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Reporting *Potentilla botschantzeviana* Adylov (Syn: *Potentilla butkovii* var. *botschantzeviana* (Adylov) Soják) (Rosaceae) as a new record species for the flora of Iran

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Abstract

Potentilla botschantzeviana (Syn: *P. butkovii* var *botschantzeviana* (Adylov) Soják is reported as a new record species from N Iran for the first time. This species is identified by petioles with suppressed erecto - patent hairs; deeply divided leaflets with sparse crispate, erect indumentum especially on underside and with flower diameter of 1.2 cm. The morphological characters of *Potentilla botschantzeviana* (especially long style thickened at the base) are similar to the species of section *Persicae* (Th. Wolf) Juz., the largest section of the genus in Iran, including 12 endemic species. The photographs of plant at the flowering stage, different parts, indumentum ultra structure and distribution map are presented.

Key words: *Potentilla*, Flora of Iran, *Persicae* (Th. Wolf) Juz.

Introduction

Potentilla L. (Rosaceae) is a genus comprising about 485 species (Soják, 2008) of mostly perennial herbs, growing in the open habitats of mountainous to alpine or arctic regions as well as xeric communities in the northern hemisphere (Dobeš and Paule, 2010). The genus is well distributed especially in north, northwest and west of Iran and forms important floristic elements of Alborz and Zagros mountains (Schiman-Czeika, 1969; Khatamsaz, 1993; Faghir, 2010).

Potentilla botschantzeviana T. A. Adylov was collected from north of Iran. This species has not been previously reported from Iran. Description of the species was compared with other species of the genus in Flora Iranica (Schiman-Czeika, 1969), Flora of URSS (Shishkin and Yuzepchuk, 1941), most recent papers of Soják (2008 and 2009) and Faghir (2010).

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Materials and Methods

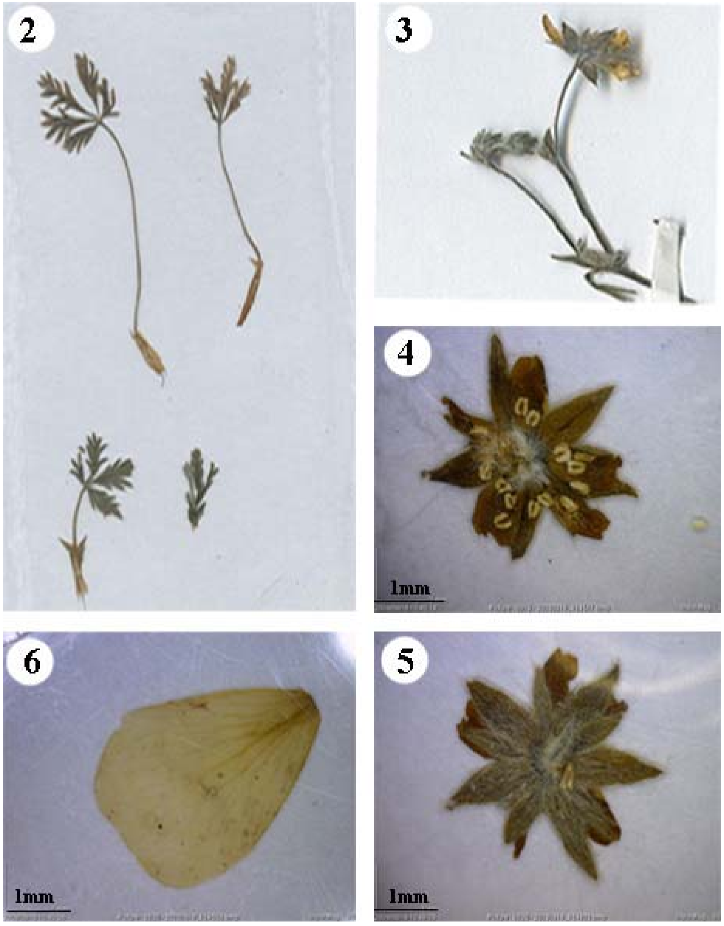
The photographs of different parts were taken by digital microscope, Dino-Lite, AN-413T model. The indumentum ultra structure was studied using scanning electron microscopy (SEM) and distribution map was presented by means of dmapw software (Morton, 2004). The collected and examined specimens are deposited in the Tehran University Herbarium (TUH).

Results

Potentilla botschantzeviana (Syn: *P. butkovii* var *botschantzeviana* (Adylov) Soják (Figure 1)

Perennial; caudex sturdy, multicipital, covered with grayish brown relics of stipules; stems ascending or decumbent, 18-25 cm, thin, pale green, without branches, covered with erect and silky hairs; leaves digitate; petioles of radical leaves 5-8 cm; radical leaves 1.8 × 0.6 cm; leaflets 2-2.5 × 0.8 cm, with 6 teeth on each side; stipules lanceolate, 2 mm; cauline leaves with short petiole or sessile (Figure 2); inflorescence with 2-4 flowers; pedicels thin 6-8 mm (Figure 3); flowers 0.7-1.2 cm in diameter; outer sepals 3 × 1 mm; inner sepals lanceolate 5 × 1.5 cm (Figures 4 and 5); covered with erect and straight indumentums; petals yellow, emarginated (Figure 6); style thickened at the base, longer than mature achenes (Figure 12); stamens with short filaments and 20 small ovate white anthers arranged in two rows (Figure 4).

Figure 1: *Potentilla botschantzeviana*



Figures 2-6: 1. *P. botschantzeviana*; 2. Radical and cauline leaves; 3. Flowers; 4. Internal view of flowers showing stamens; 5. External view clayx and calycles; 6. Petal. Scale Bars: Figures 4-6 = 1 mm.

