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FAME and RAPD analysis of selected *Vicia* taxa from eastern Anatolia, Turkey

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Fatty acid (FAs) and RAPD profiles were used to examine phenotypic and genetic relationships among 12 *Vicia* taxa. All the *Vicia* taxa were separated based on the presence and composition of 77 different FAs. Eight of the ten decamer primers examined were selected to explore the genetic variation. A total of 156 amplicons in the size range of 125–2500 bp were produced by eight different primers from the twelve *Vicia* taxa. Even though all *Vicia* species were differentiated by RAPD profiles, three genetically distinct groups were found among the species tested. This is the first study showing that RAPD and FAME analyses are useful methods for differentiation and classification of *Vicia* and perhaps other plant species and/or taxa as well.

Key words: FAMEs, genetic and phenotypic diversity, RAPD, taxonomy, *Vicia*

Introduction

Fabaceae are flowering plants comprising about 730 genera and 19 400 species (Mabberley 1997) and it is one of the largest plant families in the world. It has approximately 900 species in 68 genera in Turkey (Davis 1970, 1988, Seçmen *et al*. 1989). *Vicia* is a member of tribe Vicieae, which is divided into two subgenera, *Vicia* and *Vicilla* (Kupicha 1976). *Vicia* includes about 150 species widely distributed throughout the temperate zones of both hemispheres. Some 40 species, mainly of Eurasian origin, are cultivated (Harlan 1956). It is reported that a total of 18 varieties, 22 subspecies and 59 species of *Vicia*, belonging to six sections are present in Turkey; five species and three subspecies of them were known to be endemic in that country (Davis & Plitmann 1970).

Classification of most of the *Vicia* taxa using conventional taxonomic techniques is difficult. Molecular biology and gene technology are creating promising possibilities for a rapid and accurate determination of phenotypic and genetic variation among plant species. Fatty acid methyl esters (FAMEs) analysis and nucleic acid based techniques, such as RFLP (Restriction Fragment Length Polymorphism) and RAPD (Random

Amplified Polymorphic DNA) analyses, have been utilized for determination of phylogenetic relationships within and among plant species in addition to morphological characters since the 1990s (Williams *et al*. 1993, Wolfe & Liston 1998, Harris 1999, Wolfe & Morgan-Richards 1999, Sahin *et al*. 2000, Bagci *et al*. 2001, Bagci & Sahin 2004, Ozen *et al*. 2004). So far there have been few attempts to study the genetic variation in *Vicia* using RAPD and/or FAMEs analyses (Akpinar *et al*. 2001, Bagci *et al*. 2004). No studies have been conducted to assess phenotypic and genotypic differences in most of *Vicia* taxa, including those encountered in the eastern Anatolia region of Turkey.

The aim of the present study was to determine RAPD and FAMEs profiles to examine the genetic and phenotypic relationships of *Vicia* taxa native in eastern Anatolia, and their use for differentiation and classification purposes.

Material and methods

Plant samples of 12 *Vicia* taxa were collected at flowering stage from different locations in the vicinity of Erzurum, located in eastern Anatolia, Turkey (Table 1). The taxonomic identifications were confirmed by Dr. Meryem Sengul, in Department of Biology, Atatürk University, Erzurum. Collected plant materials were dried in shade. The leaves were detached from the stems and ground in a grinder with a 2 mm diameter mesh. The ground material was used for DNA extraction and FAMEs. Voucher specimens were deposited at the Herbarium of the Department of Biology, Atatürk University, Erzurum (Table 1).

Extraction and analysis of FAMEs

Preparation and analysis of FAMEs from whole cell fatty acids were performed according to the method described by the manufacturer (Sherlock Microbial Identification System version 4.5, MIDI, Inc., Newark, DE). Plant leaves were powdered after lyophilization in liquid nitrogen. Approximately 40 mg of powdered leaves from each sample was added to 1 ml 1.2 M NaOH in 50% aqueous methanol with 5 glass beads (3 mm in diam.) in a screw cap tube, then incubated at 100 °C for 30 min in a water bath. After the saponified samples were cooled at room temperature for 25 min, they were acidified and methylated by adding 2 ml 54% 6 N HCl in 46% aqueous methanol and incubated at 80 °C for 10 min in a water bath. After rapid cooling, methylated fatty acids were extracted with 1.25 ml 50% methyl-tert butyl ether (MTBE) in hexane. Each sample was mixed for 10 min and the bottom phase was removed with a Pasteur pipette. The top phase was washed with 3 ml 0.3 M NaOH. After mixing for 5 min, the top phase was removed for analysis. Following the base wash step, the extract (FAMEs) was cleaned in anhydrous sodium sulfate and then transferred into a GC sample vial for analysis.

