African Journal of Biotechnology Vol. 8 (15), pp. 3405-3417, 4 August, 2009
Available online at http://www.academicjournals.org/AJB
ISSN 1684–5315 © 2009 Academic Journals

Full Length Research Paper

Genetic diversity among varieties and wild species accessions of pea (*Pisum sativum* L.) based on SSR markers

J. Nasiri[1], A. Haghnazari[2]* and J. Saba[2]

[1]Department of Plant Breeding, College of Agriculture, Zanjan University, Zanjan- Iran.
[2]Department of Genetics and Plant Breeding, College of Agriculture, Zanjan University, Zanjan- Iran.

Accepted 13 April, 2009

To assess the genetic relations in *Pisum* genus and to examine putative duplicate accessions, 20 pea varieties (*Pisum sativum* L.) with 57 accessions from wild *Pisum* species *fulvum*, subspecies (subsp.) *asiaticum*, *elatius*, *thebaicum, abyssinicum, transcaucasicum* and *arvense* were analyzed using 10 out of 20 microsatellite primer pairs. We genotyped all accessions. In total, 59 alleles were identified in whole collection. The maximum number of alleles (8 alleles) was obtained from the PEACPLHPP, AF004843, and AA43090 loci. The maximum number of private alleles (4) in the wild collection was detected in AF004843 locus but in the cultivar collection, it was detected in AA430902 and PSBLOX13.2 loci. Cluster analysis and principal coordinate analysis located accessions in 3 groups and cultivated varieties were obviously separated from the wild accessions. Analysis of molecular variance (AMOVA) revealed that the intergroups component of variance (29%) is lower than the intragroups component of variance (71%). The lowest value of genetic differentiation (Φ_{PT} = 0.27) of pair wise collections between wild and variety collections, was detected in ssp. *elatius*. Assignment test on the basis of log-likelihood to estimate the likelihood that an individual belongs to a given group, showed that 96% of accessions being assigned correctly to their groups. This study showed that genetic probability profiles of accessions can corroborate clustering analyses while providing additional information as a powerful tool for assigning accessions into their related groups.

Key words: Genetic diversity, SSR, *Pisum*, cluster analysis, assignment test.

INTRODUCTION

Pea (*Pisum sativum* L.) is one of the world's oldest crops, as it was first cultivated with cereals as barley and wheat, 9000 years ago (McPhee, 2003). It is native crop of Syria, Iraq, Iran, Turkey, Israel, Jordan, Ethiopia, Lebanon and has been cultivated in Europe for several thousand years. Furthermore, pea is one of the most important food legumes in the world not only for its very old history of domestication, but also for its versatile use as vegetables, pulses and feed (Choudhury et al., 2006). Smartt and Hymowitz (1985) suggested that the *Pisum* genus is composed of 2 species, *P. sativum* L. and *P. fulvum* Sbith. and Sm. After a significant amount of study and discussion *P. sativum* has been further divided to include several subspecies, ssp. *sativum*, ssp. *elatius*, ssp. *hortense*, ssp. *humile* and *arvense* (McPhee, 2003). Zohary and Hopf (1973) indicated that ssp. *elatius* and ssp. *humile* are the progenitors of pea, *P. sativum, ssp. sativum*. Like all major crop species, cultivated *Pisum* has a condensed gene pool relative to its wild relatives and the relationships within the *Pisum* genus have still generated substantial debate. Some studies have indicated that *P. fulvum* can reasonably be considered as a distinct species, with *P. sativum* forming a subset of *P. elatius* (Vershinin et al., 2003; Baranger et al., 2004; Taran et al., 2005). Other claimed species such as *P. humile* and *P. abyssinicum* have little support from molecular studies.

Most investigators have identified only 1 or 2 legitimate species of *Pisum* including *P. fulvum* and a *P. sativum* complex comprised of 2 main races (*humile* and *elatius*),

*Corresponding author. E-mail: ahaghnazari@gmail.com. Tel.: +982415152465. Fax: +982415152546.

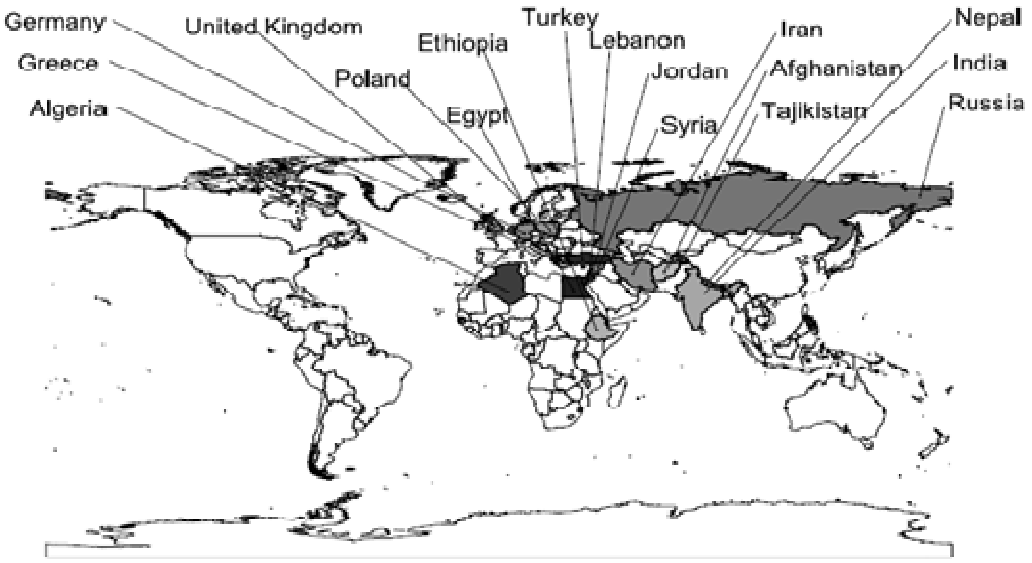

Figure 1 Origin of wild and cultivar accessions of pea.

weedy forms and cultivated varieties. Despite these distinctions, there is an unmistakably close genealogical affinity among all the wild and cultivated taxa of pea (Polans and Saar, 2000).

Most of the recent studies in pea have focused on genetic relatedness within the *Pisum* genus using morphological characters and (or) molecular techniques (Ellis et al., 1998; Hoey et al., 1996; Pearce et al., 2000; Ford et al., 2001; Burstin et al., 2001; Keneni et al., 2005; Haghnazari et al., 2005; Choudhury et al., 2006; Smykal, 2006, 2007), or comparison of different marker types to assess genetic relatedness among a limited number of accessions or specific groups of pea genotypes (Lu et al., 1996; Samec et al., 1998; Simioniuc et al., 2002; Baranger et al., 2004; Taran et al., 2005; Loridin et al., 2005; Smykal, 2008a, 2008b).

Many classes of molecular markers in pea are available, but SSRs have gained popularity because of cost effectiveness, speed, reproducibility and especially polymorphism (Lund et al., 2003; Phippen et al., 1997; Snowdon and Friedt, 2004).

Here we report analysis of SSR data for evaluation of diversity patterns and also to verify the proper classification of *Pisum* accessions in our gene bank using cluster analysis and assignment test. Assignment test uses the multi-locus genotypes of representative individuals from each accession and determines if fixed differences between accessions exist. The method was first implemented by Petkau et al. (1995) and has been used successfully in population and conservation biology studies to assign individuals to specific source populations with as few as 7 polymorphic marker loci (Primmer et al., 2000). The details of this method have been extensively reviewed (Cornuet and Luikart, 1996; Davies et al., 1999; Pritchard et al., 2000; Waser and Strobeck, 1998).

MATERIALS AND METHODS

Plant materials

20 pea varieties with 57 accessions of wild *Pisum* species *fulvum*, subspecies (subsp.) *asiaticum*, *elatius*, *thebaicum, abyssinicum, transcaucasicum* and *arvense* originated from 17 countries (Figure 1) were used in the analysis (Tables 1 and 2). Seed samples of wild accessions were kindly provided by Dr. Berner (ICARDA). Eight seeds of each variety or accession were grown in pots of 10 cm diameter in the glasshouse (20°C ± 2°C) until 3 weeks old.

DNA extraction

Total genomic DNA was isolated from young leaf tissue from 5 individuals of each accession using the CTAB method of Taylor et al. (1995) with minor modifications.

