Bangladesh J. Bot. **39**(1): 1-7, 2010 (June)

# PHENOTYPIC AND GENOTYPIC DIVERSITY AMONG *ASTRAGALUS* SPECIES GROWING IN EASTERN ANATOLIA REGION OF TURKEY

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*Keywords: Astragalus* spp., Genotypic, Phenotypic, Diversity, FAMEs, RAPD, Turkey

## Abstract

Fatty acid (FA) and RAPD profiles were used to examine phenotypic and genotypic relationships, respectively among *Astragalus atrocarpus*, *A. campylosema* subsp. *campylosema*, *A. fumosus*, *A. galegiformes*, *A. lineatus* var. *jildisianus* and *A. trichostigma*. All the taxa were separated based on the presence and composition of 33 different FAs. A set of nine FAs (14 : 0, 16 : 0, 18 : 0, 18 N alcohol, 17 : 1 iso G, 18: 1 : ω8c, C18 : 1 : ω9t alcohol, 18 : 2 : ω6c, 19 : 1: ω6c) were found in all *Astragalus* taxa. Six of the ten decamer primers examined were selected to explore the genetic variation among the taxa tested. The RAPD results suggested that *A. fumosus*, *A. lineatus* and *A. trichostigma* are closely related and completely different from the rest of the taxa. Four genetically distinct groups were found among the species. High genetic variations were present among species growing wildly in eastern Anatolia region of Turkey, which may imply differences in their origin. It appears that both RAPD and FA analyses are useful for differentiation of *Astragalus* species.

## Introduction

*Astragalus* L. (Fabaceae) is the largest genus of flowering plants, containing up to 3000 species (Lock and Simpson 1991) and traditionally classified in the tribe Galageae. The centre of development seems to be in the arid and semi-arid mountainous parts of the Northern Hemisphere (Polhill 1981). It is most diverse in the Irano- Turanian region of South-western Asia, the Sina-Himalayan Plateau of south Central Asia, the Central Asian region and the Great Basin and Colorado Plateau of western North America (Polhill 1981). It is also the largest genus in Turkey where it is represented by 400 species in 62 sections (Chamberlain and Matthews 1970). The delimitations of taxa both at the section and species levels possess considerable taxonomic problems (Davis 1988). For this reason, it is necessary to use characters other than morphology to explain the systematic relationships among *Astragalus* spp.

Fatty acid methyl esters (FAMEs) analysis and nucleic acid based techniques, such as RFLP (Restriction Fragment Length Polymorphism) analysis, RAPD (Random amplified polymorphic DNA) have been utilized to determine the phylogenetic relationship within and among plant species in addition to morphological characters, since 1990s (Williams *et al.* 1993, Wolfe and Liston 1998, Harris 1999, Wolff and Morgan-Richards 1999, Akpinar *et al.* 2001, Ozen *et al.* 2004, Adiguzel *et al*. 2006). There have been a few attempts to study the genetic variations among species of *Astragalus* such as *A. microcephale*, *A. mesoleios*, *A. keyserlingii*, *A. longistylus* and *Hololeuce* Sect. and *Acmothrix* Sect. using RAPD and/or RFLP analysis (Mehrnia *et al.* 2005, Ekici 2000). However, no study has been made on the phenotypic and genotypic differences in species of *Astragalus*, growing in the eastern Anatolia region of Turkey.

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The main goal of the present work was to study the relationships among taxa of the *Astragalus* growing in the eastern Anatolia region of Turkey using FAME and RAPD analysis.

## Materials and Methods

Plant samples of six *Astragalus* taxa were collected at flowering stage from different locations in the vicinity of Erzurum, located in the eastern Anatolia, Turkey (Table 1). Voucher specimens have been deposited at the Herbarium of the Department of Biology (ATA Herbarium), Ataturk University, Erzurum, Turkey.

