GMR Genetics and Molecular Research
Online Journal
ISSN:1676-5680

Genetic diversity studies in pea (*Pisum sativum* L.) using simple sequence repeat markers

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Genet. Mol. Res. 12 (3): 3540-3550 (2013)
Received July 27, 2012
Accepted November 1, 2012
Published March 13, 2013
DOI http://dx.doi.org/10.4238/2013.March.13.12

ABSTRACT. The genetic diversity among 28 pea (*Pisum sativum* L.) genotypes was analyzed using 32 simple sequence repeat markers. A total of 44 polymorphic bands, with an average of 2.1 bands per primer, were obtained. The polymorphism information content ranged from 0.657 to 0.309 with an average of 0.493. The variation in genetic diversity among these cultivars ranged from 0.11 to 0.73. Cluster analysis based on Jaccard's similarity coefficient using the unweighted pair-group method with arithmetic mean (UPGMA) revealed 2 distinct clusters, I and II, comprising 6 and 22 genotypes, respectively. Cluster II was further differentiated into 2 subclusters, IIA and IIB, with 12 and 10 genotypes, respectively. Principal component (PC) analysis revealed results similar to those of UPGMA. The first, second, and third PCs contributed 21.6, 16.1, and 14.0% of the variation, respectively; cumulative variation of the first 3 PCs was 51.7%.

Key words: Pea; Diversity; Molecular markers; Dendrogram; Polymorphism information content

INTRODUCTION

Legumes are important crops worldwide, and they have major impacts on agriculture, the environment, and animal and human nutrition (Graham and Vance, 2003). Legumes can interact symbiotically with specific soil-borne bacteria called rhizobia, allowing them to fix atmospheric nitrogen, improve the physical condition of the soil, and possibly protect them against certain fungal pathogens (Chakraborty et al., 2003). When grown as a preceding crop, pea increases the efficiency of organic matter utilization by subsequent crops (Makasheva, 1983). Among legumes, pea (*Pisum sativum* L., 2n = 14), belonging to family Leguminosae, is an important crop with a rich history in genetic research dating back to the classical work by the father of genetics, Gregor J. Mendel. Pea is one of the 6 major pulse crops cultivated globally and is the second highest yielding legume in the world after common bean (*Phaseolus vulgaris* L.) (Food and Agriculture Organization, 2010).

A number of studies have been carried out to study genetic diversity within the pea germplasm, including wild and cultivated species, using various approaches (Samec and Našinec, 1995; Zong et al., 2008). In addition to morphological traits and biochemical assays, molecular markers (Smýkal et al., 2008) have been used to identify genetic relationships among various accessions to explore the genetic diversity underneath. Molecular markers have the potential to explore genetic diversity by detecting polymorphisms that improve the efficiency and precision of conventional plant breeding. A variety of DNA-based marker systems have been used extensively for diversity analysis in plants, including random amplified polymorphic DNA (RAPD) markers (Williams et al., 1990), inter simple sequence repeats (Zietkiewicz et al., 1994), amplified fragment length polymorphism (AFLP; Vos et al., 1995), and simple sequence repeats (SSR; Beckmann and Soller, 1990). Among these, SSRs, or microsatellites, are the most widely applied technique for genetic diversity analyses in crop species (Blair et al., 2007). SSRs are very reliable owing to their high polymorphism level, codominant inheritance, and good reproducibility (Burstin et al., 2001; Ford et al., 2002; Baranger et al., 2004; Loridon et al., 2005).

Currently, no international center conducts pea breeding and genetic conservation (Upadhyaya et al., 2011). Moreover, a high level of duplication exists between the collections, giving a misleading impression of the true level of diversity (Maxted et al., 2010; Smýkal et al., 2011). However, the number of original pea landraces, mainly from Europe, Asia, the Middle East, and North Africa/Ethiopia, has not been documented. The much smaller collections of wild relatives of pea are less widely distributed, and more clarity exists when tracing these accessions to their origin. Important gaps remain in the collections - particularly those of wild and locally adapted materials - that need to be addressed before their genetic resources are lost forever (Maxted et al., 2010). The aim of the present study was to assess the level of genetic diversity within a collection of pea genotypes to aid in the selection and more efficient use of this germplasm in breeding programs.

MATERIAL AND METHODS

Plant material

Twenty-eight pea genotypes, including released varieties, were selected for molecular polymorphism studies (Table 1). All test genotypes were chosen from the core collection maintained at the Department of Genetics and Plant Breeding, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi, India.