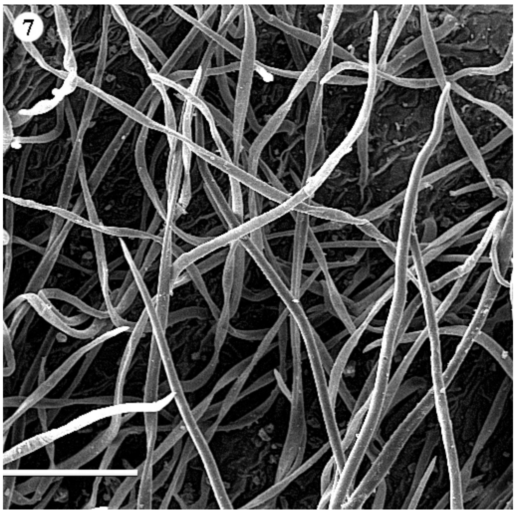
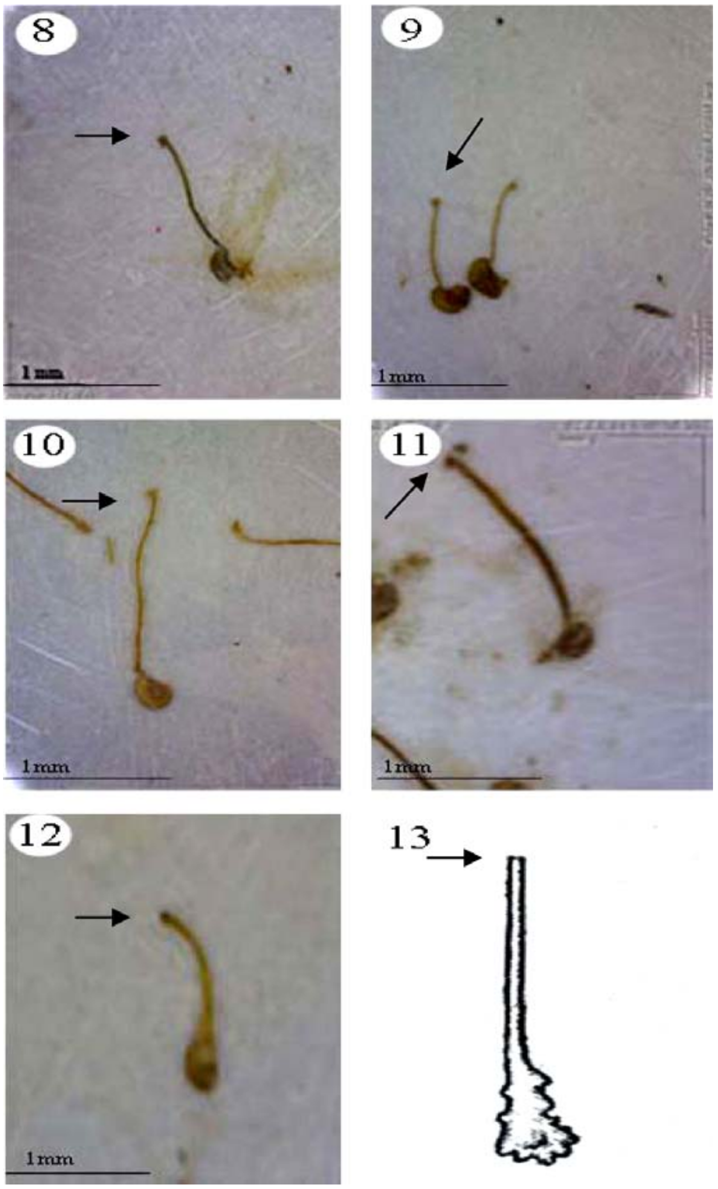


Figure 7: Scanning electron micrograph of crispate indumentum and sparse tomentum on underside of leaflets of *P. botschantzeviana*. Scale Bars = 100 µm.

Potentilla botschantzeviana is reported for the first time from Iran based on the presence of some important characters especially crispate indumentum and sparse tomentum on underside of leaflets (Figure 7); styles thickened at the base, non broadened stigma (not distinguishable from the top part of style) (Figure 12); long sepals (Figures 4 and 5) and whitish prominent anthers (Figure 4). This species was collected from Iran: Prov. Mazandaran, at 36° 27' North and 51°17' East, in Frozkouh. 2000-2500 mm altitude (36777-TUH).



Figures 8-13: 8. Style and stigma of section *Persicae* and sect. *Lipskyanae*: 8. *P. argea* Boiss. & Balansa; 9. *P. aucheriana* Th. Wolf; 10. *P. persica* Boiss. & Hausskn; 11. *Potentilla balansae* Soják; 12. *P. botschantzeviana*; 13. *P. mollissima* Lehm. (Soják, 2009). Scale Bars: Figures 8-12 = 1 mm, arrow indicates the broadened stigma in Figures 8-11 and non broadened.