FAMEs were separated using gas chromatography (HP6890) in a fused-silica capillary column (25 m by 0.2 mm) with cross-linked 5% phenyl methyl silicone. The operating parameters for the study were set and controlled automatically by a computer program. The chromatograms with peak retention times and areas were produced on the recording integrator and were electronically transferred to the computer for analysis, storage and report generation. Peak naming and column performance was achieved by using Eukary calibration standard mix (Microbial ID 1201-A) containing nC9–nC30 saturated and 2&3 hydroxy fatty acids. Cellular fatty acids were identified on the basis of equivalent chain length data. FAME profiles of each plant species tested were identified by comparing the commercial databases (Eukary) with the MIS software package.

DNA extraction

Genomic DNA was extracted from powdered plant materials using a modified method described by Lin *et al*. (2001).

RAPDs

Samples were screened for RAPD variation using standard 10-base primers supplied by Operon. Thirty μl of reaction mixture was prepared as

follows: 10 × Buffer 3.0 μl, dNTPs (10 mM) 1.2 μl, magnesium chloride (25 mM) 1.2 μl, primer (5 μM) 2.0 μl, taq polymerase (5 units) 0.4 μl, water 19.2 μl sample DNA 3.0 μl (100 ng μl^{-1}). A total of 10 RAPD primers were tested in this study.

Eight of these primers produced 156 different amplicons with all of the *Vicia* species and were selected for further studies based on the preliminary test results (Table 2).

The thermal cycle was: 2 min at 95 °C; 2 cycles of 30 sec. at 95 °C, 1 min at 37 °C, 2 min at 72 °C; 2 cycles of 30 sec. at 95 °C, 1 min at 35 °C, 2 min at 72 °C; 41 cycles of 30 sec at 94 °C, 1 min at 35 °C, 2 min at 72 °C; followed by a final 5 min extension at 72 °C then brought down to 4 °C.

Electrophoresis

The PCR products (27 μl) were mixed with 6× gel loading buffer (3 μl) and loaded onto an agarose (1.5% w/v) gel in 0.5 XTBE (Tris-Borate- EDTA) buffer and was subjected to electroforesis at 70 V for 150 min. Gels were stained in ethidium bromide solution (2 μl Etbr/100 ml 1 × TBE buffer) for 40 min. The amplified DNA products were detected by using the Bio Doc Image Analysis System with Uvisoft analysis package (Cambridge, UK).

Data analysis

PCR products were scored as presence (1) and absence (0) of band for each of the eight accessions analyzed. Only reproducible bands were scored. For FAME analysis, fatty acids of each plant species were scored as presence (0.1%–100%) and absence (0%). Data were used to calculate a Jaccard (1908) similarity index from which a UPGMA dendrogram was constructed.

All experiments were repeated at least two times.

Results and discussion

The *Vicia* taxa tested in this study were separated based on the presence and composition of 77 FAs found in all taxa (Table 2). *Vicia villosa* ssp. *villosa*, *V. canescens* ssp. *variegata*, *V. cracca* ssp. *stenophylla*, *V. canescens* ssp. *gregaria*, and *V. sativa* ssp. *nigra* had more FAs — at least 19–31 different ones — than the other species. A set of four FAs (16:0, 18:0, 18:1:ω8c, 18:2:ω6c) were found in all taxa, except for *V. hirsuta* and *V. cracca* ssp. *cracca*. The relative proportions of two fatty acids (16:0, and 18:1ω8c) were higher (7.83%–40.64%) in eleven taxa, and the amounts of four fatty acids (18:0 cis-9,10-epoxy, 20 N alcohol, 21:0 anteiso, 22 primary alcohol) were higher in *V. hirsuta* (Table 2). The concentration

Table 1. The *Vicia* material used.