SSR analysis

20 SSR primer pairs were selected from Burstin et al. (2001) and Taran et al. (2005) according to highly polymorphism for pea germplasm in previouse studies (Table 3). PCR was carried out in a Bio-Rad thermocycler. The amplification was done in a 25 µl volume containing 30 ng template DNA, 20 pM of each primer, 1 unit Taq DNA polymerase (Qiagen, Australia). 0.2 mM of each dNTP, 2.5 mM $MgCl_2$, 50 mM KCl and 10 mM Tris- HCl. Amplification was performed with the “touch down” PCR profile (Don et al., 1991) 3 min at 94°C; 10 cycle of 30 s at 94°C; 30 s at 62/66°C, decreasing by 1 per cycle, depending on the annealing temperature of the SSR primers, 45 s at 72°C and 5 min at 72°C for final extension. 10 µl of PCR products were electrophoresed on 1.7% agarose gels and were visualized by staining with ethidium bromide. 0.3 µl of this mixture were resolved on 6% polyacrylamide using an automated DNA sequencer Gel-Scan 2000 (www.Corbettresearch.com). Gels were prerun for 30 min at 1200 V, 25 mA. PCR products were run for 30 - 45 min at 1200 V, 25 mA. Images of geles were treated in 2 steps with one-Dscan software. In the first step, the height of each bands for each individual were identified and determined allele

Table 1. List of wild accessions of pisum genus used for this study.

S/N	IG*	Species	Origin
1	IG 52390	*Pisum fulvum*	Syrian Arab Republic
2	IG 52391	*Pisum fulvum*	Syrian Arab Republic
3	IG 52392	*Pisum fulvum*	Syrian Arab Republic
4	IG 52394	*Pisum fulvum*	Syrian Arab Republic
5	IG 52396	*Pisum fulvum*	Syrian Arab Republic
6	IG 52397	*Pisum fulvum*	Syrian Arab Republic
7	IG 52411	*Pisum fulvum*	Syrian Arab Republic
8	IG 52409	*Pisum fulvum*	Syrian Arab Republic
9	IG 52404	*Pisum fulvum*	Syrian Arab Republic
10	IG 52399	*Pisum fulvum*	Tajikistan
11	IG 52568	*Pisum fulvum*	Jordan
12	IG 52569	*Pisum fulvum*	Jordan
13	IG 52570	*Pisum fulvum*	Jordan
14	IG 52571	*Pisum fulvum*	Jordan
15	IG 52594	*Pisum sativum* Var. *arvense*	Algeria
16	IG 52595	*Pisum sativum* Var. *arvense*	Algeria
17	IG 52599	*Pisum sativum* Var. *arvense*	Tajikistan
18	IG 64207	*Pisum fulvum*	Jordan
19	IG 114845	*Pisum sativum* Var. *arvense*	Nepal
20	IG 122974	*Pisum sativum* subsp. *asiaticum*	Tajikistan
21	IG 52448	*Pisum fulvum*	Syrian Arab Republic
22	IG 52450	*Pisum fulvum*	Syrian Arab Republic
23	IG 52451	*Pisum fulvum*	Turkey
24	IG 52505	*Pisum sativum* subsp. *eliatus*	Turkey
25	IG 52507	*Pisum sativum* subsp. *eliatus*	Turkey
26	IG 52546	*Pisum fulvum*	Jordan
27	IG 52561	*Pisum fulvum*	Jordan
28	IG 52567	*Pisum fulvum*	Jordan
29	IG 49288	*Pisum sativum* Var. *arvense*	Iran
30	IG 49352	*Pisum sativum* subsp. *eliatus*	Greece
31	IG 49546	*Pisum sativum* subsp. *thebisum*	Rusian Federation
32	IG 49547	*Pisum sativum* subsp. *thebisum*	India
33	IG 49559	*Pisum sativum* Var. *arvense*	India
34	IG 51511	*Pisum sativum* Var. *arvense*	Ethiopia
35	IG 51496	*Pisum sativum* subsp. *abysinicum*	Ethiopia
36	IG 50759	*Pisum sativum*	Germany
37	IG 49572	*Pisum sativum* subsp. *abysinicum*	United Kingdom
38	IG 49571	*Pisum sativum* subsp. *abysinicumum*	Rusian Federation
39	IG 128788	*Pisum sativum*	Rusian Federation
40	IG 123114	*Pisum sativum* subsp. *Abysinicum*	Ethiopia
41	IG 123085	*Pisum sativum* subsp. *elatius*	Lebanon
42	IG 123079	*Pisum sativum* subsp. *asiaticum*	Syrian Arab Republic
43	IG 123022	*Pisum sativum* subsp. *asiaticum*	Afghanistan
44	IG 123073	*Pisum sativum* subsp. *asiaticum*	Algeria
45	IG 123043	*Pisum sativum* subsp. *asiaticum*	Egypt
46	IG 123030	*Pisum sativum* subsp. *asiaticum*	Afghanistan
47	IG 52439	*Pisum fulvum*	Syrian Arab Republic
48	IG 52437	*Pisum fulvum*	Syrian Arab Republic
49	IG 52432	*Pisum fulvum*	Syrian Arab Republic
50	IG 52428	*Pisum fulvum*	Syrian Arab Republic
51	IG 52421	*Pisum fulvum*	Syrian Arab Republic

Table 1. Contd.

52	IG 52420	*Pisum fulvum*	Syrian Arab Republic
53	IG 52419	*Pisum fulvum*	Syrian Arab Republic
54	IG 52416	*Pisum fulvum*	Syrian Arab Republic
55	IG 52412	*Pisum fulvum*	Syrian Arab Republic
56	IG 52441	*Pisum fulvum*	Syrian Arab Republic
57	IG 52441	*Pisum fulvum*	Syrian Arab Republic

*ICARDA gene bank identification code.

Table 2. List of pea varieties used for this study.

S/N	GB*	species	Origin
1	Pz-053	*Pisum sativum* subsp. *sativum*	Germany
2	Pz-020	*Pisum sativum* subsp. *sativum*	Germany
3	Pz-041	*Pisum sativum* subsp. *sativum*	Poland
4	Pz-043	*Pisum sativum* subsp. *sativum*	Poland
5	Pz-010	*Pisum sativum* subsp. *sativum*	Iran
6	Pz-042	*Pisum sativum* subsp. *sativum*	Poland
7	Pz-052	*Pisum sativum* subsp. *sativum*	Germany
8	Pz-009	*Pisum sativum* subsp. *sativum*	Iran
9	Pz-029	*Pisum sativum* subsp. *sativum*	Poland
10	Pz-028	*Pisum sativum* subsp. *sativum*	Poland
11	Pz-047	*Pisum sativum* subsp. *sativum*	Iran
12	Pz-058	*Pisum sativum* subsp. *sativum*	Russian Federation
13	Pz-018	*Pisum sativum* subsp. *sativum*	Germany
14	Pz-011	*Pisum sativum* subsp. *sativum*	Iran
15	Pz-005	*Pisum sativum* subsp. *sativum*	Iran
16	Pz-024	*Pisum sativum* subsp. *sativum*	Russian Federation
17	Pz-059	*Pisum sativum* subsp. *sativum*	Russian Federation
18	Pz-050	*Pisum sativum* subsp. *sativum*	Germany
19	Pz-046	*Pisum sativum* subsp. *sativum*	Iran
20	Pz-054	*Pisum sativum* subsp. *sativum*	Germany

*GB: Zanjan university gene bank identification code.

sizes. In the second step, allele sizes were scored assuming the absence (0) or presence (1).

Statistical analysis

Microsatellite allele frequencies, the number of alleles (n_o) effective number of alleles per locus (n_e) and polymorphism information content (PIC) (Botstein et al., 1980) were calculated using the Pop-Gene program (Yeh et al., 1997). Probablity of identity (PI) (Paetkau et al., 1995) was calculated according to Tessier et al. (1999). Gene diversity and private alleles were caculated using geneclass 2 (Piry et al., 2004; available at http://www.ensam.inra.fr/URLB) and GenAlex program ver.6 (Peakall and Smouse, 2006), respectively.

