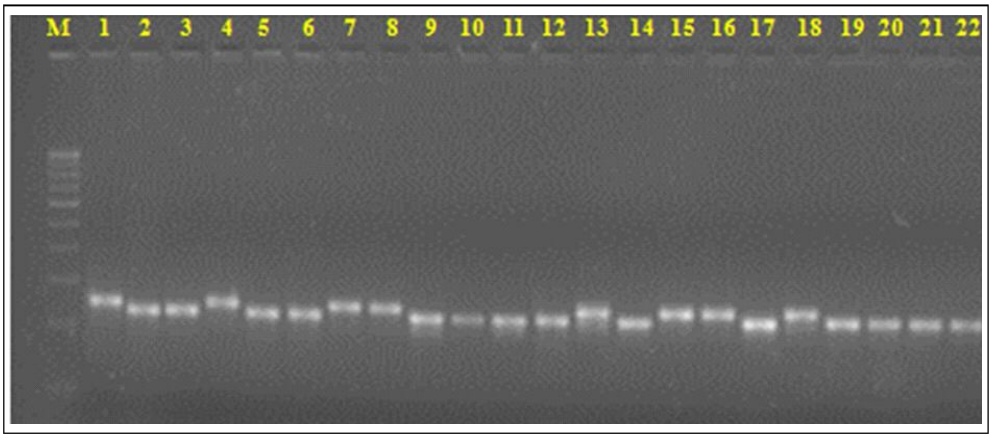

Fig 3: Electrophoretic profile of the 22 pea genotypes amplified with PSMPA9 marker (M= 1 kb molecular marker).

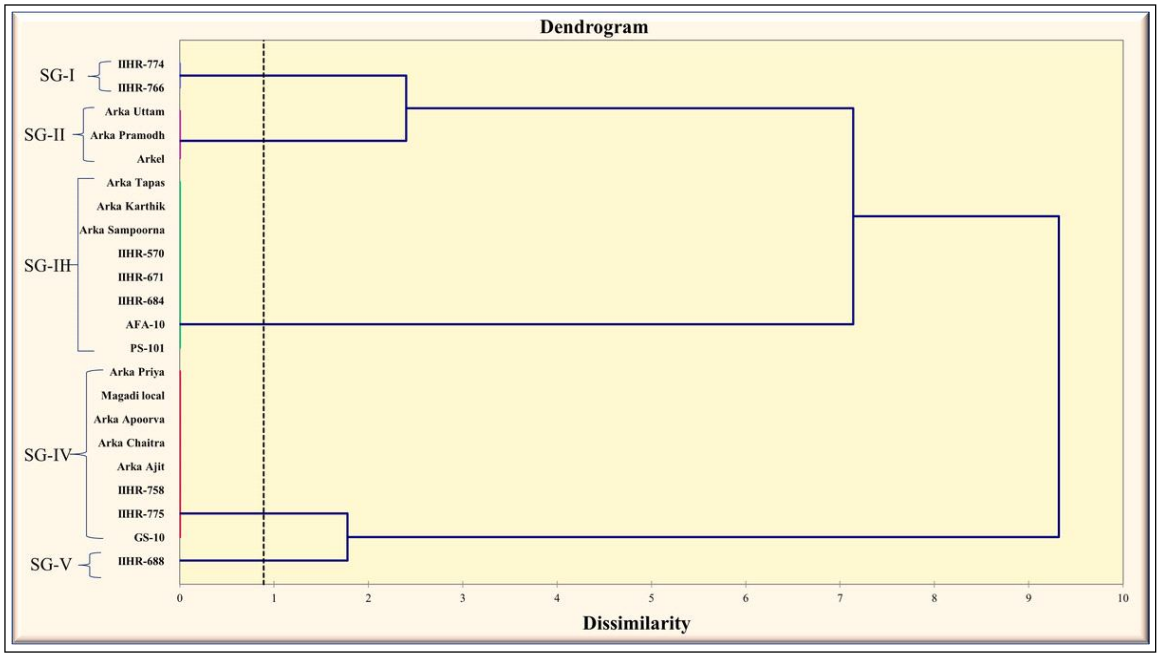

Fig 4: Molecular dendrogram of 22 pea genotypes based on their variation at 3 SSR loci using Agglomeration method: Ward's method.

CONCLUSION

The garden pea germplasm was separated into tall and dwarf plant types using molecular marker data, demonstrating that molecular markers were more reliable for determining genetic diversity. The SSR-based cluster analysis showed that the grouping did not correspond to pedigree data or breeding centers. Furthermore, as indicated by molecular markers, the relationship between the distinct genotypes was not significantly correlated with those based on morphological attributes, suggesting that the estimates of the genetic relationships between the genotypes provided by the two systems are not the same. The genotypes of clusters with the highest dissimilarity should be crossed to produce a broad spectrum of variability and isolate desirable recombinants in subsequent generations. The high discriminating power of the SSR loci suggested that a subset of the most robust SSR markers could be used to distinguish between genotypes. SSRs were found to be an efficient and reliable marker system for the genetic analysis of pea germplasm.

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Conflict of interest

All authors declared that there is no conflict of interest.

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