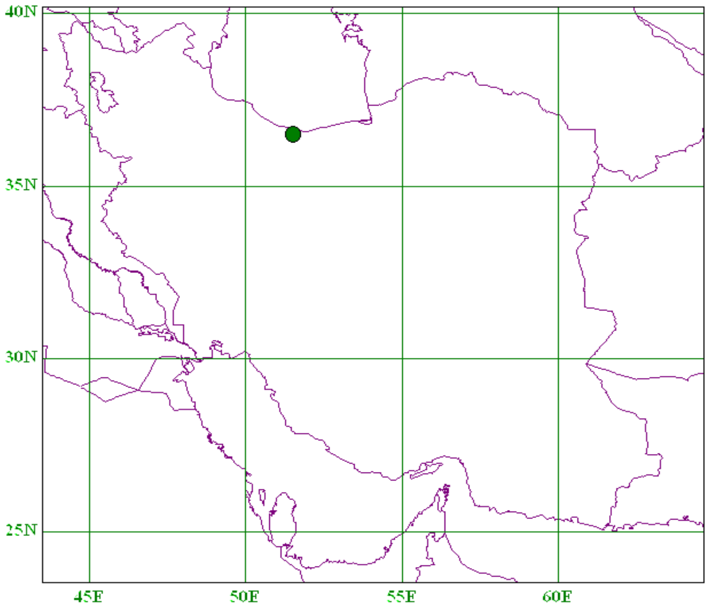


Figure 14: Distribution map of *P. botschantzeviana* (●) in Iran

Discussion

The morphological characters of *Potentilla botschantzeviana* (especially long style thickened at the base) are similar to species of section *Persicae* (Th. Wolf) Juz. Therefore authors of central Asian Flora assigned it with a group of very similar species ("*P. mollissima* Lehm group") to the section *Persicae*. This section is the largest section of the genus in Iran, including 14 species, and 12 endemic species, distributed in the north, northwest, centre and western parts of the country (Faghir *et al*., 2010a). The species of "*P. mollissima* Lehm group" are distributed in Tajikistan and adjacent regions of Turkmenistan, Afghanistan and Iran (Mesicek and Soják, 1993). These species differed from the members of sect. *Persicae* in having straight indumentum (Faghir *et al*., 2010b) and non broadened stigma (not differing morphologically from top of the style) (Figures 8-13). Based on this important differences *Potentilla botschantzeviana*, *P. mollissima* Lehm., (Syn: *P. komaroviana* Wolf., *P. lipskyana* Wolf.), *P. Butkovii* Botsch. and *P. rvazica* Juz. ex. Botsch. were referred in to section *Lipskyanae* Czevtajbva (Soják, 2009).

Further morphological survey of section *Lipskyanae* revealed that these species are so close to each other that they could be considered as varieties. According to Soják (2009) *Potentilla botschantzeviana* is referred to as *P. butkovii* var. *botschantzeviana* (Adylov) Soják. This species is the closest relative to *P. butkovii* Botsch (Syn: *P. butkovii* Botsch var. *butkovii*) so that both species posseses non broadened stigma and petioles with suppressed or erecto-patent hairs). However presence of important difference especially deeply divided leaflets, crispate indumentum and sparse tomentum on underside of leaflets; flowers diameter (1.2 cm) separates it from *P. butkovii* Botsch var. *butkovii* (Soják, 2004 and 2009).

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A taxonomic revision of the genus *Astragalus* L. (Fabaceae) in Zanjan province, Iran and describing a new species

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Abstract
Zanjan province in NW Iran, with diverse climatic and topographic conditions includes a considerable part of *Astragalus* gene pool. From 2007 through 2009, a collection as complete as possible was made in this area by the authors. As a results of this study, we added 41 species to the already 75 known species for this province. Furthermore, 9 new species were described for the area from which one new species (*Astragalus fausicola* Podlech ex Bagheri, Maassoumi & F. Ghahrem. from sect. *Incani* DC.) is introduced.
Key words: *Astragalus*, Fabaceae, New species, Taxonomy, Zanjan province

Introduction

The genus *Astragalus* L. with nearly 3000, species is probably the largest genus among the flowering plants (Lock and Simpson 1991). Iran, possessing nearly 800 species, is one of the most important centre of diversity for this genus (Maassoumi, 1998). This study following previous investigations which focused on the genus *Aastragalus* in Iran (Maassoumi, 1986-2005; Podlech, 1999 and 2010; Podlech *et al*., 2001; Zarre *et al*., 2008) aims to increase our knowledge about the Mega genus in Zanjan area. Based on previous local revision, this province with about 75 species belonging to 25 sections (Maassoumi, 1986-2005; Podlech, 1999 and 2010; Podlech *et al*., 2001; Zarre *et al*., 2008) was distinguished as an important area with respect to the genetic diversity of this genus. According to this new collection, the realistic number of *Astragalus* species occurring in Zanjan province was increased to 116 belonging to 28 sections.

Material and Methods

Zanjan province is situated in the NW Iran, with an approximate area of 2,216,400 hectars. The province with seven other provinces make a rich floristic zone presenting high number of endemic species. Climate of this province usually is cold and snowy in the mountain areas and moderate climate in the plains in winter. This province belongs to Irano-Turanian floristic region (Zohary, 1973) and presents its specific geographic,

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topographic and climate variation, also possesses suitable condition for the growth of several *Astragalus* sections including *Hymenostegis* Bunge, *Incani* DC., *Malacothrix* Bunge, and *Caprini* DC. (Podlech *et al*., 2001; Maassoumi, 1986-2005; Maassoumi, 2001; Bagheri, 2009; Ghahremaninejad and Bagheri, 2009; Ghahremaninejad *et al*., 2011; Bagheri *et al*., 2011; Maassoumi *et al*., 2011). This study was focused on the genus *Astragalus* especially sections *Hemenostegis*, *Incani* and *Malacothrix.* All materials are deposited in TARI and FAR herbaria. In this research, more than 1200 herbarium specimens were collected within about 40 localities as demonstrated in table 1 (Figure 1).