All DNA marker data were processed by NTSYS-pc version 2.2 software (Rohlf, 2006), using SIMQUAL module with the Jaccard genetic similarity coefficient (GSj). Cluster analysis was conducted based on similarity estimates, by using the unweighted pair group method on arithmetic averages (UPGMA). The resulting clusters were expressed as a dendrogram. Furthermore, UPGMA clustering method on the basis of Φ_{PT} (genetic differentiation) values (Maguire et al., 2002), was applied on 8 groups consisting of cultivars, *P. fulvum* species and 6 different subspecies of *P. sativum*.

Principal co-ordinates analysis

In order to more-effectively view, the patterns of grouping, principal co-ordinates analysis (PCOA) was performed with GenAlex ver.6 (Peakall and Smouse, 2006). This multivariate approach was used to complement the information generated from cluster analysis, because cluster analysis is more sensitive to closely related individuals, whereas PCOA is more informative regarding distances among major groups (Hauser and Crovelo, 1982; Sun et al., 2001).

Assignment test

The assignment tests to estimate the likelihood that an individual belongs to a given group by calculating the expected frequency of the observed genotype in each of the groups were carried out using

Table 3. List of 20 SSR primers used for this study with primer sequences and characteristics of these markers

S/N	Locus	Accession No.	Forward and reverse primers	Length (bp)	T_m (ºC)	Motif	Repeats	Alleles
1	PSBLO X13.1[a]	X78581	5'GAACTAGAGCTGATAGCATGT3' 5'GCATGCAAAAGAACGAAACAGG3'	21 22	54	AT	17	6
2	PSGAP A1[a]	X15190	5'GACATTGCCAATAACTGG3' 5'GGTTCTGTTCTCAATACAAG3'	21 20	51	AT	17	7
3	PSADH 1[a]	X06281	5'GATGTGATAGGCCTAGAACAAGC3' 5'CAGTCACACACTACAAGAGATC3'	23 22	54	AT	10	-
4	PEACP LHPPS[a]	L19651	5'GTGGCTGATCCTGTCAACAA3' 5'CAACAACCAAGAGCAAAGAAAA3'	20 22	58	AT	6	8
5	PSRBC S3C[a]	X04334	5'CCCAGTGAAGAAGGTCAACA3' 5'CAATGGTGGCAAATAGGAAA3'	20 20	58	AT	6	3
6	AF016458[a]	AF016458	5'CACTCATAACATCAACTATCTTTC3' 5'CGAATCTTGGCCATGAGAGTTGC3'	24 23	54	TC	9	-
7	AF004843[a]	AF004843	5'CCATTTCTGGTTATGAAACCG3' 5'CTGTTCCTCATTTTCAGTGGG3'	21 21	54	TC	7	8
8	PSP4O SG[a]	X51594	5'CAACCAGCCATTATACACAAACA3' 5'GGCAATAAAGCAAAAGCAGA3	23 20	58	AAT	36	-
9	AA430942[a]	AA430942	5'CTGGAATTCTTGCGGTTTAAC3' 5'CGTTTTGGTTACGATCGAGCAT3'	21 22	54	AAT	7	8
10	PSBLO X13.2[a]	X78581	5'CTGCTATGCTATGTTTCACATC3' 5'CTTTGCTTGCAACTTAGTAACAG3'	22 23	54	CAT	8	-
11	PSCAB 66[a]	M64619	5'CACACGATAAGAGCATCTGC3' 5'GCTTGAGTTGCTTGCCAGCC3'	20 20	55	CAT	5	3
12	PEAPH TAP[a]	M37217	5"TGGATTGGATTGGATGATGA3' 5"TGGAGCCCTTAGTCCACAAC3'	20 20	60	AAT	4	3
13	PSAJ22 3318[a]	AJ223318	5'CAGTGGTGACAGCAGGGCCAAG3' 5'CCTACATGGTGTACGTAGACAC3'	22 22	58	CAT	6	6
14	PSMPS AA278[b]	-	5'CCAAGAAAGGCTTATCAACAGG3' 5'TGCTTGTGTCAAGTGATCAGTG3'	22 22	60	-	-	7
15	PSMPS AD141[c]	-	5'AATTTGAAAGAGGCGGATGTG3' 5'ACTTCTCTCCAACATCCAACGA3'	21 22	50	-	-	-
16	PSMPS AD237[d]	-	5'AGATCATTTGGTGTCATCAGTG3' 5'TGTTTAATACAACGTGCTCCTC3'	22 22	60	-	-	-
17	PSMPS AD270[d]	-	5'CTCATCTGATGCGTTTGGATTAG3' 5'AGGTTGGATTTGTTGTTTGTTG3'	23 22	60	-	-	-
18	PSMPS AA456[e]	-	5'TGTAGAAGCATAAGAGCGGGTG3' 5'TGCAACGCTCTTGTTGATGATT3'	22 22	60	-	-	-
19	PSMPS AA476[e]	-	5'TAGTTTTGAACTTTGGCCGTAT3' 5'CACACCCTAATCTAGGCTATCC3'	22 22	60	-	-	-
20	PSMPS AA473[e]	-	5'CAATCGATCAGACAGTCCCCTA3' 5'AAGCTCACCTGGTTATGTCCCT3'	22 22	60	-	-	-

[a]Burstin et al. (2001).
[b]USDA/ARS, Washington State University, USA.
[c]Universi y of Melbourne, Australia.
[d]Lochow Petkus, Germany.
[e]University of Saskatchewan, Canada. (b, c , d, e, from Taran et al. 2005)

the Geneclass 2 software program (Piry et al., 2004). We used the "Frequency" option (and not the "Bayesian" option). Simulation algorithm was based on Paetkau et al. (2004) and the number of simulated individuals was set on 10000 to compute the allele frequencies. This method is more similar to the Buchanan et al. (1994) method, which employs the allele frequencies observed in

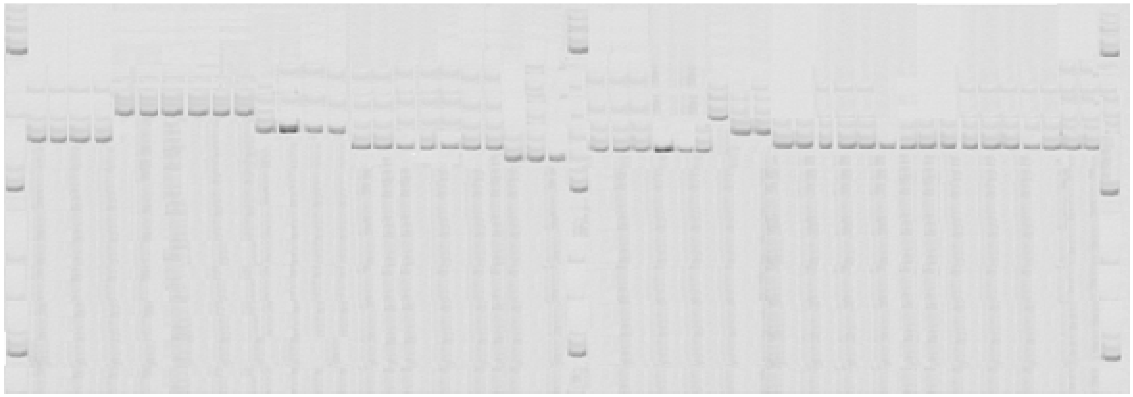

Figure 2. Microsatellite banding patterns generated by the SSR primer pair PSAJ3318 (L - 50 bp ladder) using an automated DNA sequencer Gel-Scan 2000.