OTUs	Herbarium number of the vouchers	Locality	Distribution	Altitude (m)
V. villosa ssp. *villosa*	9783	Tortum, Erzurum	Widespread	1640
V. sativa ssp. *nigra*	9599	Aşkale, Erzurum	–	2100
V. faba ssp. *minor* var. *minor*	9786	Erzurum	Culture form	1950
V. pannonica var. *pannonica*	9782	Aşkale, Erzurum	–	2150
V. cracca ssp. *cracca*	9522	Aşkale, Erzurum	Euro-Siberian	2150
V. canescens ssp. *variegata*	9521	Aşkale, Erzurum	Irano-Turanian	1950
V. cracca ssp. *stenophylla*	9550	Pasinler, Erzurum	Euro-Siberian	2000
V. noeana var. *noeana*	9787	Aşkale, Erzurum	Irano-Turanian	1950
V. hirsuta	9785	Erzincan	–	1640
V. sativa var. *cordata*	9784	Refahiye, Erzincan	–	1820
V. canescens ssp. *gregaria*	9788	Çat, Erzurum	Irano-Turanian	1880
V. sativa ssp. *sativa*	9789	Erzurum	–	1850

Table 2. Composition of fatty acids in the *Vicia* taxa.

Fatty acids	*V. villosa* ssp. *villosa*	*V. sativa* ssp. *sativa*	*V. faba* ssp. *minor*	*V. pannonica* var. *pannonica*	*V. cracca* ssp. *cracca*	*V. canescens* ssp. *variegata*	*V. cracca* ssp. *stenophylla*	*V. noeana* var. *noeana*	*V. hirsuta*	*V. sativa* var. *cordata*	*V. canescens* ssp. *gregaria*	*V sativa* ssp. *nigra*
8 Dicarboxylic	–	–	–	–	–	0.12	0.26	–	–	–	0.14	0.44
9 Dicarboxylic	0.74	0.54	0.92	–	–	1.29	2.32	4.95	–	–	1.02	2.41
10 Dicarboxylic	–	–	–	–	–	0.11	1.11	–	–	–	–	1.07
9:0	–	–	–	–	–	–	–	–	–	–	0.06	–
10:0	–	–	–	–	–	0.14	–	–	–	–	0.20	0.40
10:0 3OH	–	–	–	–	–	–	–	–	–	–	–	0.20
11:0 2OH	–	–	–	–	–	0.14	–	–	–	–	–	–
12:0	–	–	–	–	–	1.53	1.77	–	–	–	0.98	2.99
12:0 2OH	–	–	–	–	–	–	–	–	–	–	–	0.32
12:0 iso 3OH	–	–	–	–	–	–	–	–	–	–	–	0.38
12:0 ALDE	–	–	–	–	–	–	0.83	–	–	–	0.64	–
13:0	–	–	–	–	–	0.23	–	–	–	–	–	1.90
13:0 anteiso	–	–	–	–	–	0.42	0.80	–	–	–	–	0.77
14:0	0.51	0.67	–	–	–	1.44	2.44	–	–	–	2.23	6.23
14:0 2OH	–	–	–	–	–	–	–	–	–	–	–	–
14 N Alcohol	–	–	–	–	–	–	1.18	–	–	–	–	–
15:0	0.53	0.66	0.63	–	–	0.26	0.28	–	–	–	0.54	0.26
15:0 anteiso	–	–	–	–	–	0.09	–	–	–	–	–	–
16:0	20.31	23.37	23.98	20.75	–	15.25	18.93	37.34	–	40.64	25.16	20.28
16:0 2OH	1.48	1.40	1.14	1.55	–	0.25	0.38	–	–	–	0.44	–
17:0	–	–	–	–	–	0.29	0.73	–	–	–	0.38	–
17:0 anteiso	–	–	–	–	–	0.23	0.45	–	–	–	–	–
18:0	1.32	1.95	1.99	1.62	5.24	2.50	2.16	6.79	–	9.46	4.55	3.58
18:1 2OH	–	–	–	–	–	1.70	–	–	–	–	–	–
18:0 cis 9,10 epoxy	–	–	–	–	–	0.68	1.35	–	13.26	–	0.32	0.93
18 N Alcohol	0.60	–	–	2.14	–	1.72	3.47	–	–	–	3.17	2.35
19:0 iso	–	–	–	–	–	0.68	–	–	–	–	–	–
19:0 N Alcohol	–	–	–	–	–	1.35	0.42	–	–	–	–	–
20:0	0.43	–	0.86	–	–	0.75	0.70	–	–	–	1.32	1.89
20:0 3OH	–	–	–	–	–	–	0.37	–	–	–	–	–
20 N Alcohol	0.62	–	–	–	–	0.72	0.85	–	20.27	–	0.79	–
21:0	–	–	–	–	–	–	–	–	–	–	0.58	–
21:0 iso	–	–	–	–	–	1.65	1.94	–	–	–	1.63	1.94
21:0 anteiso	–	–	–	–	–	–	–	–	11.24	–	–	–
22:0	0.55	–	–	–	–	0.56	0.46	–	–	–	1.25	1.60
22 Primary Alcohol	–	–	–	–	–	0.30	0.45	–	50.48	–	0.69	–