Table 1: Locality information for *Astragalus* taxa examined

No.	**Locality**	**Elevation(m)**
1.	Gheydar, Gheydar Mt.	2000-2500
2.	Gheydar, Paskohan, mountainously area	2100-2200
3.	Gheydar, Yenghikand	2000-2050
4.	10-20 km on the road from Zanjan to Bijar	1900
5.	25-30 km on the road from Dandi to Tekab	2400-2500
6.	33-37 km on the road from Dandi to Tekab, Belgheis Mt.	2400-2600
7.	10-20 km on the road from Abhar to Gheydar	1700-1900
8.	20-35 Km on the road from Abhar to Gheydar	1800-2000
9.	From Gheydar to Hamedan, Pirmarzeban	2050
10.	Sultaniyeh, Arjin, Sheikhsari Mt.	2100-2250
11.	10-15 km on the road from Zanjan to Meianeh	1400-1500
12.	20-40 km on the road from Zanjan to Meianeh	1300-1400
13.	60 Km from Zanjan to Mahneshan, Andabad	1750
14.	From Mahneshan to Pari, Alamkandi	2240
15.	20-50 km on the road from Zanjan to Bijar	1800-2000
16.	20-35 km on the road from Zanjan to Tarom	2105-2280
17.	35-55 km on the road from Zanjan to Tarom	2200-2400
18.	From Gheydar to Gharmab, 2 km before Gharmab	1600
19.	45 km from Gheydar to Khorkhoreh	1500
20.	14 km on the road from Mahneshan to Pari	2120
21.	80-84 Km on the road from Zanjan to Bijar	1550
22.	From Gheydar to Bijar, 2 km after Karasf, Salehabad	1750-1900
23.	Gheydar, from Arghin to Gheydar Mt.	2000-2100
24.	Zanjan, Sohrein, mountainous area	1950
25.	Zanjan, Taham, mountainous area	1900
26.	Gheydar, Dehjalal, mountainous area	2150-2200
27.	Anghuran, Belgheis Mt.	2250-2500
28.	From Gheydar to Zanjan, Mazidabad	1850-1900
29.	Gheydar, Zarand, mountainous area	2100-2200
30.	From Zanjan to Tarom, Badamestan	1850-1900
31.	20-30 km from Mahneshan to Pari	1850-1900
32.	63 km from Zanjan to Mahneshan	1700
33.	Zanjan, Ghavazangh, mountainous area	2200
34.	Gheydar, Akbarabad, mountainous area	2100-2200
35.	From Sultaniyeh to Gheydar	1850-1900
36.	75 km from Zanjan to Mahneshan, mountainous area	2120-2200
37.	45-50 km on the road from Mahneshan to Pari	1900-2000
38.	30-50 km on the road from Dandi to Zanjan	2100-2300
39.	Gheydar, Kahla, mountainous area	1850-1900
40.	From Zanjan to Mahneshan, Sheikhlar	1950-2000

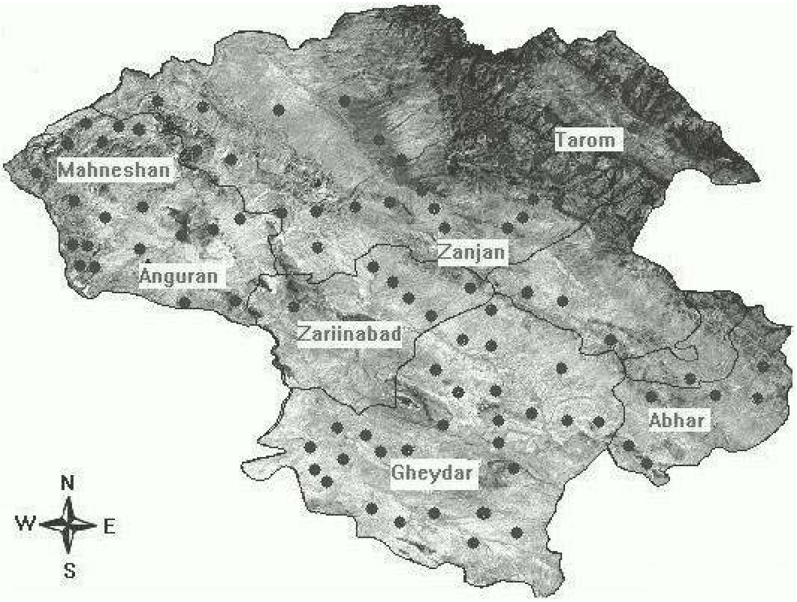

Figure 1: Geographical map of Zanjan province with main localities

Results

Abbreviations: A (Annuals species); **H** (Perennial herbaceous species); **WO** (Perennial woody species); **EP** (Endemic of Zanjan province); **SP** (Share with other province); **SC** (Share with other countries); **NC** (Not collected in this research, but have been reported in published literatures for Zanjan province); **D** (species which were recently described); **RP** (New record for Zanjan province); **RI** (New record for Iran).

Annuals species

Annual species grow on limited parts of the province.

1. Section *Ankylotus* Bunge
A. commixtus Bunge (18) A, SC
2. Section *Annulares* DC.
***A. campylorhynchus* Fisch. & C.A.Mey.** (9, 12, 13, 18, 19, 20, 21) A, SC
3. Section *Heterodontus* Bunge
A. guttatus [Soland.] (18) A, SC
4. Section *Oxyglottis* Bunge
A. crispocarpus Na'belek A, SC, NC
A. oxyglottis Steven ex M.Bieb. (1, 11, 12, 22) A, SC
A. schmalhausenii Bunge A, SC, NC
A. vicarius Lipsky, (12, 15, 19, 22) A, SC, RP
5. Section *Platyglottis* Bunge
A. comptoceras Bunge (7, 17, 22, 24, 30, 35) A, SC, RP
6. Section *Sesamei* DC.
A. coronilla Bunge, subsp. ***coronilla*** (7, 22) A, SC, RP

Perennial herbaceous species

7. Section *Alopecuroidei* DC.
A. echinops Auch. ex Boiss. (1) H, SC, RP
A. foliosus Podlech, Maassoumi & Ranjbar H, SP, NC