Table 1. Pea genotypes used for genetic diversity analysis.

No.	Genotypes	Source	Pedigree	Plant type
1	Pant P-11	G.B.P.U.A.&T., Pantnagar	T 163 x FC 1	Normal foliaged
2	Pant P-13	G.B.P.U.A.&T., Pantnagar	HFP 4 x EC 1	Normal foliaged
3	Pant P-14	G.B.P.U.A.&T., Pantnagar	HFP 4 x Longitee	Normal foliaged
4	Pant P-25	G.B.P.U.A.&T., Pantnagar	(EC 324110 x FC 1) x FC 1	Normal foliaged
5	KPMR-615	C.S.A.U., Kanpur	DMR-37 x P-1463	Normal foliaged
6	KPMR-660	C.S.A.U., Kanpur	Rachna x KPMR-157	Normal foliaged
7	KPMR-662	C.S.A.U., Kanpur	Rachna x KPMR-62	Normal foliaged
8	KPMR-678	C.S.A.U., Kanpur	KPMR 186 x KPMR-157	Semi leafless
9	KPMR-675	C.S.A.U., Kanpur	KPMR 65 x HUP 2	Normal foliaged
10	DDR-23	I.A.R.I., New Delhi	HFP 4 x Pusa 10	Normal foliaged
11	IPF 98-1	I.I.P.R., Kanpur	HUP 11 x KPSD 1	Normal foliaged
12	IPF 2-13	I.I.P.R., Kanpur	KPMR 10 x KFPD 9	Normal foliaged
13	IPF 1-22	I.I.P.R., Kanpur	HUP-2 x DPF-62	Semi leafless
14	IPFD-1-9	I.I.P.R., Kanpur	HFP-8909 x HUDP-7	Normal foliaged
15	IPFD-1-10	I.I.P.R., Kanpur	PDPD-8 x HUDP-7	Normal foliaged
16	HFP-9426	H.A.U., Hisar	KPMR 84 x EC 109195	Normal foliaged
17	HUDP-18	B.H.U., Varanasi	HUDP 2 x FC 1	Semi leafless
18	HUDP-15	B.H.U., Varanasi	PG3(PG3 x S 143) x FC 1	Semi leafless
19	HUDP-9	B.H.U., Varanasi	PG3(PG3 x S 143) x FC 1	Semi leafless
20	HUDP-7	B.H.U., Varanasi	HUP 2 x FC 1	Semi leafless
21	HUDP-8	B.H.U., Varanasi	HUP 2 x FC 1	Semi leafless
22	HUDP-27	B.H.U., Varanasi	(PG3 x Pusa 10) x FC 1	Semi leafless
23	HUVP 1	B.H.U., Varanasi	(Bonneville x 5064) x S 143	Acacia with normal stipule
24	FC 1	I.I.H.R., Bangluru	(Bonneville x HR 209) x IIHR 656	Normal foliaged
25	VL-40	V.P.K.A.S., Almora	T-163 x VL-1	Normal foliaged
26	S 143	-	Mutant	Pleofila with reduced stipule
27	Pant P-138	G.B.P.U.A.&T., Pantnagar	Pant P 14 x FC 1	Normal foliaged
28	Pant P-31	G.B.P.U.A.&T., Pantnagar	EC 1 x FC 1	Normal foliaged

Polymerase chain reaction (PCR) procedure

Very young, healthy, fresh leaves (approximately 100 mg) from each pea genotype was taken and stored at -20°C. DNA extraction was carried out using a DNA extraction kit (Qiagen, Düsseldorf, Germany). DNA quality was visualized using 0.8% agarose gel electrophoresis, and DNA quantity was determined using a NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA). The PCR was carried out in a 15-µL volume of a master mixture containing 20-25 ng genomic DNA, 200 µM deoxyribonucleotide triphosphates, 2 mM $MgCl_2$, 1 U Taq DNA polymerase (Fermentas, Pittsburgh, PA, USA), 1X Taq buffer, and 0.6 mM reverse and forward primers. DNA amplification was carried out in a thermal cycler (Mastercycler gradient, Eppendorf, USA). The PCR program included an initial denaturation step at 94°C for 3 min; this was followed by 40 cycles of denaturing at 94°C for 30 s, annealing at optimum annealing temperature for 30 s, and extension at 72°C for 1 min. After the last cycle, samples were kept at 72°C for 5 min for final extension.