22:0 2OH	0.62	–	–	–	–	–	–	–	–	–	0.62	–
23:0	–	–	–	–	–	–	–	–	–	–	0.32	–
23:0 2OH	–	–	–	–	–	0.11	–	–	–	–	0.29	–
24:0	–	–	–	–	–	0.50	0.75	–	–	–	1.95	0.81
24:0 2OH	–	–	–	–	–	–	0.60	–	–	–	–	–
24:0 3OH	1.25	–	–	–	–	3.78	2.67	–	–	–	–	7.13
25 N Alcohol	–	0.85	–	–	–	0.41	0.68	–	–	–	1.03	1.38
28:0	–	–	–	–	–	0.31	0.58	–	–	–	0.67	–
30:0	–	–	–	–	–	0.93	1.48	–	–	–	2.66	–
12:1:ω9c	–	–	–	–	–	0.04	–	–	–	–	–	–
13:1:ω3c	–	–	–	–	–	–	0.59	–	–	–	–	–
13:1:ω6c	–	–	–	–	–	0.51	1.39	–	–	–	–	–
14:1:ω6c	–	–	–	–	–	–	0.33	–	–	–	–	–
14:1:ω7c	–	–	–	–	–	–	0.41	–	–	–	–	–
15:1:ω6c	–	–	–	–	–	–	–	–	–	–	0.32	0.81
16:1:ω6c	–	–	–	–	3.48	1.53	5.25	–	–	–	2.81	2.10
16:1:ω7c	0.80	–	–	–	–	0.13	0.37	–	–	–	–	–
16:1:ω9c	–	–	–	–	–	0.23	–	–	–	–	–	–
16:1:ω7c Alcohol	–	–	–	–	–	0.67	1.27	–	–	–	1.89	1.32
16:1 cis 7 DMA (ω9)	–	–	–	–	–	2.07	2.01	–	–	–	2.53	1.65
17:1 iso G	0.52	0.77	0.81	–	5.03	3.31	5.56	–	–	–	7.23	3.91
17:1:ω3c	–	–	–	–	–	0.16	–	–	–	–	–	–
17:1:ω8c	–	–	–	–	–	–	0.85	–	–	–	–	–
18:1:ω5c	–	–	–	–	–	–	–	–	–	–	–	1.36
18:1:ω8c	29.22	23.23	26.43	27.59	26.06	28.53	10.59	21.06	–	27.11	7.83	16.10
18:1:ω 9c DMA	–	–	–	–	–	1.19	–	–	–	–	–	–
18:1:ω9t Alcohol	1.06	1.04	0.97	–	8.22	3.34	6.33	9.84	–	7.54	8.64	4.37
18:2:ω6c	38.51	42.44	40.89	44.80	14.11	7.76	3.43	9.09	–	15.24	3.73	6.16
18:3:ω6c	–	–	–	–	–	–	–	–	4.75	–	–	–
19:1:ω6c	0.68	2.15	–	1.55	10.40	6.01	8.47	10.94	–	–	8.72	–
19:1:ω8c	–	–	–	–	–	–	–	–	–	–	0.91	–
19:2:ω6c	–	–	–	–	–	0.87	0.26	–	–	–	–	0.93
20:1:ω3c	–	–	–	–	–	0.17	–	–	–	–	–	–
20:5:ω3c	–	–	–	–	–	0.85	0.65	–	–	–	–	–
22:1:ω9c	–	–	–	–	–	0.33	–	–	–	–	–	–
18.473	–	–	–	–	–	0.45	–	–	–	–	–	–
20.343 “D”	–	–	–	–	–	0.89	1.08	–	–	–	0.90	0.91
20.588	–	–	–	–	–	–	0.44	–	–	–	–	–
24.407 “D”	–	–	–	–	–	0.05	–	–	–	–	–	–
25.052	0.26	–	–	–	–	0.47	0.30	–	–	–	–	–
25.339	–	0.93	1.37	–	–	–	–	–	–	–	0.86	1.13