A. macrocephalus Willd. subsp. ***macrocephalus*** (1) H, SC
A. megalotropis C.A.Mey. ex Bunge SP, NC
8. Section *Astragalus*
A. caraganae Fisch. & C.A.Mey. (1, 4, 15, 20, 22, 24, 25) H, SC
9. Section *Caprini* DC.
A. aegobromus Boiss. & Hohen (1, 22, 23, 29, 34) H, SC, RP
A. angustiflorus K. Koch subsp. ***angustiflorus*** (1, 2, 17, 20, 29, 34) H, SC
A. apricus Bunge (1, 15, 23, 32) H, SC, RP
A. chrysanthus Boiss. (1) H, SP
A. citrinus Bunge subsp. ***citrinus*** (17) H, SC
A. kirpicznikovii Grossh. (24, 30) H, SC, RP
A. macropelmatus Bunge subsp**. *macropelmatus*** (4, 7, 10, 11, 13, 21, 22, 28) H, SC
A. multijugus DC. (1, 2, 16, 29) H, SP
A. ovinus Boiss. (1, 7, 9, 14, 27, 29) H, SC
A. pseudoutriger Grossh. H, SC, NC
A. semilunatus Podlech H, EP, NC, RP
A. shemachensis Karjagin, (6, 9) H, SC, RP
A. urmiensis Bunge (1, 2, 5, 15, 22, 29, 34) H, SC
10. Section *Corethrum* Bunge
A. dendroproselius Rech.f. H, SC, NC
11. Section *Dissitiflori* DC.
A. argyroides Beck (21, 22, 32) H, SC
12. Section *Erioceras* Bunge
A. pentanthus Boiss. (20, 22) H, SP
13. Section *Hololeuce* Bunge
A. alyssoides Lam. (1, 2, 4, 7, 16, 19, 22, 23, 28, 29, 32, 34, 35) H, SC
Based on our collection, this species is extensively distributed at high altitude in several localities of the province.
14. Section *Incani* DC.
A. abharensis Maassoumi & Podlech (7, 15) H, EP
A. alavaanus Podlech (1) H, SP
A. andabadensis Maassoumi, Bagheri & F.Gharem. (13) H, EP, D
A. askius Bunge (1, 5) H, SP, RP
A. curvirostris Boiss. (1, 2, 5, 7, 9, 11, 16, 17, 20, 22, 24 28, 29, 30, 34, 37) H, SC
A. delutulides Maassoumi, F.Ghahrem., & Bagheri (6) H, EP, D
A. delutulus Maassoumi (23) H, SP, RP
A. fausicola Podlech ex Bagheri, Maassoumi & F.Ghahrem., ***sp.nov***. *e* sect. *Incani* DC. (Fig. 2).

Typus: Zanjan: Gheidar, Gheidar Mt., 2430m, 2009/07/10, Bagheri 9064. (Holotype, TARI; Isotypes, FAR, MSB).

Sed differt ab A. askius Bunge bracteis glabris vel margin remotiusculi glandulosi vel raro sparse ciliatis (nec manifest ciliatis), foliolis angust ovatis vel rhomboidis, magnis c. 40-50 mm longis apicem versus long acutis vel subaristatis (nec obtusis vel breviter acutis), corolla intense violacea (nec lutea), legumnibus dorso manifest rotundatum (nec sulcatum) non purpureo punctato provisum.

Plants 30-50 cm tall, with symmetrically medifixed deppressed hairs 0.3-0.5 mm long. Caudex 8-11 mm in diameter, with few short branches, covered with remnants of old leaves and stipules. Stipules triangular to narrowly triangular-acuminate, 8-14 mm lang, adnate to the petiole for 5-7 mm, hairy. Leaves 10-25 cm long; petiole 3-11 cm long, like the rachis finely striate, loosely to rather densely hairy, later on glabrescent. Leaflets in 8-13 pairs, narrowly ovate, 10-50 × 4-20 mm, sharply acute to apex. Mostly aristate, sparsely to loosely, rarely rather densely hairy on both sides. Peduncle 15-30 cm long, thick, finely striate, glabrous. Raceme loosely many flowered, 15-20 cm long. Bracts brownish, linear-acute, 3-4 mm long, sparsely ciliate, glabrous, glandulosa or rarely sparsely ciliate on the margin. Pedicels ca. 3 mm long, Flowers erect, later becoming spreading or deflexed. Bracteoles whitish, linear, ca. 1 mm long, at the base of the calyx. Calyx 8-13 mm long, tubular, obliquely acute at the mouth, covered with deppressed black hairy; teeth narrowly triangular, acute, unequal, 1-2 mm long. Corolla violet. Standard c. 20 mm long; blade minutely narrowed, 10 mm wide, elliptic, at the base abruptly narrowed into a cuneate claw. Wings 15-17 mm long; blades narrowly oblong, rounded to obliquely retuse, at apex 8 × 3 mm; auricle 1.5 mm long, claw 9-10 mm long. Keel 15mm long; blades elliptic, acute at the apex, 8 × 4 mm; auricle very short, claw 9-10 mm long. Ovary with a short or distinct stipe up to 4 mm long, linear, scarcely hairy to glabrous. Legume with a 3-4 mm long stipe, pendulous, linear, straight or slightly upcurved, 18-35 mm long, 2-3 mm high und 3-5 mm wide, carinate ventrally, distinctly rotundate dorsally, at the apex narrowed into a straight beak 2-4 mm long; valves yellowish, without dark spots, glabrous. Fruit fully bilocular. Seeds rectangular, 3-5 × 2-3 mm, dark brown.