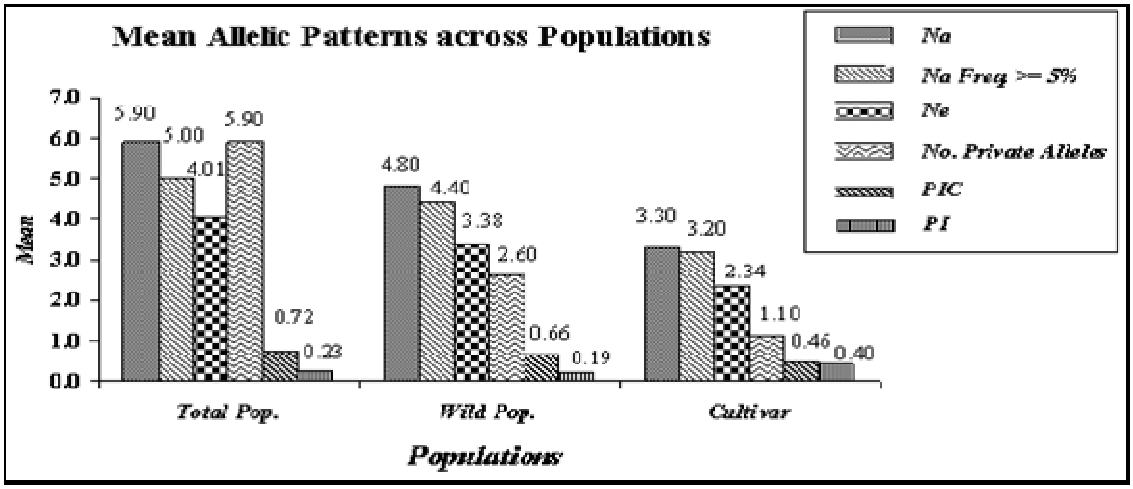

Figure 3. Mean allelic pattern for total, wild and cultivar groups

the population sample to calculate assignment probabilities. Also we used GenAlex software ver.6 (Peakall and Smouse, 2006) to produce graphical output for assignment test results.

RESULTS

Only 10 microsatellite primer pairs amplified scorable bands in the *Pisum* accessions. In whole samples, these 10 microsatellite markers were used for analysis of 77 genotypes of pea (Figure 2). In total, 59 alleles were detected by 10 SSR markers. The number of alleles per locus varied from 2 to 8, with a mean of 5.9 alleles/locus. 10 loci displayed polymorphism among the total accessions analyzed. In the total accessions, the most variable loci, that is PEACPLHPPS, AF004843, AA430902 had 8 alleles, and PSRBCR3C, PEAPHTAP and PSCAB66 loci had the lowest number of alleles so that produced only 3 alleles across total accessions, the remaining loci showed more than 3 alleles each.

In the wild and cultivar collections, 48 and 33 alleles were detected by 10 markers, respectively. In the wild accessions, the number of alleles per locus varied from 2 to 7, with a mean of 4.8 alleles/locus but in cultivars the number of alleles varied from 1 to 6, with a mean of 3.3 alleles/locus (Figure 3). In the wild accessions, PSGAPA 1, PEACPLHPPS, AF004843 and PSmpsaa278c loci gave the maximum number of alleles (that is, 7 alleles) but in the cultivars only PSGAPA1 locus gave the maximum number of alleles (that is 5 alleles). In the wild accessions, the maximum number of private alleles (4) was detected in AF004843 locus but in the cultivars, the maximum number of private alleles were detected in AA430902 and PSBLOX13.2 loci. The calculated mean of private alleles in total, wild and cultivar collections were 5.9, 2.6 and 1.1, respectively (Figure 3).

Probablity of Identity (PI) is defined as the probability with which 2 random genotypes display the same SSR profile. The calculated PI value for each locus across all accessions (Table 4) varied from 0.416 for PSCAB66 to 0.083 in AA430902. The higher PI values reflect the low

Table 4. The number of samples (N), number of alleles (n_a), effective number of alleles per locus (n_e), polymorphism information content (PIC) and probability of identity (PI) for total population.

Locus	N	Na	Ne	PI	PIC
PSGAPA1	77	7.000	5.620	0.095	0.822
PEACPLHPPS	77	8.000	4.680	0.127	0.790
PSRBCR3C	77	3.000	2.352	0.385	0.580
AF004843	77	8.000	5.072	0.119	0.803
AA430902	76	8.000	6.201	0.083	0.840
PSBLOX13.1	77	6.000	3.337	0.233	0.700
PEAPHTAP	77	3.000	2.917	0.340	0.670
PSCAB66	77	3.000	2.250	0.416	0.560
PSAJ3318	77	6.000	2.696	0.352	0.630
PSmpsaa278c	77	7.000	4.945	0.120	0.800
Total	-	59.000	40.068	2.270	7.200
Mean	-	5.900	4.010	0.227	0.720
Standard error	-	0.674	0.460	0.062	0.034

power of differentiation between 2 random genotypes using a specific SSR marker. The high and low PI values (that is 0.376 and 0.095) in the wild accessions group was detected in PEAPHTAP and PSGAPA1 loci, respectively, and in the cultivars group the highest and the lowest PI values (that is, 1 and 0.093) were observed in PEAPHTAP and PSCAB66 loci, respectively (Figure 3).

The polymorphism information content (PIC) in whole collection, varied from 0.556 to 0.839 and averaged as 0.72. In total accessions the highest PIC (that is 0.839) was observed in AA430902 locus, but in both wild and cultivar populations the highest PIC (that is, 0.818 and 0.765 respectively) was detected only PSGAPA1 locus. Average of calculated PIC values for both wild and cultivar collections were 0.66 and 0.46, respectively (Figure 3). The relationship between the polymorphism information content (PIC) and probability of identity (PI) of 10 microsatellite markers was calculated for whole accessions and a significant correlation was detected ($R^2 = 0.983$; $P < 0.01$).

Cluster analysis

Cluster analysis of the genetic similarity data grouped the 77 accessions into 3 major clusters with 2 sub-groups in the first and second clusters (1A, 2A and 1B, 2B; respectively, Figure 4). The UPGMA procedure grouped most of the wild accessions into their subspecies. The majority of *P. fulvum* accessions (28 accessions) were well separated from other subspecies and cultivar accessions so they were replaced into the first major group. The sub-group 1A consisted 27 accessions of *P. fulvum* originated from Syria, Jordan, Turkey and Tajikistan and the sub-group 2A included only one accession of *P. fulvum* originated from Syria.

All *P. sativum* ssp. accessions with 4 accessions of *P. fulvum*, one originated from Syria and 3 accessions from Jordan were placed in the second group. Whole accessions of ssp. *elatius* and *arvense* with one accession of ssp. *asiaticum* originated from Tajikistan and 1 accession of *P. fulvum* originated from Syria were clustered in sub-group 1B. Three accessions of *P. fulvum* with same origin (Jordan) and 2 accessions of ssp. *transcacasicum* originnated from Germany and Russia with all accessions of *ssp. asiaticum* were placed in sub-group 2B. This sub-group also included 3 accessions of ssp. *abyssinicum* originated from Russia, Ethiopia and United Kingdom. Accessions from ssp. *thebaicum* originated from Russia and India with one genotype of ssp. *abyssinicum* were clustered into sub-group 2C. Finally whole accessions of cultivars were placed in the third major group.

Cluster analysis of groups

To assess the efficiency of microsatellites for differentiation of *Pisum* ssp, pairwise estimates of Φ_{PT} values were obtained for all groups, using the polymorphism detected by 10 pea microsatellites. The highest value of Φ_{PT} was observed between ssp. *transcaucasicum* and ssp. *abyssinicum* (0.82), whereas, the lowest value was detected between ssp. *asiaticum* and ssp. *arvense* (0.02). Otherwise, when we compared genetic differentiation (Φ_{PT}) values between cultivar collection and other collections, interesting result was obtained; the lowest PhiPT (0.27) was observed between cultivar and ssp. *elatius*. This represent that the similarity of genetic background of ssp. *elatius* and cultivar accessions is high (Table 5).