The SSR primers used were synthesized by Eurofins Genomics (India). To identify the primers that produced clear amplified bands, we screened the amplification products using electrophoresis on 2.5% agarose gels containing 0.002 ng/mL ethidium bromide in 1X Tris-acetate buffer solution. The amplification products were examined under ultraviolet light and photographed using a gel documentation system (Gel Doc™ XR+, Bio-Rad, USA).

Statistical analyses

SSR markers that generated clear and unambiguous bands of various molecular weight

sizes were scored for the presence (1) and absence (0) of the corresponding band among the genotypes in the form of a binary matrix, and the data matrix was subjected to further analysis using NTSYS-pc version 2.11W (Rohlf, 1997). The SIMQUAL program was used to calculate Jaccard's similarity coefficients. The resulting similarity matrix was used for unweighted pair-group method with arithmetic mean (UPGMA)-based dendrogram construction. Polymorphism information content (PIC) for SSR markers was calculated using the following formula:

$$PIC_i = 1 - \Sigma P ij^2$$

where PIC_i is the PIC of marker i; Pij is the frequency of the j^{th} pattern for marker i, and the summation extends over n patterns. Principal component (PC) analysis (PCA) was also carried out to check the results of UPGMA-based clustering using the EIGEN module of NTSYSpc.

RESULTS

Of the 32 SSR primers used, 21 displayed reproducible and polymorphic patterns (Table 2). These 21 primers yielded a total of 44 polymorphic bands; the number of polymorphic bands per primer ranged from 2 to 4, with an average of 2.1 (Table 3). Primers AA504, AA205, and AD79 were the most informative primers, with the highest PIC of 0.657. SSR primer AA446 had the lowest PIC value of 0.309 (see Table 2). Gel images obtained from the SSR banding profile of primers AA355 and AC58 are shown in Figure 1. These 21 highly polymorphic primers produced an average PIC of 0.493 (see Table 3). The average GC content of the polymorphic primers was 52.8%.

Table 2. Details of the pea SSR primers used in the present study.

SSR primer	Tm (°C)	Linkage group	Distance (cM)	Allele size (bp)	PIC
C20	64	I	26	225 (M)	0.0
AD70	51	I	57.4	180 (M)	0.0
AD147	61	I	78.3	300-325	0.334
D21	51	I	134.9	200-300	0.477
AD148	54	II	39.3	350-375	0.459
AA233	61	II	92.3	Not amplified	-
AB149	61	II	146.1	Not amplified	-
AA504	61	II	184.8	375-400	0.657
AA205	51	II	217.5	190-215	0.657
AB25	51	III	27.2	Not amplified	-
AA5	61	III	183.8	225-240	0.477
AB30	61	III	198.9	Not amplified	-
AD174	51	III	217.5	190-215	0.375
AA355	51	III	238.6	200-215	0.500
AD270	51	III	254.3	245-290	0.460
AA122	61	IV	116.1	180-210	0.458
AB45	51	IV	131.5	140-230	0.337
AD171	61	IV	139	125 (M)	0.0
AD61	51	IV	165.8	120-300	0.519
AB23	61	V	36.8	200-225	0.640
AD79	56	V	98.6	300-325	0.657
AC58	61	V	167.3	200-225	0.500
AB71	61	VI	69.1	Not amplified	-
AD60	51	VI	75.5	550 (M)	0.0
AB91	56	VI	142.9	140 (M)	0.0
AA416	61	VII	47.1	240-290	0.575
AD56	61	VII	88.6	200-225	0.497
AD146	51	VII	96.1	375-450	0.490
AA446	51	VII	136.6	315-900	0.309
AA505	55	VII	151.5	140-210	0.519
AD237	54	VII	152.1	275-300	0.458
AB36	56	VII	186	Not amplified	-

Tm = melting temperature; PIC = polymorphic information content. Linkage group and distance (cM) of SSR primers were according to Loridon et al. (2005).

Table 3. Summary of polymorphism pattern generated by the SSR primers used.