**Table 1. Details of voucher specimens of *Astragalus* species used.**

| Taxa | Abbre-viations | Herbarium No. of vouchers | Locality | Status | Altitude (m) |
|---|---|---|---|---|---|
| *A.* sect. *onobrychium* | | | | | |
| *A fumosus* | FU | 9781 | Oltu, Erzurum | End | 1700 |
| *A. atrocarpus* | AT | 9786 | Aşkale, Erzurum | End | 1900 |
| *A.* sect. *grammocalyx* | | | | | |
| *A. lineatus var. jildisianus* | LI | 9782 | Çat, Erzurum | End | 1680 |
| *A.* sect. *erinotus* | | | | | |
| *A. trichostigma* | TR | 9783 | Aşkale, Erzurum | End | 1950 |
| *A.* sect. *galegiformes* | | | | | |
| *A. galegiformes* | GA | 9784 | Tortum, Erzurum | Eur-Sib | 1550 |
| *A.* sect. *proselius* | | | | | |
| *A. campylosema* ssp. *campylosema* | CA | 9785 | Aşkale, Erzurum | End | 1860 |

*Extraction and analysis of FAMEs*: Fatty acid composition was analyzed following Adiguzel *et al*. (2006) and fatty acids were separated by gas chromatography (HP6890, Hewlett Packard, Palo Alto, CA, USA) with a fused-silica capillary column (25 m × 0.2 mm) and cross-linked 5% phenyl methyl silicone.

*DNA extraction and PCR amplification*: Genomic DNA was extracted from powdered plant materials using a modified method of Lin *et al*. (2001). Ten primers (Operon Technologies Inc., Alameda, CA, USA) were used to generate preliminary RAPD profiles. Six of these primers produced amplicons with all of the *Astragalus* species tested were selected and used in further studies based on the preliminary test results (Table 2). PCR amplification reactions were carried out in 30 µl final volume of reaction mixture, containing 3.0 µl 10x Buffer, 1.2 µl dNTPs (10 mM), 1.2 µl magnesium chloride (25 mM), 2.0 µl primer (5 µM), 0.4 µl Taq polymerase (5 units), 19.2 µl water, and 3.0 µl sample DNA (100 ng/µl). A thermalcycler (Eppendorf Company, Hamburg, Germany) was programmed to run at two min at 95°C, followed by two cycles of 30 sec. at 95° C, 1 min at 37° C, and 2 min at 72° C, 2 cycles of 30 sec. at 95° C, 1 min at 35° C, and 2 min at 72° C, 41 cycles of 30 sec. at 94° C, 1 min at 35° C, and 2 min at 72° C, and a final 5 min extension at 72° C, then cooled to 4° C. The amplified fragments were separated on a 1.5% agarose gel in 0.5XTBE (Tris-Borate-EDTA) buffer at 70 V for 150 min and then stained with ethidium-bromide (2 µl Etbr/100ml 1xTBE buffer). The amplified DNA products were detected by using the Bio Doc Image Analysis System with Uvisoft analysis package (Cambrige, UK).

**Table 2. Number of amplification products generated with six arbitrary oligonucleotide primers. *Aa = A. atrocarpus, Ac = A. campylosema, Af = A. fumosus, Ag = A. galegiformes, Al = A. lineatus, At = A. trichostigma.***

| Primers | Total No. of RAPD products/ primer | Total No. of polymorphic RAPD products | Number of polymorphic amplification products | | | | | | Sequence of primers |
|---|---|---|---|---|---|---|---|---|---|
| | | | *Af* | *Al* | *At* | *Ag* | *Ac* | *Aa* | |
| OPA011 | 5 | 5 | 0 | 3 | 2 | 2 | 1 | 3 | 5'-CAATCGCCGT-3' |
| OPC05 | 10 | 10 | 5 | 3 | 7 | 2 | 2 | 2 | 5'-GATGACCGCC-3' |
| OPC015 | 10 | 10 | 2 | 2 | 2 | 3 | 4 | 7 | 5'-GACGGATCAG-3' |
| OPD03 | 15 | 15 | 5 | 3 | 6 | 7 | 3 | 5 | 5'-GTCGCCGTCA-3' |
| OPD07 | 11 | 11 | 3 | 4 | 3 | 2 | 2 | 6 | 5'-TTGGCACGGG-3' |
| OPD08 | 22 | 22 | 7 | 5 | 5 | 6 | 5 | 7 | 5'-GTGTGCCCCA-3' |
| Total % polymorphism | | | 30.1 | 27.3 | 34.2 | 30.1 | 23.2 | 41.0 | |

*Data analysis*: PCR products were scored as presence (1) and absence (0) of bands 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 of the experiments in this study were repeated at least twice.