Dendrogeram of 8 collections indicated that the *P. fulvum* accessions were completely separated from the others (Figure 5). Both collections of ssp. *arvense* and

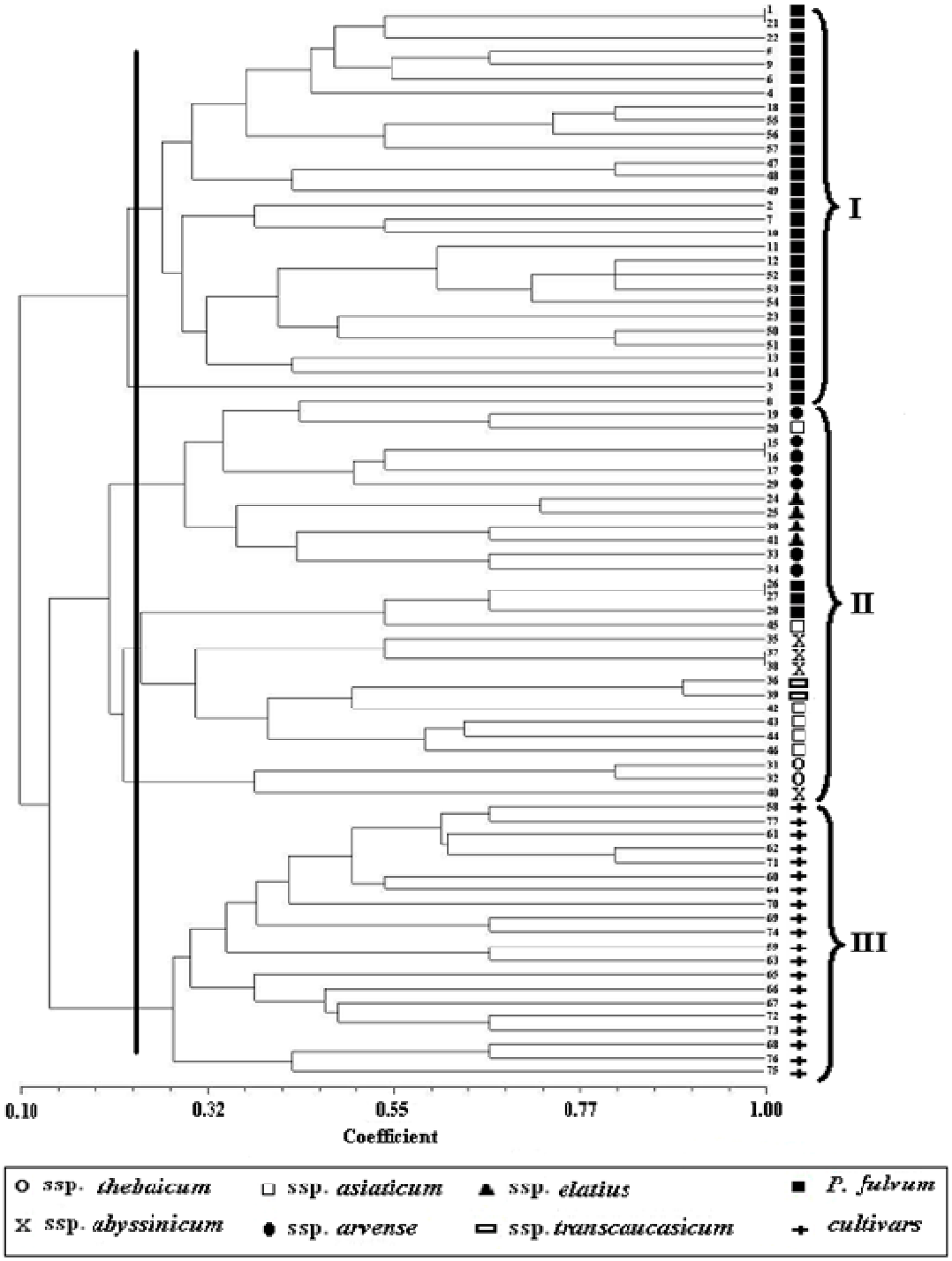

Figure 4. Dendrogeram for 57 wild Pisum accessions and 20 pea varieties based on UPGMA clustering method with genetic similarity from 10 SSR markers.

ssp. *asiaticum* were placed in the same group. The collections of ssp. *elatius* with cultivars formed a distinct cluster and finally, the collections of ssp. *thebaicum,* ssp. *transcacasicum* and ssp. *abyssinicum* were placed in the separate clusters.

Associations among 57 wild *Pisum* accessions and 20 varieties were also examined by means of PCOA. The results of PCO analysis of the pairwise individual genetic distance matrices for SSRs are shown in Figure 7. The first 2 principal coordinates, PC1 and PC2 explained 40.06 and 20.86% of the total variation, respectively (Figure 6). The PCOA generated a good separation of

Table 5. Calculated genetic differentiation (Φ_{PT}) values for eight collections.

Collections	*P. fulvum*	ssp. *arvense*	ssp. *asiaticum*	ssp. *elatius*	ssp. *transcaucasicum*	ssp. *thebaicum*	ssp. *abyssinicum*	Cultivars
P. fulvum	0.00							
ssp. arvense	0.26	0.00						
ssp. asiaticum	0.34	0.02	0.00					
ssp. elatius	0..33	0.06	0.11	0.00				
ssp. transcaucasicum	0.48	0..29	0.45	0.16	0.00			
ssp. thebaicum	0..38	0..25	0.25	0..20	0..50	0.00		
ssp. abyssinicum	0.41	0..37	0.44	0..39	0.82	0..33	0.00	
Cultivars	0.40	0..33	0..35	0..27	0..37	0..33	0..35	0.00

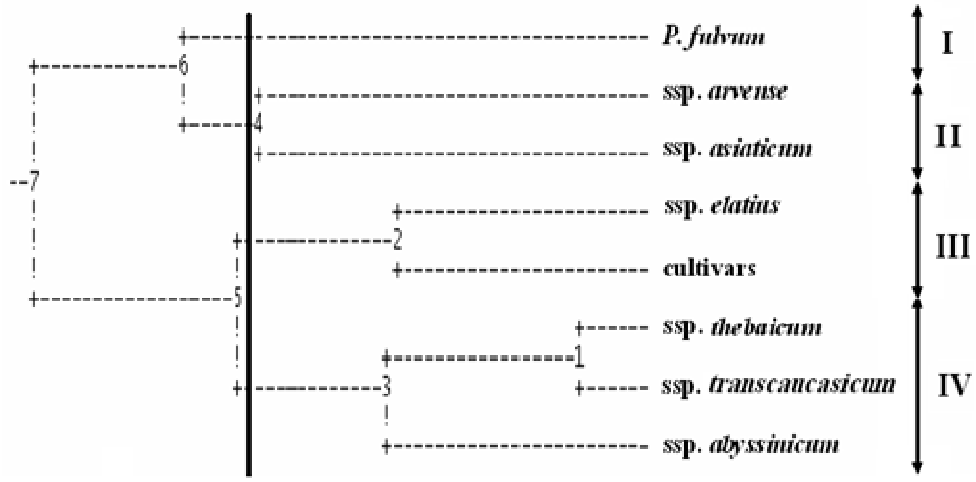

Figure 5. Dendrogeram of eight collections of pea based on Φ_{PT} (genetic differentiation) values.

the cultivated varieties from the wild accessions. The PC2 distinctly separated whole accessions of *P. fulvum* from the wild subspecies of *Pisum* and were intermixed into a large group. On the other hand, most of the wild subspecies were clubbed in to one group.

Assignment test

In general, in all *Pisum* accessions there was concordance between relationships revealed in the dendrograms, the probabilities of assignment derived from analysis and the pairwise tests of population differenttiation. The dendrograms clustered accessions with similar probability profiles. The unique components of these accessions did not cluster with those that included misassigned accessions. Similarly, tests of population differentiation support the relationship among accessions as indicated by the dendrogram. Accessions that are not significantly different are located in the same cluster. Based on the allele combination at all loci, on average, 96% of the accessions were correctly assigned to the group that they had been sampled (species, subspecies and cultivars) and the remaining accessions (4%) of these were generally assigned to neighbouring groups. Two accessions of ssp. *asiaticum* (No. 20 and 45) originnated from Tajikistan and Egypt, respectively, were assigned to the ssp. *arven-se* collection. Also, one accession of ssp. *abyssinicum* from Ethiopia (No. 40) was assigned to ssp. *asiaticum* collection (Figure 7).

DISCUSSION

The current study was conducted to assess the pattern of genetic relations among 57 wild accessions of *Pisum* genus and 20 pea varieties based on SSR loci. Furthermore, we used clustering method and assignment test to identify the group of origin of an individual and allocate individual accessions into their real groups.