Total number of primers screened with 28 pea genotypes	32
Number of primers not amplified	6
Number of primers that produced monomorphic bands	5
Number of primers that produced polymorphic bands	21
Total number of bands amplified by the polymorphic primers	44
Average number of polymorphic bands per primer	2.10
Average size (bp) of the fragments amplified by polymorphic primers	280
Average GC content (%) of the polymorphic primers	52.80
Average polymorphic information content (PIC) of primers	0.493

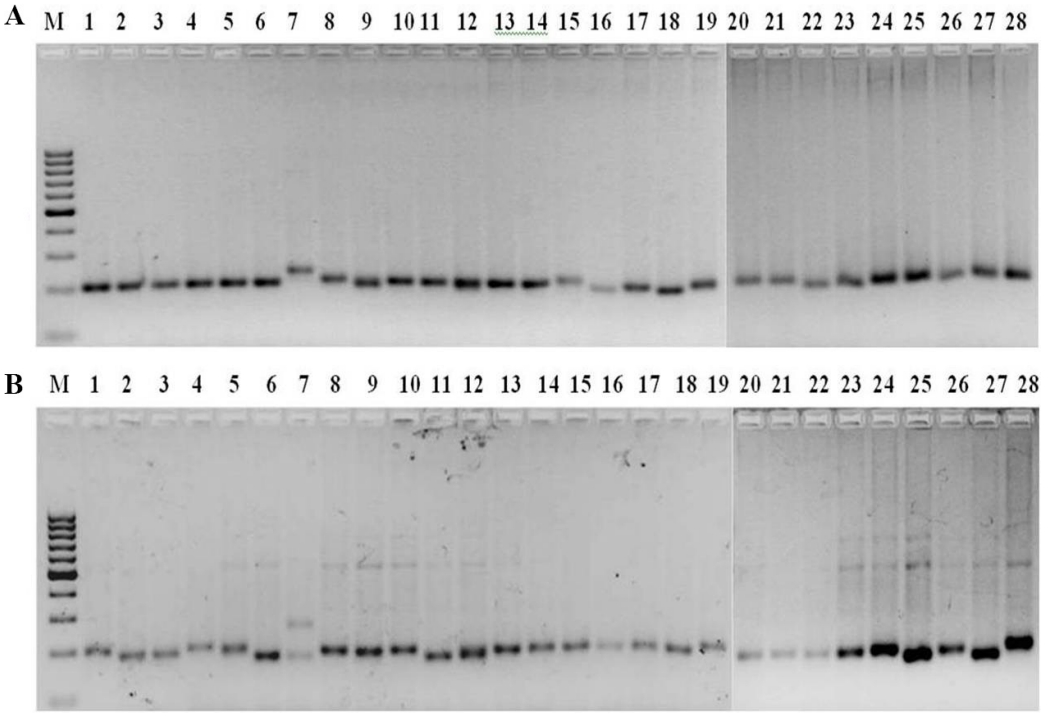

Figure 1. Gel images showing SSR banding profile obtained by (**A**) AA355 and (**B**) AC58 primers. *Lanes 1* to *28* = pea genotypes as listed in Table 1; *lane M* = 100-bp DNA size marker.

The genetic coefficients measured from molecular data on 21 polymorphic SSR markers revealed varying degrees of genetic relatedness among the pea genotypes. The Jaccard similarity coefficient ranged from 0.11 to 0.73 owing to diversification in morphology and pedigree among the genotypes. Pea genotype pairs Pant P-11 and HUDP-7 revealed the maximum similarity of 0.73, followed by KPMR-660 and IPF2-13 (0.72), and KPMR-678 and IPFD-1-9 (0.71). Genotype pairs IPF1-22 and KPMR-660 showed the least genetic similarity of 0.11, followed by KPMR-675 and IPF2-13 (0.13), and 0.14 between KPMR-615, HUVP 1 and FC 1 (Table 4).

Table 4. Jaccard's similarity coefficient of the 28 pea genotypes based on 21 polymorphic SSR markers.