## Results and Discussion

*Astragalus* taxa tested in this study were separated based on the presence and composition of 33 FAs found in all taxa (Table 3). A set of nine FAs (14 : 0, 16 : 0, 18 : 0, 18 N alcohol, 17 : 1 iso G, 18 : 1 : ω8c, 18 : 1 : ω9t alcohol, 18 : 2 : ω6c, 19 : 1 : ω6c) were found in all taxa tested. The relative proportions of the FA (18 : 1 : ω6c) was the higher (31.12 - 46.96%) in all taxa, except *A. atrocarpus*, comparing with those of other FAs identified. The presence of FAs 15 : 0 anteiso and 20 : 1 : ω7c; 20 : 0 3OH; 22 : 5ω3c and 23 : 0; 18 : 3 : ω6c, and 24 : 0 were only observed in *A. fumosus, A. lineatus* var. *jildisianus, A. galegiformes*, and *A. atrocarpus*, respectively. Three FAs, 20 : 0; 16 : 1 cis 7 DMA (ω9) and 22 : 2 : ω6c were absent only in *A. lineatus* var. *jildisianus, A. fumosus*, and *A. campylosema* subsp *campylosema*, respectively.Results suggest that concentration and composition of fatty acids might be useful in assessing chemotaxonomic relationships of *Astragalus* species and higher plants (Adiguzel *et al.* 2006, Agar *et al*. 2006, Agar *et al*. 2008, Agar *et al*. 2009, Sunar *et al.* 2009)

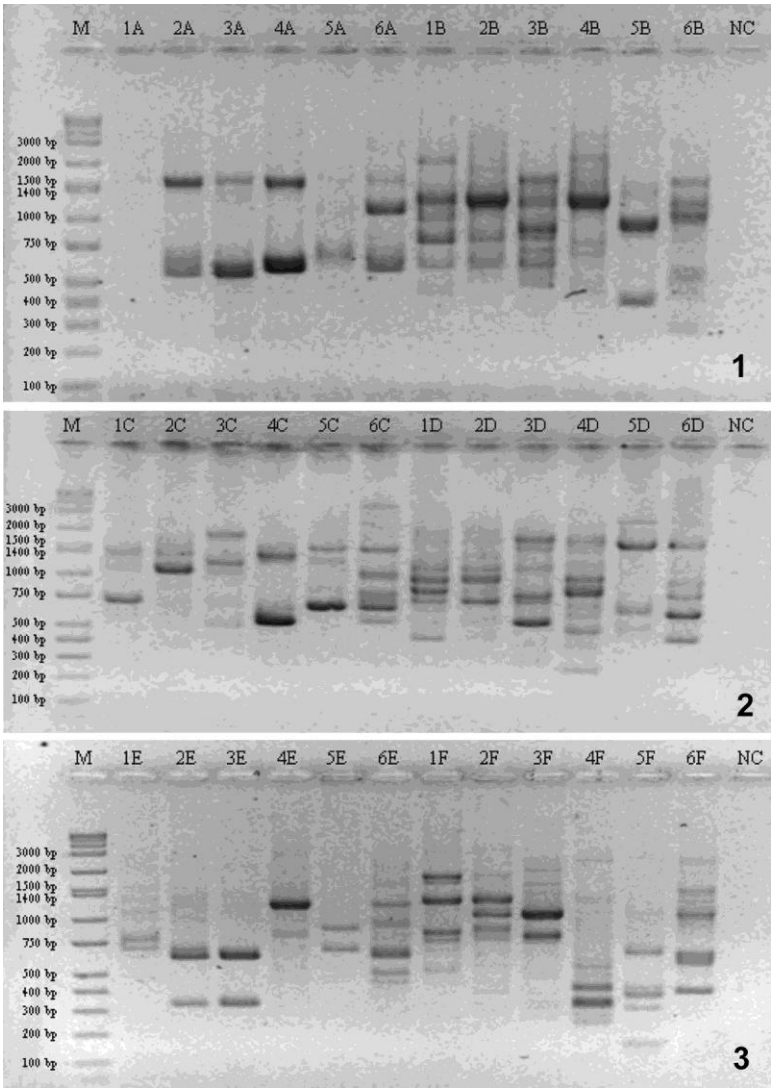
The RAPD results are summarized in Table 2 and Figs 1-3. The size of the amplicons varied from150 to 4000 bp. *A. campylosema* compared with the other species gave at least DNA banding with all six primers tested (Figs 1-3, Table 2). Primers OPD08 and OPA011 gave the highest and lowest number of RAPD products, respectively. The RAPD results showed the presence of four clusters with genetic similarity. Each cluster is represented by a different species of *Astragalus*, except for one cluster that is divided into three subclusters and includes *A. fumosus, A. lineatus* and *A. trichostigma* which were the most genetically related species (Fig. 4). The remaning taxa (*A. atrocarpus, A. galagiformes* and *A. campylosema*) showed completely different RAPD patterns using the same primers (Figs 1-3).