Among 20 SSR loci only 10 were selected for analysis on the basis of their high polymorphism. The number of observed alleles, showed that in whole collection, AF004 843 $(TC)_7$, AA430902 $(AAT)_7$ and PEACPLHPPS $(AT)_6$

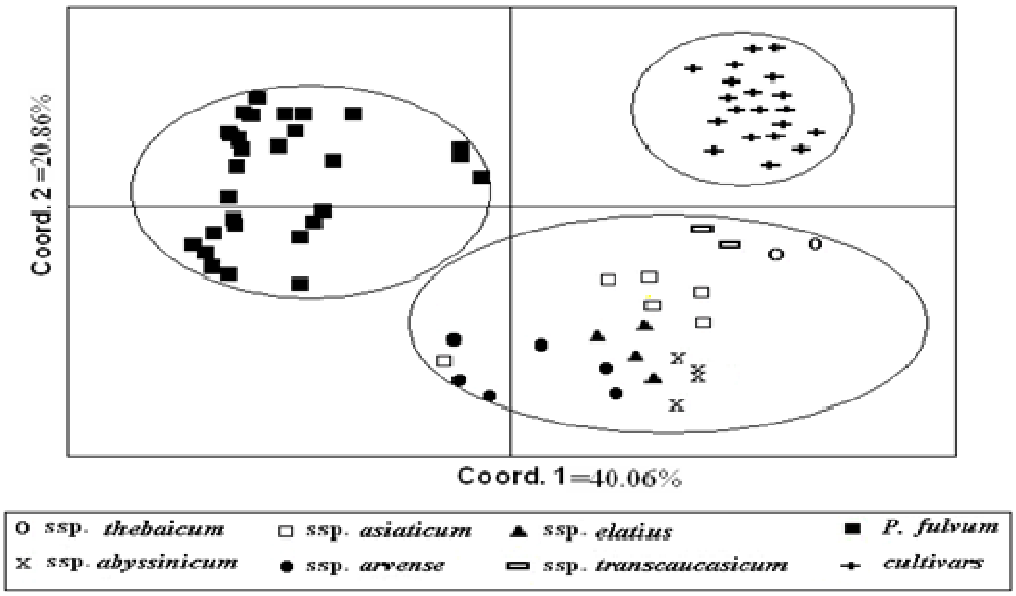

Figure 6. Biplot distribution pattern of 20 pea varieties and 57 wild Pisum accessions according to the first 2 principal coordinates (PC1 and PC2).

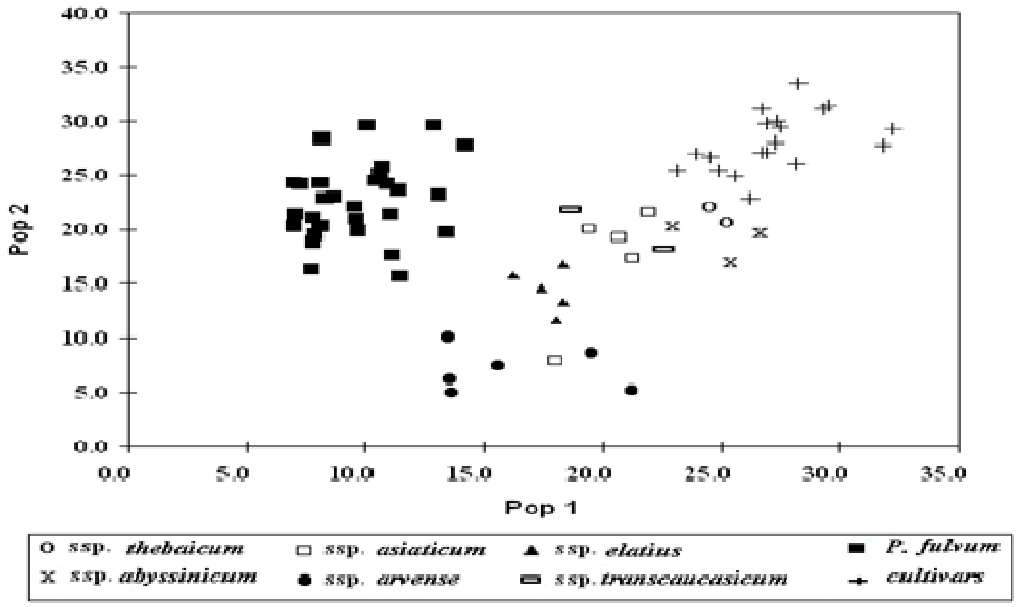

Figure 7. Distribution of the 57 wild Pisum accesions with 20 pea cultivars based on assignment test

loci possess the highest levels of polymorphism and out of 59 alleles, 41% were detected at these 3 loci. PSGAPA1 $(AT)_{17}$ locus was not one of the loci that had maximum number of alleles, but when we analyzed accessions in 2 separate groups (that is wild accessions and cultivars) this locus gave the maximum number of alleles. Furthermore, in both wild accessions and cultivars the highest value of PIC (that is 0.818 and 0.765, respectively) was detected only in PSGAPA1 locus. This locus had a high level of polymorphism in pre-vious studies as well (Burstin et al., 2001; Baranger et al., 2004; Haghnazari et al., 2005). In previous studies, AF004843 and AA430902 loci had low levels of polymorphism. For example, Burstin et al. (2001) reported 4 alleles for both loci and Haghnazari et al. (2005) reported 4 and 3 alleles, respectively whereas here, these loci

showed higher polymorphism. The level of polymorphism as estimated by PIC values were significantly correlated with PI values (R^2 = 0.983, P < 0.01). These indices estimate markers level of polymorphism and allow the most useful markers to be chosen for genetic studies in pea (Loridin et al., 2005). Furthermore, the negative correlation between PIC and PI in our study (r = -0.99 , P < 0.01) show that usually high PIC values are obtained by low PI values, therefore these results help us in detection of the most informative markers to reduce the number of loci needed for reliable genotype distinction. For this purpose the usefulness of this set of markers was assessed as the probability of identity (PI) overall accessions which was varied from 0.416 for PSCAB66 locus to 0.083 in AA430902 locus with an average of 0.29. A low PI value for a locus shows the high discriminating power of specified primers. It is difficult to compare level of diversity between different studies, because both the number of alleles detected per marker and the genetic diversity of the markers depend on the number of genotypes analyzed (Burstin et al., 2001). However, in pea, Burstin et al. (2001), Ford et al. (2001), Baranger et al. (2004), Haghnazari et al. (2005), Loridin et al. (2005), Taran et al. (2005), Choudhury et al. (2006), Smykal et al. (2008a,b) reported the mean number of alleles per polymorphic marker as 3.6, 5.0, 4.0, 5.0, 3.8 ,4.0, 9.65, 3.8 and 5.3, respectively. Also in pea, Loridin et al. (2005), Haghnazari et al. (2005), Smykal et al. (2008a,b) reported mean value of PIC as 0.62, 0.53, 0.52 and 0.89, respectively. In this study mean number of alleles per locus was obtained as 5.9. These levels of polymorphism, when compared to data obtained in other studies for other species, indicate that pea represents a rather polymorphic autogamous species (Loridin et al. 2005). In barley, 15 SSRs revealed a mean number of 3.5 alleles per polymorphic marker and an average PIC value of 0.45 among 26 accessions (Hamza et al., 2004). In sunflower, 170 SSRs revealed 3.5 alleles per locus with a mean PIC value of 0.55 in 16 accessions (Paniego et al., 2002) and 300 SSRs revealed a mean number of 3.6 alleles per polymorphic marker among 24 accessions (Tang et al., 2003). In tomato, 65 polymorphic SSRs revealed 2.7 alleles per locus with a mean PIC value around 0.35 among 19 accessions (He et al., 2003). In *Oryza sativa* L., 22 SSRs revealed an average PIC value of 0.73 among 13 diverse accessions (Coburn et al., 2002).

Some authors reported a significant correlation between the number of variants for a given SSR and the length of these SSRs (Innan et al., 1997; Udupa et al., 1999; Burstin et al., 2001). In our study this correlation (R^2 = 0.35, P > 0.05) was not significant, which is in agreement with Haghnazari et al. (2005).

Also, there was no significant correlation between the number of motif repeats and number of alleles observed at SSR locus (R^2 = 0.153, P> 0.05). Rongwen et al. (1995), Szewc-Mcfadden et al. (1996), Struss and Plieske (1998) and Haghnazari et al. (2005) working with soybean, rapessed, apple, barley and pea, respectively, reported similar results.

The molecular-marker-based UPGMA cluster analysis and PCOA demonstrated that all the cultivated genotypes were clearly separated from the wild *Pisum* genus accessions. These results are in agreement with previous studies of Hoey et al. (1996) Ellis et al. (1998), Ford et al. (2001), Burstin et al. (2001), Baranger et al. (2004) and Taran et al. (2005). PCO analysis separated completely all *P. fulvum* accessions (100%) from the other accessions. This is in agreement with Ford et al. (2001) as in their study, *P. fulvum* accessions were distinctly separated from other accessions.