	1*	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28
1	1.00																											
2	0.61	1.00																										
3	0.56	0.73	1.00																									
4	0.64	0.40	0.54	1.00																								
5	0.26	0.19	0.21	0.23	1.00																							
6	0.25	0.26	0.32	0.27	0.58	1.00																						
7	0.19	0.23	0.22	0.20	0.22	0.25	1.00																					
8	0.31	0.37	0.30	0.29	0.26	0.21	0.58	1.00																				
9	0.38	0.26	0.28	0.27	0.17	0.17	0.38	0.33	1.00																			
10	0.44	0.31	0.29	0.48	0.33	0.37	0.26	0.34	0.32	1.00																		
11	0.52	0.54	0.44	0.33	0.30	0.38	0.31	0.23	0.43	0.44	1.00																	
12	0.32	0.33	0.31	0.30	0.50	0.72	0.28	0.24	0.13	0.50	0.41	1.00																
13	0.37	0.33	0.27	0.35	0.31	0.11	0.42	0.61	0.22	0.36	0.28	0.25	1.00															
14	0.28	0.29	0.31	0.34	0.24	0.26	0.58	0.71	0.39	0.40	0.24	0.29	0.48	1.00														
15	0.23	0.24	0.36	0.40	0.27	0.18	0.32	0.28	0.26	0.23	0.23	0.18	0.38	0.38	1.00													
16	0.33	0.30	0.32	0.41	0.46	0.56	0.18	0.21	0.14	0.41	0.25	0.59	0.26	0.30	0.30	1.00												
17	0.43	0.39	0.52	0.46	0.41	0.35	0.33	0.38	0.27	0.32	0.43	0.34	0.44	0.39	0.44	0.31	1.00											
18	0.44	0.46	0.60	0.42	0.21	0.37	0.34	0.26	0.28	0.18	0.34	0.35	0.19	0.27	0.31	0.32	0.52	1.00										
19	0.37	0.57	0.41	0.35	0.23	0.34	0.37	0.48	0.18	0.31	0.42	0.43	0.38	0.38	0.29	0.26	0.50	0.52	1.00									
20	0.73	0.54	0.56	0.64	0.22	0.29	0.31	0.31	0.33	0.39	0.46	0.37	0.37	0.32	0.28	0.29	0.43	0.50	0.48	1.00								
21	0.57	0.59	0.48	0.36	0.19	0.12	0.29	0.33	0.36	0.28	0.44	0.18	0.46	0.26	0.25	0.15	0.31	0.32	0.35	0.57	1.00							
22	0.44	0.46	0.38	0.37	0.25	0.32	0.34	0.22	0.28	0.25	0.39	0.35	0.31	0.27	0.31	0.37	0.24	0.38	0.31	0.44	0.48	1.00						
23	0.34	0.52	0.54	0.28	0.14	0.24	0.30	0.26	0.37	0.18	0.30	0.24	0.19	0.35	0.41	0.24	0.24	0.38	0.27	0.34	0.32	0.48	1.00					
24	0.44	0.41	0.54	0.48	0.14	0.24	0.26	0.34	0.46	0.21	0.26	0.17	0.23	0.45	0.46	0.32	0.37	0.48	0.31	0.39	0.28	0.43	0.54	1.00				
25	0.29	0.34	0.37	0.27	0.32	0.31	0.33	0.33	0.27	0.28	0.21	0.39	0.30	0.39	0.34	0.40	0.20	0.24	0.22	0.38	0.31	0.41	0.58	0.37	1.00			
26	0.34	0.31	0.25	0.37	0.43	0.32	0.26	0.34	0.32	0.33	0.26	0.35	0.27	0.31	0.27	0.32	0.32	0.38	0.41	0.34	0.23	0.33	0.33	0.25	0.37	1.00		
27	0.26	0.31	0.43	0.23	0.29	0.41	0.34	0.18	0.37	0.21	0.44	0.31	0.19	0.27	0.46	0.32	0.37	0.38	0.23	0.26	0.23	0.48	0.54	0.54	0.46	0.18	1.00	
28	0.27	0.28	0.34	0.33	0.30	0.29	0.27	0.41	0.33	0.26	0.23	0.17	0.28	0.46	0.54	0.25	0.38	0.26	0.32	0.31	0.20	0.18	0.39	0.56	0.29	0.26	0.34	1.00

*Name of 1-28 pea genotypes listed in Table 1.

In the dendrogram, 28 genotypes were grouped into 2 main clusters consisting of 6 and 22 genotypes, respectively (Figure 2). Cluster I consisted of 6 genotypes, namely, KPMR-615, KMPR-660, IPF2-13, HFP-9426, DDR-23, and S 143. Cluster II was further divided into 2 subclusters: IIA consisted of a maximum of 12 genotypes (KPMR-675, IPFD-1-10, FC 1, Pant P-31, HUDP-27, HUVP 1, VL-40, Pant P-138, KPMR-662, KPMR-678, IPFD-1-9, and IPF1-22), whereas subcluster IIB consisted of 10 genotypes that, with the exception of IPF-98-1, belonged mainly to the Pant P (Pant P-11, Pant P-25, Pant P-13, and Pant P-14) and HUDP (HUDP-7, HUDP-8, HUDP-18, HUDP-15, and HUDP-9) series pea genotypes (see Figure 2).