A. khadem-kandicus Maassoumi & Podlech (2) H, SP
A. montis-queydari F.Ghahrem.,Maassoumi & Bagheri (1) H, EP, D
A. qeydarensis Podlech (7) H, EP (ined)
A. refractus C.A.Mey. (14) H, SC, RP
A. supervisus Sheld. (1, 28, 29, 32, 35, 40) H, SC
A. zanjanensis Podlech & Maassoumi (9, 33) H, EP
15. Section *Laxiflori* Kirchhof
A. dictyolobus C.A.Mey. ex Bunge (9) H, SP
A. tawilicus C.C.Towns. H, SC, NC
16. Section *Malacothrix* Bunge
A. beckii Bornm. (1, 23, 29) H, SP
A. belgheisicoides Podlech & Maassoumi (7, 16, 33, 34) H, EP
A. belgheisicus Maassoumi (27, 32) H, EP
A. comosus Bunge (1, 32) H, SC
A. singarensis Boiss. & Hausskn. ex Boiss. (1, 22) H, SP
A. eriocarpus DC. H, SC, NC
A. eriopodus Boiss.(20, 24) H, SC
A. hendelanicus Maassoumi (11, 18) H, SP
A. iranicus Bunge (7, 11, 12, 35) H, SC
A. macrourus Fisch & C.A. Mey. (1, 2, 23) H, SC
A. meshkinensis Podlech H, EP, NC

A. patrius Maassoumi (1, 17, 25, 35) H, SP
A. pileh-khasehensis Podlech & Maassoumi H, EP, NC
A. podocarpus C.A. Mey.(17) H, SC
A. saccatus Boiss. (28) H, SP
A. senilis Bornm. (1, 2, 4, 8, 11, 15, 20, 22, 23, 28, 32) H, SP

17. Section *Onobrychoidei* DC.

A. aduncus Willd. H, SC, NC
A. brevipes Bunge H, SP, NC
A. effusus Bunge H, SP, NC
A. pendulipodus Ranjbar & Karamian H, SP, NC
A. vegetus Bunge (1, 7, 8, 9, 32, 40) H, SC

18. Section *Ornithopodium* Bunge

A. brachyodontus Boiss. (1, 7, 8, 9, 12, 15, 17, 18, 19, 27, 30, 38) H, SP
A. glochideus Boriss. (1, 2, 4, 9, 10, 13, 16, 17, 23, 24, 27, 32, 33) H, SC

19. Section *Stereothrix* Bunge

A. mahneshanensis Maassoumi & Mousavi H, EP, NC

20. Section *Theiochrus* Bunge

A. siliquosus Boiss. subsp. ***siliquosus*** (1, 8, 15, 35) H, SC

21. Section *Uliginosi* Gray

A. odoratus Lam. (6) H, SC

22. Section *Trachycercis* Bunge

A. barnasariformis Maassoumi, F.Ghahrem. & Bagheri (5) H, EP, D

Perennial woody species

23. Section *Adiaspastus* Bunge

A. aureus Willd. WO, SC, NC
A. caspicus M. Bieb. subsp. ***caspicus*** (1, 2, 3, 4, 5, 7, 9, 10, 11, 13, 14, 29, 39) WO, SC
A. michauxianus Boiss. (1, 2, 4, 5, 8, 16, 34) WO, SC
A. polyanthus Bunge (1, 7, 10) WO, SC, RP

24. Section *Anthylloidei* DC.

A. ebenoides Boiss. subsp. ***ebenoides*** WO, SP, NC
A. halicacabus Lam. (22) WO, SC
A. submitis Boiss. & Hohen. subsp. ***submitis*** (1, 2, 7, 10, 23) WO, SP, RP

25. Section *Cremoceras* Bunge

A. campylanthoides Bornm. (7, 10, 32) **WO, SC**

26. Section *Hymenostegis* Bunge

A. anguranensis Podlech & Maassoumi (6, 27) WO, EP
A. austromahneshanensis F.Ghahrem.,Maassoumi, & Bagheri (32) WO, EP, D
A. bounophilus Boiss. & Hohen. (5, 6) WO, SP
A. chrysostachys Boiss. (4, 5, 9, 11, 21, 22, 32, 37) WO, SC

Within the sect. *Hymenostegis*, this species shows a wide-ranging distribution in the major parts of the province from other related species.

A. demonstratus Maassoumi (37) WO, EP
A. glumaceus Boiss. (4, 7, 15, 21, 22, 32, 38) WO, SP
A. kapherrianus Fisch. WO, SC, NC
A. lagopoides Lam. (17) WO, SC, RP
A. marivanensis Maassoumi & Podlech (4, 32) WO, SP, RP
A. melanostictus Freyn (4) WO, SC
A. nervistipulus Boiss. & Hausskn. ex Boiss. (1, 3, 4, 7, 8, 9, 17, 22, 35) WO, SP, RP
A. paralurges Bunge (1, 3, 7, 10, 15, 16, 21, 22, 24, 26, 30, 35) WO, SP
Widely distributed in the mountainous area mixed with Tragacanthic species.
A. pauxillis Maassoumi & F. Ghahrem. (1) WO, SP
A. pediculariformis Maassoumi (1, 3, 7, 23, 29) WO, EP
A. qorvehensis Podlech (1) WO, SP, RP
A. qeydarnabiensis Bagheri, F.Ghahrem. & Maassoumi (1) WO, EP, D
A. rubrostriatus Bunge (1, 7, 9, 10, 17, 24, 25, 29, 33, 35) WO, SP
A. sciureus Boiss. & Hohen. (1) WO, SP
A. sosnowskyi Grossh. (21) WO, SC, RI
Type species previousely reported from Iraq, based on new collection, this species as a new record for Iranian territory.
A. subkohrudicus Maassoumi, F.Ghahrem. & Bagheri (1) WO, EP, D
A. subrecognitus Bagheri, Maassoumi & F.Ghahrem. (20) WO, EP, D
A. tabrizianus Buhse (16, 17, 30, 32) WO, SP