Assignment tests (Paetkau et al., 1995) are used to estimate the likelihood that an individual belongs to a given population by calculating the expected frequency of the observed genotype in each of the populations. The individual is then assigned to the population that has the highest likelihood of containing a member with the observed genotype. Rejection and acceptance of membership are based on a likelihood-ratio test (Siegismund 1995). All cultivars and *P. fulvum* accessions were correctly assigned to their own groups, and only 3 accessions assigned to the neighboring groups. Two genotypes of ssp. *asiaticum* originated from Tajikistan and Egypt, respectively, were assigned to the ssp. *arvense* group and only 1 genotype of ssp. *abyssinicum* from Ethiopia was assigned to ssp. *asiaticum* group (Figure 7). In the study of Scef et al. (1999) using 9 SSRs, 66% of grapevine accessions were correctly assigned to their populations. In another study by Jepsen et al. (2001), by 5 SSRs, 62% of native caribou were correctly assigned to their populations.

Our results confirm that SSR markers can successfully differentiate between wild accessions and cultivars of pea. On the other hand, based on cluster analysis, PCOA and assignment test, it was revealed that the genetic basis of *P. fulvum* is different than other wild ssp. of *Pisum* genus and varieties of *P. sativum.* This is in agreement with the report of Smartt et al. (1985). These literatures indicated that the *Pisum* genus is composed of 2 species: *P. sativm* L. and *P. fulvum* Sbith. and Sm.

Also, Hoey et al. (1996), Saar and Polans (2000), Ford et al. (2001), Baranger et al. (2004) and Kosterin et al. (2008) reported similar results, as in their studies, whole accessions of *P. fulvum* were placed in separate group. In fact, along with a large and very variable species *Pisum sativum* L., all authors recognize a clear-cut and peculiar species *P. fulvum* Sibth. and Sm., which is almost completely isolated from *P. sativum* (Kosterin et al., 2008).

Based on our results and literature (Hoey et al., 1996; Ford et al., 2001; Baranger et al., 2004; Kosterin et al., 2008), genetic background of *P. fulvum* is more different from another species and subspecies of *P. sativum* and may, consequently, have been distantly related. To mini-

mize the negative effects of distant relatedness in unadapted germplasm, Sorrells and Wilson (1997) suggested the concept of parent building, the gradual incurporation of a few traits through some form of back-crossing or marker-assisted selection to make use of allelic variation. In addition, suggested that to remove or decrease of negative effects arise genetic distance of wild *P. fulvum* and pea cultivars, gene transformation can be useful.

On the other hand, it is necessary also to mention that the lowest Φ_{PT} value (0.27) was observed between cultivar and ssp. *elatius* show that the similarity of genetic background of ssp. *elatius* and cultivar accessions is high. This is in agreement with the report of Zohary and Hopf (1973). These reseachers indicated that ssp. *elatius* and ssp. *humile* are the progenitors of pea *P. sativum* ssp. *sativum.* In the research of Hoey et al. (1996) and Saar and Polans (2000) whole accessions of ssp. *elatius* were the closest relative to the cultivated pea and in study of Kosterin et al. (2008), among 15 accessions which were originally designated as '*Pisum elatius* Bieb.' or '*P. sativum subsp. elatius* (Bieb.) Schmalh.', only 8 accessions combined to cultivated pea. Threfore, according to our results and reports Hoey et al. (1996), Saar and Polans (2000) and Kosterin et al. (2008), the genetic basis of *P. sativum ssp. elatius* and cultivars is high than another ssp. of *sativum* and *P. fulvum.*

This study showed that genetic probability profiles of accessions can corroborate clustering analyses while providing additional information as a powerful tool for assigning accessions into their related groups.

REFERENCES

Baranger A, Aubert G, Arnau G, Laine´ AL, Deniot G, Potier J, Weinachter C, Lejeune-He´ naut I, Lallemand J, Burstin J (2004) Genetic diversity within *Pisum sativum* using proteinand PCR-based markers. Theor. Appl. Genet. 108: 1309-1321.

Botstein D, White RL, Skolnick M, Davis RW (1980). Construction of a genetic linkage map in man using restriction fragment length polymorphisms. Am. J. Hum. Genet. 32: 314-331.

Buchanan FC, Adams LJ, Littlejohn RP, Maddox JF, Crawford AM (1994). Determination of evolutionary relationships among sheep breeds using microsatellites. Genomics, 22: 397-403.

Burstin J, Deniot G, Potier J, Weinachter C, Aubert G, Baranger A (2001) Microsatellite polymorphism in *Pisum sativum*. Plant Breed, 120: 311-317

Coburn JR, Temnykh SV, Paul EM, McCouch SR (2002). Design and Application of microsatellite marker panels for semi-automated genotyping of rice (*Oryza sativa* L.). Crop Sci. 42: 2092-2099.

Cornuet JM, Luikart G (1996). Description and evaluation of two tests for detecting recent bottlenecks, Genet. 144: 2001-2014.

Davies N, Villablanco FX, Roderick GK (1999). Determining the source of individuals: multilocus genotyping in nonequilibrium population genetics, TREE, 14(1): 17-21.

Don RH, PT Cox, Wainwright BJ, Baker K, Mattick JS (1991). Touchdown PCR to circumvent spurious priming during gene amplification. Nucl. Acid Res. 19: p. 4008.

Ellis THN, Poyser SJ, Knox MR, Vershinin AV, Ambrose MJ (1998). Polymorphism of insertion sites of Ty1-copia class retrotransposons and its use for linkage and diversity analysis in pea. Mol. Gen. Genet. 260: 9-19.

Ford R, Le RK, Itman C, Jan Bert B, Paul WJT (2001). Diversity analysis and genotyping in *Pisum* with sequence tagged microsatellite site (STMS) primers. Euphytica, 124: 397-405.

Haghnazari A, Samimifard R, Najafi J, Mardi M (2005). Genetic diversity in pea (*Pisum sativum* L.) accessions detected by sequence tagged microsatelite markers. Genet. Breed. 59: 145-152.

Hamza S, Hamida WB, Rebai¨ A, Harrabi M (2004) SSR-based genetic diversity assessment among Tunisian winter barley and relationship with morphological traits. Euphytica, 135: 107-118.

Hauser LA, Crovello TJ (1982). Numerical analysis of genetic relationships in *Thelypodiae* (*Brassicaceae*). Sys. Bot. 7: 249-268.

He C, Poysa V, Yu K (2003). Development and characterization of simple sequence repeat (SSR) markers and their use in determining relationships among *Lycopersicon esculentum* cultivars. Theor. Appl. Genet. 106: 363-373.

Hoey BK, Crowe KR, Jones VM, Polans NO (1996) Aphylogenetic analysis of *pisum* based on morphological characters,and allozyme and RAPD markers. Theor. Appl. Genet. 92: 92-100.

Innan I, Terauchi R, Miyashita NT (1997). Microsatellite polymorphism in natural populations of the wild plant *Arabidopsis thaliana*., Genet. 146: 1441-1452.

Jepsen BI, Siegismund HR, Fredholm M (2001). Population genetics of the native caribou (*Rangifer tarandus groenlandicus*) and the semi-domestic reindeer (*Rangifer tarandus tarandus*) in Southwestern Greenland: Evidence of introgression. Conservation Genet., 3: 401-409.

Keneni G, Jarso M, Wolabu T, Dino G (2005). Extent and pattern of genetic diversity for morpho-agronomic traits in Ethiopian highland pulse landraces: I. Field pea (*Pisum sativum* L.). Genet. Resour. Crop Evol. 52: 539-549.

Kosterin OE, Bogdanova VS (2008). Relationship of wild and cultivated forms of *Pisum* L. as inferred from an analysis of three markers, of the plastid, mitochondrial and nuclear genomes. Genet. Resour. Crop Evol. 55: 735-755.

Loridin K, McPhee K, Morin J, Dubreuil P, Pilet-Nayel ML, Aubert G, Rameau C, Baranger A, Coyne C, Lejeune-He`naut I, Burstin J (2005). Microsatellite marker polymorphism and mapping in pea (*Pisum sativum* L.). Theor. Appl. Genet. 111: 1022-1031.