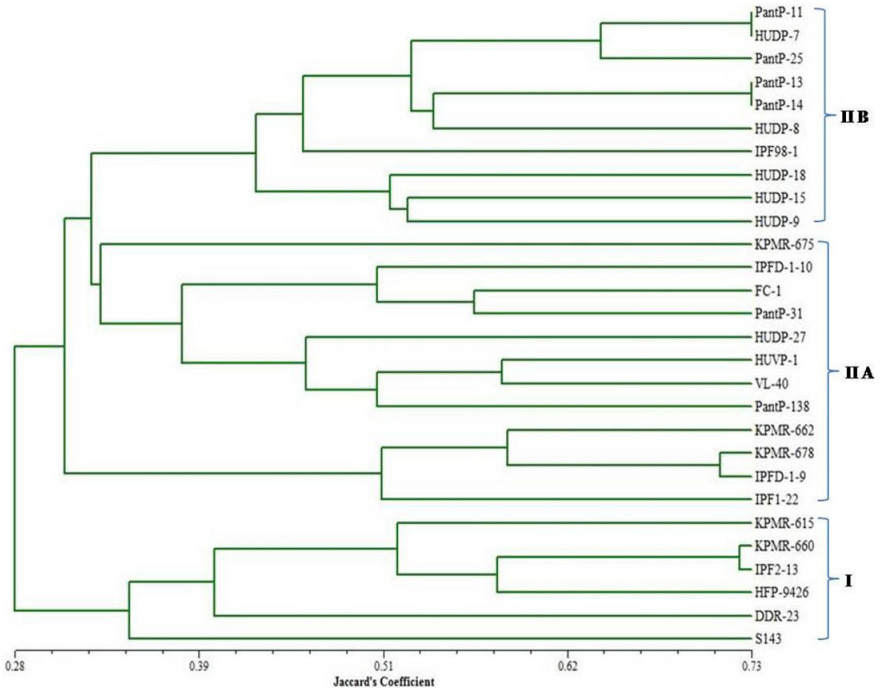

Figure 2. Dendrogram of 28 pea genotypes based on genetic similarity.

PCA revealed that PC 1, PC 2, and PC 3 accounted for 21.6, 16.1, and 14.0% of the total variation, respectively. Together, the first 3 PCs accounted for 51.7% of the total variation. Two-dimensional (2-D; Figure 3) and three-dimensional (3-D; Figure 4) plots were prepared using the first 2 and 3 PCs, respectively. In the 2-D plot, the genotypes were grouped into 4 clusters, whereas most of the UPGMA cluster IIA genotypes were grouped into 2 clusters, i.e., clusters II and III. Three clusters were obtained from the PCA 3-D plot, which corresponded to UPGMA clustering, with the exception of genotype KPMR-675 from UPGMA cluster IIA. Genotype KPMR-675 was included in cluster I of the PCA 3-D plot, and most of the UPGMA cluster IIA and IIB genotypes were together grouped into cluster II of the PCA 3-D plot.