27. Section *Rhacophorus* Bunge

A. andalanicus Boiss. & Hausskn. ex Boiss. (1, 2, 23) WO, SC, RP
A. compactus Lam. (1, 2, 4, 5, 16, 22, 23, 26, 29, 32, 38) WO, SC
A. denudatus Steven WO, SC, NC
A. eriosphaerus Boiss. & Hausskn. ex Boiss. (9, 16, 22, 25, 36, 37, 38, 39) WO, SP
A. floccosus Boiss. subsp. ***floccosus*** (9) WO, SP
A. gossypinus Fisch. (1, 3, 7, 21, 22, 23, 29, 32, 36) WO, SC
A. microcephalus Willd. subsp**. *microcephalus*** (1, 2, 3, 7, 10, 11, 12, 15, 16, 17, 22, 23, 29, 32, 36) WO, SC
This species shows a wide-ranging distribution in the mountainous area, up to 1800 m and with other tragacanthic species such as: *A. eriosphaeru*s, *A. gossypinus*, and *A. campactus* create a mixed associations.
A. paralipomenus Bunge (1, 4, 5, 7, 8, 14, 15, 16, 17, 20, 29, 36) WO, SC
A. psilostylus Bunge WO, SP, NC
A. rhodosemius Boiss. & Hausskn. (23, 40) WO, SP, RP
A. verus Oliver, (16, 27, 34) WO, SC

28. Section *Tricholobus* Bunge

A. tricholobus DC.
subsp. ***tricholobus*** (1, 2, 5, 7, 9, 10, 16, 17, 20, 22, 23, 26, 27, 29) WO, SP
subsp. ***hohenackeri*** (Boiss.) Tietz (16, 27) WO, SC

Figure 2: *A. fausicola* Podlech ex Bagheri, Maassoumi, & F.Ghahrem.; (after Holotype, Bagheri 9064)

Discussion

In total, 116 distinct species of *Astragalus* were recognized in Zanjan province. They include 9 annuals species (7.75%), 65 perennial herbaceous species (56.03%) and 42 perennial woody species (36.20%).

Among 116 species, 22 spp. (18.96%) belong to sect. *Hymenostegis,* 16 (13.79%) species to sect. *Malacothrix*, 14 (12.06%) species to sect. *Incani,* 13 (11.20%) species to sect. *Caprini*, 11 (9.48%) species to sect. *Rhacophorus*, and 40 (34.48%) species belonging to the other sections which are illustrated in fig. 4. In the point of endemism percentage the province are divided into 3 groups: 1- containing 21 spp. (18.10%) are endemics for the province; 32 spp. (27.58%) are shared with other provinces; and 63 spp. (54.31%) are shared with other neighboring countries.

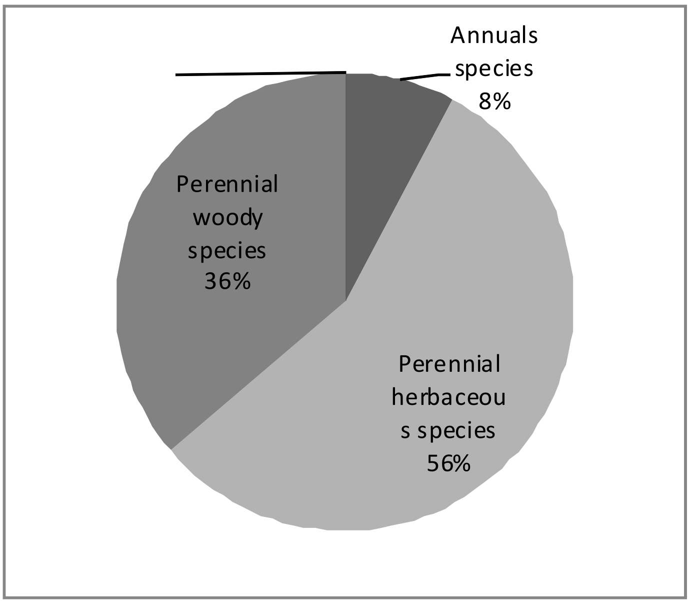

Figure 3: Life form of *Astragalus* species in Zanjan province

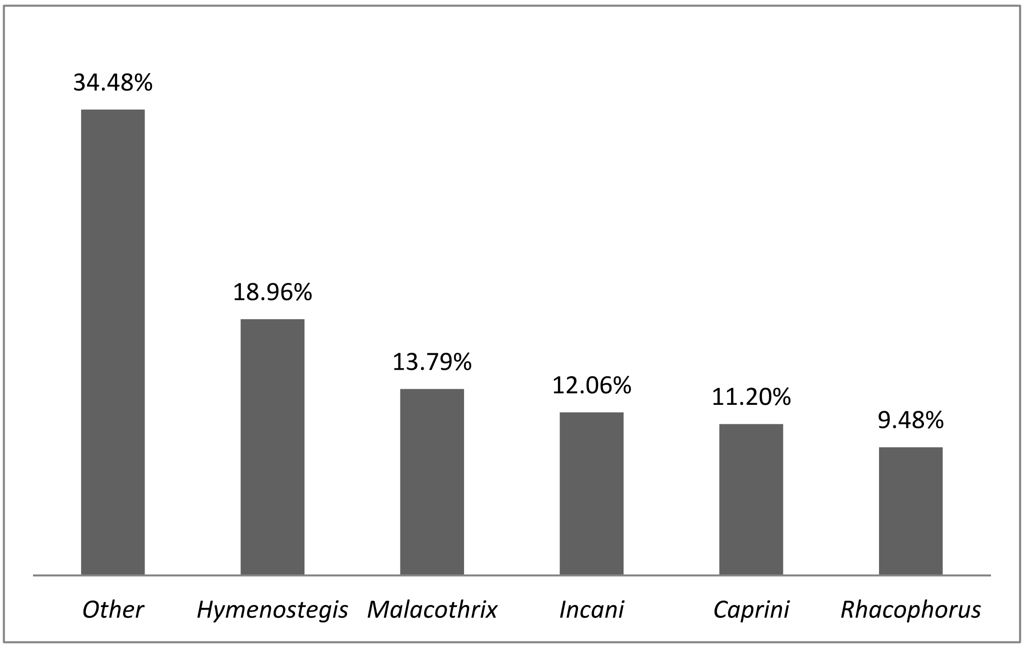

Figure 4: Sectional profile of *Astragalus* species in Zanjan province

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Cytological study of *Hordeum bulbosum* L. in Iran