**Table 3. Composition of fatty acids in *Astragalus* taxa. *Aa = A. atrocarpus, Ac = A. campylosema, Af = A. fumosus, Ag = A. galegiformes, Al = A. lineatus, At = A. trichostigma.***

| | Fatty acid concentration (%) | | | | | |
|---|---|---|---|---|---|---|
| Fatty acids | *Af* | *Al* | *At* | *Ag* | *Ac* | *Aa* |
| 12 : 0 | - | - | - | 1.27 | 1.44 | 1.27 |
| 14 : 0 | 1.80 | 1.32 | 1.16 | 1.43 | 1.80 | 0.98 |
| 14 : 0 3OH | - | - | - | 1.35 | - | - |
| 15 : 0 anteiso | 2.43 | - | - | - | - | - |
| 16 : 0 | 16.48 | 11.98 | 13.45 | 13.38 | 22.33 | 11.30 |
| 17 : 0 cyclo | - | - | - | 0.74 | - | - |
| 18 : 0 | 1.90 | 1.06 | 1.48 | 1.40 | 2.40 | 1.55 |
| 18 N alcohol | 4.71 | 1.93 | 2.65 | 1.26 | 4.13 | 2.53 |
| 19 : 0 iso | - | - | - | - | - | 0.70 |
| 20 : 0 | 1.68 | - | 0.85 | 0.71 | 0.46 | 0.90 |
| 20 : 0 3OH | - | 0.55 | - | - | - | - |
| 21 : 0 anteiso | 0.60 | - | - | - | - | 1.67 |
| 22 : 0 | 2.60 | 0.87 | 0.63 | - | 0.41 | 0.98 |
| 22 primary alcohol | 2.34 | 0.71 | - | - | 1.13 | 0.52 |
| 22 : 0 2OH | - | - | - | - | - | 0.49 |
| 23 : 0 | - | 0.19 | - | - | - | - |
| 24 : 0 | - | - | - | - | - | 0.58 |
| 25 : 0 3OH | - | 13.06 | 10.50 | - | - | - |
| 16 : 1 : ω7c | 2.80 | - | - | 2.47 | - | - |
| 16 : 1 : ω7c alcohol | - | - | 1.17 | 0.75 | 1.57 | 0.57 |
| 16 : 1 cis 7 DMA (ω9) | - | 1.47 | 2.06 | 1.27 | 1.69 | 1.53 |
| 17 : 1 iso G | 2.55 | 1.85 | 2.13 | 2.37 | 4.17 | 1.89 |
| 18 : 1 : ω8c | 38.53 | 31.12 | 46.96 | 44.14 | 41.62 | 17.01 |
| 18 : 1 : ω9c DMA | - | - | - | 0.47 | 1.63 | - |
| 18 : 1 : ω9t alcohol | 4.33 | 2.88 | 3.74 | 2.74 | 6.37 | 2.34 |
| 18 : 2 : ω6c | 11.46 | 9.35 | 8.84 | 7.42 | 6.29 | 2.56 |
| 18 : 3 : ω6c | - | - | - | 1.60 | - | - |
| 19 : 1 : ω6c | 3.39 | 2.24 | 3.34 | 1.32 | 2.56 | 3.60 |
| 20 : 1 : ω7c | 0.74 | - | - | - | - | - |
| 21 : 1 : ω5c | - | 18.29 | - | 11.87 | - | 46.24 |
| 22 : 1 : ω3c | - | - | - | - | - | 0.30 |
| 22 : 2 : ω6c | 1.65 | 0.80 | 1.04 | 0.45 | - | 0.48 |
| 22 : 5 : ω3c | - | 0.31 | - | - | - | - |