Table 3. Number of amplification products generated with eight arbitrary oligonucleotide primers.

Primer	Total number of RAPD products per primer	Total number of polymorphic RAPD products	Number of polymorphic amplification products											Sequence of primer
			1	2	3	4	5	6	7	8	9	10	11	12	
OPK04	29	29	6	8	12	6	4	5	7	6	9	4	0	10	5′-CCGCCCAAAC-3′
OPK06	25	25	5	4	5	1	0	1	6	4	7	3	0	1	5′-CACCTTCCC-3′
OPK09	20	20	2	2	4	4	4	2	5	3	6	4	0	2	5′-CCCTACCGAC-3′
OPK13	10	10	2	2	2	1	1	0	1	3	5	1	0	2	5′-GGTTGTACCC-3′
OPK19	23	23	3	6	5	3	3	1	3	5	8	3	8	8	5′-CACAGGCGGA-3′
OPL09	9	9	1	1	0	1	0	0	1	5	4	1	0	1	5′-TGCGAGAGTC-3′
OPL14	22	22	5	7	5	6	2	2	4	6	7	5	5	4	5′-GTGACAGGCT-3′
OPL15	18	18	3	3	3	3	0	1	8	6	6	4	2	2	5′-AAGAGAGGGG-3′
Total polymorphism (%)			17.3	21.1	23.0	16.0	8.9	7.6	22.4	24.3	33.3	16.0	9.6	19.2	

Taxa are indicated as: 1: *V. villosa* ssp. *villosa*, 2: *V. sativa* ssp. *nigra*, 3: *V. faba* ssp. *minor* var. *minor*, 4: *V. pannonica* var. *pannonica*, 5: *V. cracca* ssp. *cracca*, 6: *V. canescens* ssp. *variegata*, 7: *V. cracca* ssp. *stenophylla*, 8: *V. noeana* var. *noeana*, 9: *V. hirsuta*, 10: *V. sativa* var. *cordata*, 11: *V. canescens* ssp. *gregaria*, 12: *V. sativa* ssp. *sativa*.

Fig. 1. RAPD profiles generated with the primer OPK04, respectively. Lanes: 1: *Vicia villosa* ssp. *villosa*, 2: *V. sativa* ssp. *nigra*, 3: *V. faba* ssp. *minor* var. *minor*, 4: *V. pannonica* var. *pannonica*, 5: *V. cracca* ssp. *cracca*, 6: *V. canescens* ssp. *variegata*, 7: *V. cracca* ssp. *stenophylla*, 8: *V. noeana* var. *noeana*, 9: *V. hirsuta*, 10: *V. sativa* var. *cordata*, 11: *V. canescens* ssp. *gregaria*, 12: *V. sativa* ssp. *sativa*. N = negative control, M = molecular marker (10 kb).

of fatty acid (18:2:ω6c) was found to be higher (38.51%–44.80%) in *V. villosa* ssp. *villosa*, *V. sativa* ssp. *sativa*, *V. faba* ssp. *minor* var. *minor*, and *V. pannonica* var. *pannonica*.