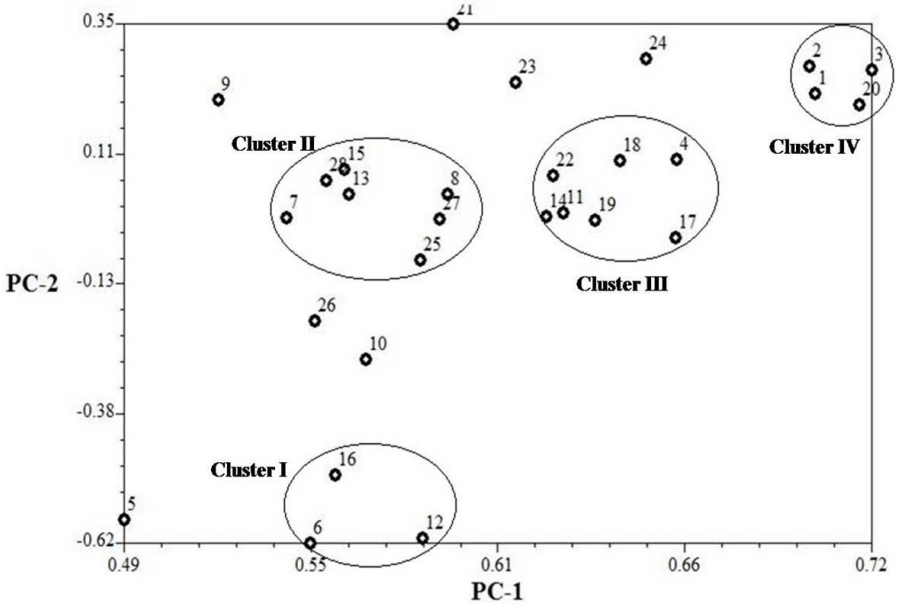

Figure 3. Two-dimensional plot of principal components (PC) 1 and 2 based on pea SSR markers. Name of the 1-28 genotypes is listed in Table 1.

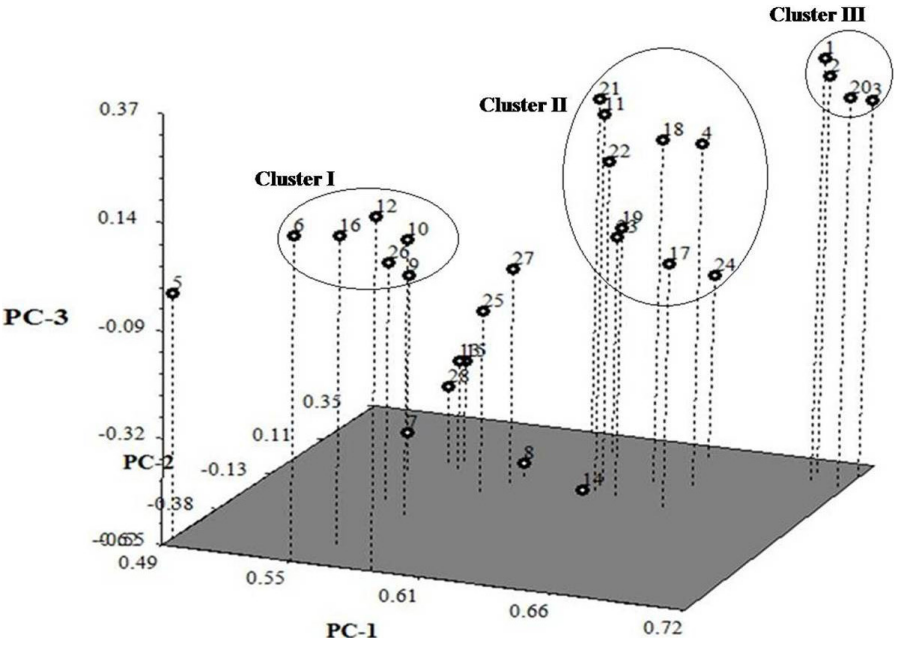

Figure 4. Three-dimensional plot of principal components (PC) 1, 2, and 3 based on pea SSR markers. Name of the 1-28 genotypes is listed in Table 1.

DISCUSSION

Modern molecular markers have an array of applications, including marker-assisted selection (Ek et al., 2005), identification of regions affecting quantitative trait loci (Tar'an et al., 2005), and estimates of diversity (Baranger et al., 2004; Smýkal et al., 2008) in the study of pea. The current composite microsatellite map has 229 codominant SSR molecular markers (216 anonymous and 13 genic) spread over a 1430-cM (Haldane) map, yielding an average 6.2-cM distance between these reliable markers (Loridon et al., 2005). In the present study, the high polymorphism rate (average PIC = 0.502; maximum PIC = 0.650) is owing to the efficiency of the selected SSR primers. Because a marker's importance is based on its proximity to a gene of interest, expressed sequence tag/gene-derived microsatellites are inherently more valuable than random sequence-based markers (De Caire et al., 2012). However, the efficacy of other marker systems, e.g., restriction fragment length polymorphism, RAPD, and AFLPs, cannot be ruled out in pea diversity analyses (Simioniuc et al., 2002; Choudhury et al., 2007; Yadav et al., 2007).