The least variation was observed between *A. fumosus* and *A. lineatus* (Table 3, Fig. 4). The RAPD analysis suggested that there were distinct genetic differences among species. All the six *Astragalus* taxa were clearly distinguished in a dendrogram constructed using Jaccard UPGMA.

Both FAME and RAPD profiles have been used to study phenotypic and genetic diversity in many plant species (Li *et al.* 1999, Ozen *et al.* 2004, Adiguzel *et al.* 2006, Agar *et al.* 2006, Agar *et al*. 2008, Agar *et al*. 2009, Sunar *et al.* 2009). RAPD markers have been used to determine




Figs 1-3: 1. RAPD profiles generated with the primer A: OPA11; B: C05, respectively. Lanes: 1A-1B) *A. fumosus;* 2A-2B) *A. lineatus;* 3A-3B) *A. trichostigma;* 4A-4B) *A. galegiformes;* 5A-5B) *A. campylosema;* 6A-6B) *A. atrocarpus;* NC: Negative control; M: Molecular marker (10 kb). 2. RAPD profiles generated with the primer C: C15; D: D03, respectively. Lanes: 1C-1D) *A. fumosus*; 2C-2D*) A. lineatus* ; 3C-3D*) A. trichhosigma;* 4C-4D) *A. galegiformes ;* 5C-5D) *A. campylosema;* 6C-6D) *A. atrocarpus;* NC: Negative control; M: Molecular marker (10 kb). 3. RAPD profiles generated with the primer E: D07; F: D08, respectively. Lanes: 1E-1F) *A. fumosus;* 2E-2F) *A. lineatus;* 3E-3F) *A. trichostigma;* 4E-4F) *A. galegiformes;* 5E-5F) *A. campylosema;* 6E-6F) *A. atrocarpus;* NC: Negative control; M: Molecular marker (10 kb).

genetic relationships at the species and subspecies level. It is particularly useful for resolving relationship between closely related species and populations of genetically variable species (Yu and Pauls 1993).

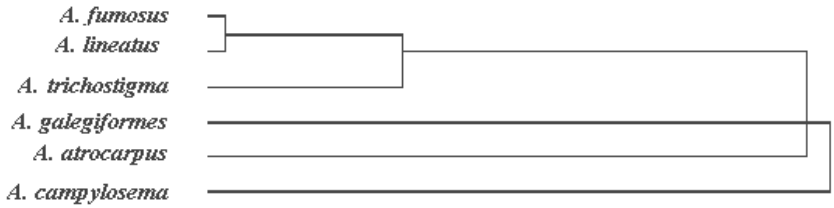



Fig. 4. UPGMA dendrogram showing the relationship of *Astragalus* species.

The results demonstrated that RAPD and FAME analyses are useful as chemical and genetic marker for differentiation and classification of *Astragalus* taxa tested in the present study. For the identification and characterization of each tested *Astragalus* taxa in the future studies it is necessary to determine the sequences of the polymorphic RAPD bands and particular delineation of the world-wide fatty acid patterns of this species.

**Acknowledgements**

This study was supported by grants from the research funds appropriated to Ataturk University and The Scientific and Technological Research Council of Turkey (TUBITAK).

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*(Manuscript received on 29 November 2006; revised on 13 May 2010)*