FAMEs have been used to explore phenotypic diversity in plant taxa such as *Lathyrus*, *Hypericum* and *Vicia* (Akpinar *et al*. 2001, Bagci *et al*. 2001, Ozen *et al*. 2004) but not in *Vicia*. Hence, this is the first study of determination of FAME profiles in *Vicia*, demonstrating that the profiles can be used to determine phenotypic differences among closely related *Vicia* taxa. Our data supported a previous study suggesting that fatty acids are powerful tools for the chemotaxonomic characterization and evolutionary relationships among the tribes and genera of Fabaceae (Sahin *et al*. 2000, Bagci *et al*. 2001, Bagci & Sahin 2004).

A total of 156 amplicons in the size range of 125–2500 bp were produced by eight different primers from twelve taxa (Figs. 1–3). *Vicia cracca* ssp. *cracca and V. canescens* ssp. *gregaria* produced fewer amplicons than the other taxa with all eight tested primers (Figs. 1–8 and Table 3). Primers OPK04 and OPL09 gave the highest and lowest number of RAPD products, respectively (Figs. 1–8 and Table 3). *Vicia hirsuta* produced the highest number of DNA bands with all eight primers (Figs. 1–8 and Table 3). The RAPD results showed the presence of three clusters with genetic similarity. The first cluster,

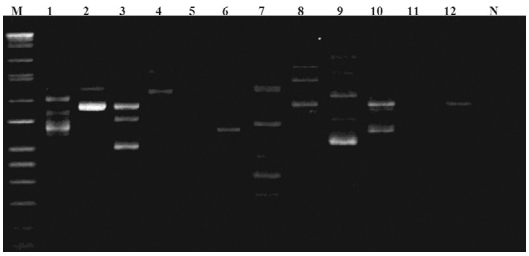

Fig. 2. RAPD profiles generated with the primer OPK06, respectively. Lanes: 1: *Vicia villosa* ssp. *villosa*, 2: *V. sativa* ssp. *nigra*, 3: *V. faba* ssp. *minor* var. *minor*, 4: *V. pannonica* var. *pannonica*, 5: *V. cracca* ssp. *cracca*, 6: *V. canescens* ssp. *variegata*, 7: *V. cracca* ssp. *stenophylla*, 8: *V. noeana* var. *noeana*, 9: *V. hirsuta*, 10: *V. sativa* var. *cordata*, 11: *V. canescens* ssp. *gregaria*, 12: *V. sativa* ssp. *sativa*. N = negative control, M = molecular marker (10 kb).

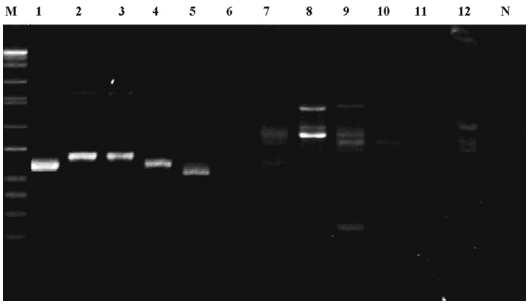

Fig. 4. RAPD profiles generated with the primer OPK13, respectively. Lanes: 1: *Vicia villosa* ssp. *villosa*, 2: *V. sativa* ssp. *nigra*, 3: *V. faba* ssp. *minor* var. *minor*, 4: *V. pannonica* var. *pannonica*, 5: *V. cracca* ssp. *cracca*, 6: *V. canescens* ssp. *variegata*, 7: *V. cracca* ssp. *stenophylla*, 8: *V. noeana* var. *noeana*, 9: *V. hirsuta*, 10: *V. sativa* var. *cordata*, 11: *V. canescens* ssp. *gregaria*, 12: *V. sativa* ssp. *sativa*. N = negative control, M = molecular marker (10 kb).