The coefficient of genetic similarity obtained in the present study ranged from 0.11 to 0.73, indicating that a high level of genetic diversity existed among the 28 pea genotypes. Samec and Našinec (1996) have reported a narrow diversity (0.69-0.88) between cultivars of *P. sativum* ssp *sativum* and *P. sativum* ssp *arvense*, whereas a much higher range (0.49-0.98) was obtained between the wild species *P. sativum* ssp. *elatius* and *P. sativum* ssp *humile*. Simioniuc et al. (2002) reported a relatively higher similarity range (0.80-0.94) with RAPD markers compared with that obtained using AFLP markers in pea cultivars (0.85-0.94). Baranger et al. (2004) obtained a very wide range of similarity (0.0-1.0) in 148 *Pisum* genotypes using protein and PCR-based markers. In this study, the estimated genetic diversity (0.05-0.82) among pea accessions based on SSR markers was higher than that reported by Tar'an et al. (2005) (0.0-0.66) and Ford et al. (2002) (0.05-0.48) but similar to those published by Cupic et al., (2009) (0.24-0.84). The higher estimated genetic distance could be ascribed to differences between accessions owing to diversification in the pedigree of the genotypes.

In the present study, the PCA results were similar to those of UPGMA cluster analysis. The genotypes of PCA cluster I formed cluster I of the UPGMA dendrogram with the exception of DDR-23 and S 143 in 2-D PCA. Similarly, most of the cluster IIA and IIB genotypes in the UPGMA dendrogram formed PCA cluster II. Notably, 4 genotypes - Pant P-11, Pant P-13, Pant P-14, and HUDP-7 of cluster IIB in the UPGMA dendrogram - formed an exclusive cluster in PCA. Similarities between some of the genotypes could be explained by common parents in the immediate pedigree. FC 1 was one of the parents of HUDP-7, HUDP-8, HUDP-9, HUDP-15, HUDP-18, HUDP-27, Pant P-11, Pant P-25, Pant P-31, and Pant P-138. Genotypes HUDP-7 and HUDP-8 shared HUP-2 as a common parent. However, cluster I consisted of 6 genotypes with uncommon parents in their pedigree, but all were grouped together. Conversely, KPMR-660 (cluster I) and KPMR-678 (cluster IIA) share a common parent, KPMR-157, but these lines fell into separate clusters. Three KPMR-series pea genotypes (KPMR-662, KPMR-678, and KPMR-675) have uncommon parents in their immediate pedigree but were grouped together in cluster IIA.

Immediate parentage explained some of the clustering but could not account entirely for some of the groupings. In previous studies on pea, the dendrogram obtained from molecular data agreed with the pedigree data (Simioniuc et al., 2002; Baranger et al., 2004) with some notable exceptions. For example, Simioniuc et al. (2002) have observed that genotypes

Baccara and Grafila and genotypes Laser and Algarve cluster together in the UPGMA dendrogram, although they are quite distantly related. Similarly, the cultivar 'Swing', which is a direct progeny of Bohatyr did not cluster with genotypes that have Bohatyr as a parent.

Thus, the clustering patterns generated by UPGMA and PCA were mostly congruent. Messmer et al. (1993) have suggested that for extraction of the maximum information from molecular marker data, ordination methods (PCA and principal coordinates analysis) can be used in combination with cluster analyses, particularly when the first 2 or 3 PCs explain >25% of the original variation. In this study, the first 3 PCs accounted for 51.7% of the total variation. Several studies have been performed for the evaluation of genetic diversity in peas using UPGMA and PCA for analysis of molecular marker data; in general, the results obtained from PCA agreed with UPGMA clustering (Samec et al., 1998; Simioniuc et al., 2002; Baranger et al., 2004; Ta'ran et al., 2005). Baranger et al. (2004) performed PCA on pooled data from several molecular markers (RAPD, ISSR, SSR, sequence tagged sites, allozymes, storage protein) in pea; both PCA and UPGMA classifications resolved the pea genotypes according to their end uses.

The results in the present study enable the selection of some diverse pea genotypes, e.g., HUDP-9, HUDP-15, HUDP-27, Pant P-25, Pant P-31, and S 143. Upon hybridization, these selected genotypes can yield desirable transgressive segregants to improve yield and other related traits in pea. Furthermore, most of the pea genotypes belonging to cluster III have FC 1 as a common parent in their pedigree. FC 1 is a genotype well known to be resistant to 2 major diseases of pea: rust (*Uromyces fabae*) and powdery mildew (*Erysiphe pisi*). Therefore, the inclusion of such FC 1-derived genotypes along with other identified diverse genotypes in pea improvement programs will be useful.

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