Lu J, Knox MR, Ambrose MJ, Brown JKM, Ellis THN (1996). Comparative analysis of genetic diversity in peaassessed by RFLP- and PCR-based methods. Theor. Appl. Genet. 93: 1103-1111.

Lund B, Ortiz R, Skovgaard IM, Waugh R, Anderson SB (2003). Analysis of potential duplicates in barley gene bank collections using re-sampling of microsatellite data. Theor. Appl. Genet. 106: 1129-1138.

Maguire TL, Peakall R, Saenger P (2002). Comparative analysis of genetic diversity in the mangrove species *Avicennia marina* (Forsk.) Vierh. (*Avicenniaceae*) detected by AFLPs and SSRs. Theor. Appl. Genet. 104: 388-398.

McPhee K (2003). Dry pea production and breeding, a mini-review. Food Agric. Environ. 1(1): 64-69.

Paetkau D, Calvert W, Stirling I, Strobek C (1995). Variation in genetic diversity across the range of North American brown bears. Conserv. Biol. 12: 418-429.

Paetkau D, Slade R, Burden M, Estoup A (2004). Genetic assignment methods for the direct, real-time estimation of migration rate: a simulationbased exploration of accuracy and power. Mol. Ecol. 13: 55-65.

Paniego N, Echaide M, Munoz M, Fernandez L, Torales S, Faccio P, Fuxan I, Carrera M, Zandomeni R, Suarez EY, Hopp HE (2002). Microsatellite isolation and characterization in sunflower (*Helianthus annuus* L.). Genome, 45: 34-43.

Peakall R, Smouse PE (2006). GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research. Mol. Ecol. Notes, 6: 288-295.

Pearce SR, Knox M, Ellis THN, Flavell AJ, Kumar A (2000) Pea *Ty*1-copia group retrotransposons: transcriptional activity and use as markers to study genetic diversity in Pisum. Mol. Gen. Genet. 263: 898-907.

Phippen WB, Kresovich S, Candelas FG, McFerson JR (1997). Molecular characterization can quantify and partition genetic variation among gene bank holdings: a case study with phenotypically similar accessions of *Brassica oleracea* var. capitata L. (cabbage) Golden

Acre. Theor. Appl. Genet. 94: 227-234.
Piry S, Alapettte A, Cornuet JM, Paetkau D, Baudouin L, Estoup A (2004). GENECLASS2: A Software for Genetic Assignment and First-Generation Migrant Detection. J. Hered. 95(6): 536-539.
Polans NO, Saar DE (2000). ITS sequence variation in wild species and cultivars of pea. Pisum Genet. 32: 42-45.
Primmer CR, Koskinen MT, Piironen J (2000). The one that did not get away: individual assignment using microsatellite data detects a case of fishing competition fraud. Proc. R Soc. Lond. 267: 1699-1701.
Pritchard JK, Stephens M, Donnelly P (2000). Inference of population structure using multilocus genotype data., Genet. 155: 945-959.
Choudhury Ray P, Tanveer H, Dixit GP (2006). Identification and detection of genetic relatedness among important varieties of pea (*Pisum sativum* L.), grown in India. Genetica, 130: 183-191.
Rohlf F (2006). NTSYSpc: Numerical Taxonomy System (ver. 2.2). Exeter Publishing, Ltd., Setauket
Rongwen J, Akkaya MS, Bhagwat AA, Lavi U, Cregan PB (1995). The use of microsatellite DNA markers for soybean genotype identification. Theor. Appl. Genet. 90: 43-48.
Samec P, Posvec Z, Stejskal J, Nasinec V, Griga M (1998). Cultivar identification and relationships in *Pisum sativum* L. based on RAPD and isozymes. Biol. Plant, 41: 39-48.
Sefc KM, Lopes MS, Lefort F, Botta R, Roubelakis-Angelakis KA, Ibáñez J, Peji´c I, Wagner HW, Glössl J, Steinkellner H (1999). Microsatellite variability in grapevine cultivars from different European regions and evaluation of assignment testing to assess the geographic origin of cultivars. Theor. Appl. Genet. 100: 498-505.
Siegismund HR (1995). G-stat version 3.1, Genetical Statistical Programs for the Analysis of Population Data, Department for Ecology, Botanical Institute, University of Copenhagen.
Simioniuc D, Uptmoor R, Friedt W, Ordon F (2002). Genetic diversity and relationships among pea cultivars revealed by RAPDs and AFLPs. Plant Breed, 121: 429-435.
Smartt J, Hymowitz T (1985). Domestication and evolution of grain legumes. p. 37–72. In: R.J. Summerfield and E.H. Roberts (eds.), Grain legume crops, (Eds RJ Summerfield, EH Roberts) pp. 37-72.
Smykal P (2006). Development of an efficient retrotransposon-based fingerprinting method for rapid pea variety identification. Theor. Appl. Genet. 47(3): 221-230.
Smykal P, Valledor L, Rodrı´guez R, Griga M (2007). Assessment of genetic and epigenetic stability in long-term in vitro shoot culture of pea (*Pisum sativum* L.). Plant Cell Rep., 26: 1985-1998.
Smykal P, Horacek J, Dostalova R, Hybl M (2008a). I. Variety discrimination in pea (*Pisum sativum* L.) by molecular, biochemical and morphological markers. Theor. Appl. Genet. 49(2): 155-166.
Smykal P, Hybl M, Corander J, Jarkovsky J, Andrew JF, Griga M (2008b). II. Genetic diversity and population structure of pea (*Pisum sativum* L.). varieties derived from combined retrotransposon, microsatellite and morphological marker analysis. Theor. Appl. Genet. 117(3): 413-424.
Snowdon RJ, Friedt W (2004). Molecular markers in *Brassica* oilseed breeding: current status and future possibilities. Plant Breed, 123: 1-8.
Struss D, Plieske J (1998). The use of microsatellite markers for detection of genetic diversity in barley populations. Theor. Appl. Genet. 97: 308-315.
Sun GL, William M, Liu J, Kasha KJ, Pauls KP (2001). Microsatellite and RAPD polymorphisms in Ontario corn hybrids are related to the commercial sources and maturity ratings. Mol. Breed, 7: 13-24.
SzewcMcFadden AK, Kresovich S, Bliek SM, Mitchell SE, McFerson JR (1996). Identification of polymorphic, conserved simple sequence repeats (SSRs) in cultivated *Brassica* species. Theor. Appl. Genet. 93: 534-538.
Taylor PWJ, Fraser TA, Ko HL, Henry RJ (1995). RAPD analysis of sugarcane during tissue culture. In: Terzi M, Cella R and Falavigan A (Eds), Kluwer Academic Int., Dordrecht, The Netherlands, Curr. Issues Plant Mol. Cell. Biol. pp. 241-246.
Tang S, Kishore VK, Knapp SJ (2003). PCR-multiplexes for a genome-wide framework of simple sequence repeat marker loci in cultivated sunflower. Theor. Appl. Genet. 107: 6-19.
Taran B, Zhang C, Warkentin T, Tullu A, Vandenberg A (2005). Genetic diversity among varieties and wild species accessions of pea (Pisum sativum L.) based on molecular markers, morphological and physiological characters. Genome, 48: 257-272.
Tessier C, David J, This P, Boursiquot JM, Charrier A (1999). Optimization of the choice of molecular markers for varietal identification in Vitis vinifera (L.). Theor. Appl. Genet. 98: 171-177.
Treuren R, Hintum TJL (2003). Marker-assisted reduction of redundancy in germplasm collections: genetic and Economic aspects. Acta Hort. 623: 139-149.
Udupa SM, Robertson LD, Weigand F, Baum M, Kahl G (1999). Allelic variation at (TAA)n microsatellite loci in a world collection of chickpea (Cicer arietinum L.) germplasm. Mol. Gen. Genet. 261: 354-363
Waser PM, Strobeck C (1998). Genetic signatures of interpopulation dispersal. TREE, 13(2): 43-44.
Yeh FC, Yang RC, Boyle TBJ, Ye ZH, Mao JX (1997). POPGENE the user friendly shareware for population genetic analysis. Molecular Biology and Biotechnology Centre, University of Alberta, Canada
Zohary D, Hopf M (1973). Domestication of pluses in the old world. Sci. 182: 887-849.