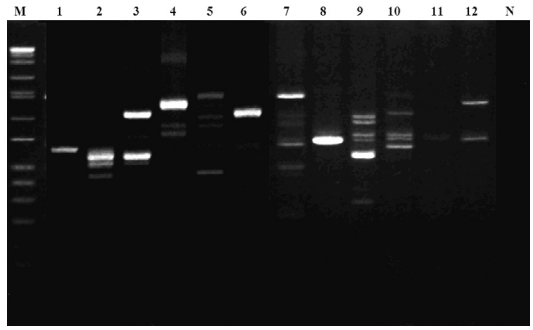

Fig. 3. RAPD profiles generated with the primer OPK09, respectively. Lanes: 1: *Vicia villosa* ssp. *villosa*, 2: *V. sativa* ssp. *nigra*, 3: *V. faba* ssp. *minor* var. *minor*, 4: *V. pannonica* var. *pannonica*, 5: *V. cracca* ssp. *cracca*, 6: *V. canescens* ssp. *variegata*, 7: *V. cracca* ssp. *stenophylla*, 8: *V. noeana* var. *noeana*, 9: *V. hirsuta*, 10: *V. sativa* var. *cordata*, 11: *V. canescens* ssp. *gregaria*, 12: *V. sativa* ssp. *sativa*. N = negative control, M = molecular marker (10 kb).

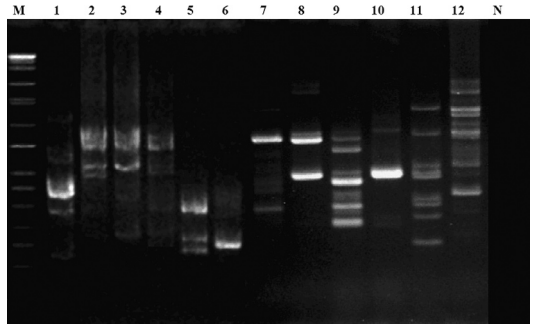

Fig. 5. RAPD profiles generated with the primer OPK19, respectively. Lanes: 1: *Vicia villosa* ssp. *villosa*, 2: *V. sativa* ssp. *nigra*, 3: *V. faba* ssp. *minor* var. *minor*, 4: *V. pannonica* var. *pannonica*, 5: *V. cracca* ssp. *cracca*, 6: *V. canescens* ssp. *variegata*, 7: *V. cracca* ssp. *stenophylla*, 8: *V. noeana* var. *noeana*, 9: *V. hirsuta*, 10: *V. sativa* var. *cordata*, 11: *V. canescens* ssp. *gregaria*, 12: *V. sativa* ssp. *sativa*. N = negative control, M = molecular marker (10 kb).

represented by *V. faba* ssp. *minor*, was genetically distinct from the other taxa. The second cluster was represented by *V. cracca* ssp. *stenophylla* and *V. cracca* ssp. *cracca*. The remaining taxa (*V. sativa* ssp. *sativa*, *V. pannonica* var. *pannonica*, *V. canescens* ssp. *variegata*, *V. noeana* var. *noeana*, *V. sativa* var. *cordata*, *V. canescens* ssp. *gregaria*, *V. sativa* ssp. *nigra* and *V. villosa* ssp. *villosa*) were grouped together with eight subclusters with a similarity range of 75%–80%. *Vicia sativa* ssp. *nigra* and *V. sativa* var. *cordata* were found to be closely related (98%). The RAPD analysis suggested that there were distinct genetic differences between species. All of the twelve *Vicia* taxa were clearly distinguished in a dendrogram constructed using Jaccard UPGMA.

RAPD profiles have been used to explore genetic diversity in many plant taxa such as *Triticum* accessions, *Astragalus*, *Cicer*, *Leucaena*, *Tripsacum* and *Ixora* (Rajaseger *et al*. 1997, Ahmad 1999, Coa *et al*. 1999, Harris 1999, Li *et al*. 1999, Adiguzel *et al*. 2006). RAPD markers have been used to determine genetic relationships at the species and subspecies level.

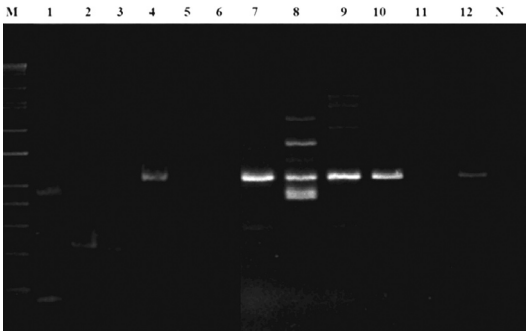

Fig. 6. RAPD profiles generated with the primer OPL19, respectively. Lanes: 1: *Vicia villosa* ssp. *villosa*, 2: *V. sativa* ssp. *nigra*, 3: *V. faba* ssp. *minor* var. *minor*, 4: *V. pannonica* var. *pannonica*, 5: *V. cracca* ssp. *cracca*, 6: *V. canescens* ssp. *variegata*, 7: *V. cracca* ssp. *stenophylla*, 8: *V. noeana* var. *noeana*, 9: *V. hirsuta*, 10: *V. sativa* var. *cordata*, 11: *V. canescens* ssp. *gregaria*, 12: *V. sativa* ssp. *sativa*. N = negative control, M = molecular marker (10 kb).

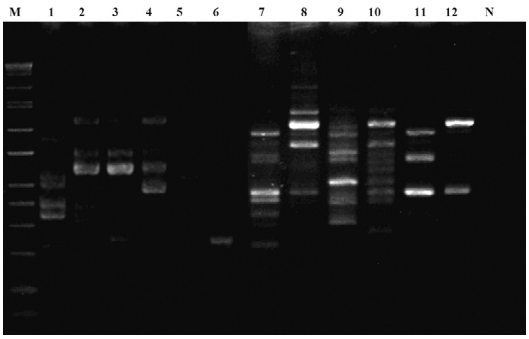

Fig. 8. RAPD profiles generated with the primer OPL15, respectively. Lanes: 1: *Vicia villosa* ssp. *villosa*, 2: *V. sativa* ssp. *nigra*, 3: *V. faba* ssp. *minor* var. *minor*, 4: *V. pannonica* var. *pannonica*, 5: *V. cracca* ssp. *cracca*, 6: *V. canescens* ssp. *variegata*, 7: *V. cracca* ssp. *stenophylla*, 8: *V. noeana* var. *noeana*, 9: *V. hirsuta*, 10: *V. sativa* var. *cordata*, 11: *V. canescens* ssp. *gregaria*, 12: *V. sativa* ssp. *sativa*. N = negative control, M = molecular marker (10 kb).

They are particularly useful for resolving relationships between closely related species and populations of genetically variable species (Yu & Pauls 1993). The present study provided further evidence of genetic differences among the *Vicia* taxa based on RAPD profiles.

In conclusion, the results demonstrated that RAPD and FAME analyses are useful for differentiation and classification of the *Vicia* taxa tested in the present study. A further study is necessary to determine the sequences of the polymorphic RAPD bands for each *Vicia* taxa

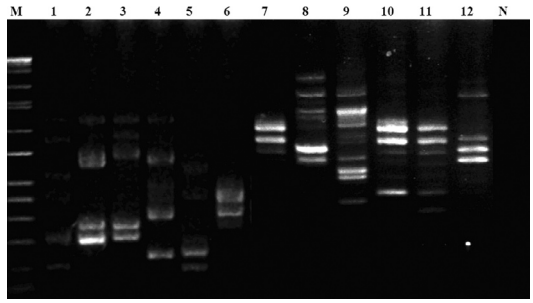

Fig. 7. RAPD profiles generated with the primer OPL14, respectively. Lanes: 1: *Vicia villosa* ssp. *villosa*, 2: *V. sativa* ssp. *nigra*, 3: *V. faba* ssp. *minor* var. *minor*, 4: *V. pannonica* var. *pannonica*, 5: *V. cracca* ssp. *cracca*. 6: *V. canescens* ssp. *variegata*, 7: *V. cracca* ssp. *stenophylla*, 8: *V. noeana* var. *noeana*, 9: *V. hirsuta*, 10: *V. sativa* var. *cordata*, 11: *V. canescens* ssp. *gregaria*, 12: *V. sativa* ssp. *sativa*. N = negative control, M = molecular marker (10 kb).

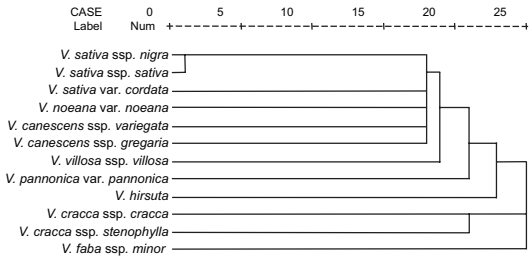

Fig. 9. UPGMA dendrogram of the studied *Vicia* taxa.

tested for use in their identification and characterization in the future.

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