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Abstract

Hordeum bulbosum L. (Poaceae) is considered to be sources of useful alleles which can be used in cereal improvement. Thirty two native Iranian *H. bulbosum* were collected from different localities and were studied by karyotype analysis. We assessed the karyotype asymmetry of the Iranian bulbous barley populations and analyzed the data to look for their geographic distribution correlations. All of the studied populations were tetraploid (2n=4x=28) and the analysed parameters of karyotype of *H. bulbosum* support the autopolyploidy origin of the species with nearly symmetric karyotype. The results showed the most asymmetric karyotypes within northeast (Golestan) and northwest (Gardane-e Heiran) populations and the most symmetric karyotyps in populations from the west of Iran. Therefore, it can be assumed that the oldest populations are in the slopes of Zagros Mountains and the youngest germplasms occur in the northeast of this country. It can be concluded that the species originated from the west of Iran and distributed towards east and northeast.

Key words: *Hordeum bulbosum* L., Iran, Karyotype symmetry, Tetraploid

Introduction

The genus *Hordeum* consists of 32 species (45 taxa in total, including subspecies and cytotypes) including diploid (2n=2x=14), tetraploid (2n=4x=28) and hexaploid (2n=6x=42) cytotypes with a basic chromosome number of x=7 (Bothmer *et al.,* 1995). The genus is classified into five genome groups, namely H, I, X, Y and XI (Taketa *et al.,* 1999). In this study, genome designation followed that of Taketa *et al.* (2001), namely, *H. vulgare* and *H. bulbosum* both carry the H genome, so that *H. marinum* carries the X genome, while *H. murinum* has the Y genome, and the 25 remaining species share variants of the I genome (Taketa *et al.,* 2005). *H. bulbosum* has been recognized as one of the two separate allogamous species of the genus, possessing a sporophytic incompatibility system (Bothmer *et al.,* 1995). This species include two well-known cytotypes, diploid and tetraploid, with the latter being more widespread. The tetraploid cytotype is commonly considered as an autopolyploid (HHHH) (Xu and Snape, 1988; Chin, 1941; Papes and Bosiljevac, 1984).

The populations of bulbous barley grow widely in the mountainous and sub mountainous regions of Iran in the north, northeast, northwest, west, southwest and the south (except in

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the Central Plateau, northern Persian Gulf and southern Caspian Sea shores) (Bor, 1970) with different and under stressful environmental conditions.

Symeonidis *et al.,* (1985) claimed that the chromosome set of bulbous barley originated from Greece which contains 16 metacentric including 4 satellited, 8 sub-metacentric and 4 telo-centric chromosomes. Nasirzadeh and Mirzaie Nadoushan (2005) reported that bulbous barley in north of Fars province is tetraploid with karyotype formulae (6m+1sm).

The aim of the present work was the evaluation of the cytotypes of *H. bulbosum* in Iran, characterization of the cytological and karyotypic details (numerical parameters) and their correlations with the geographic distribution of *H. bulbosum.*

Materials and Methods

Plant materials

A total of 32 specimens of *H. bulbosum* were randomly collected from various regions of Iran by the authors and were identified morphologically according to Bothmer *et al.,* (1995) and analysed cytologically (Table 1).

Chromosome spread preparation

The seeds were germinated on paper tissue in pertidishes and the root tips selected for cytological experiments. Somatic chromosomes of meristematic root tip cells were treated from germinated seeds based on Agayev (1996) protocol with minor modifications. Briefly, pretreatment was carried out in saturated solution of Monobromonaphthalene, washed in distilled water for 30 min, fixed in Chromic Acid/Formaldehyde mixture (1/1) at about 4 ºC for 24 h, and finally washed under tap water for 3 h. Then the materials were transferred into 70% ethanol solution and kept refrigerated till staining process. For staining, the materials were transferred into distilled water for about 5-6 min and treated with 1N NaOH at 60 ºC for 10 min, washed in distilled water thoroughly for 30 min then stained in aceto-iron hematoxylin at 30 ºC for 24 h, washed in distilled water for at least 30 min, and macerated for 10-15 min in cellulase-pektinase enzyme solution at 37 ºC.

The roots were gently squashed in 45% acetic acid, on a slide glass and were observed and photographed under an Olympus AX-40 light microscope. At least, five cells were screened and the cells with good spread were used for analyzing and constructing karyograms. In order to characterize the karyotypic asymmetry, 5- 10 chromosome spreads from different individuals of each accession were examined. All chromosome sizes were measured with computer–aided program Image Tool 3.0. The parameters measured for each metaphase chromosome spread included Total Chromosome Length of the haploid complement (TCL), Mean Chromosome Length of the haploid complement (MCL), and Total Form percent (TF%: Ratio between the shortest arms of the chromosomes and their total length); the TF% value was considered to be close to 50% in most symmetric karyotypes and less than 50% based on the degree of asymmetry, (Huziwara, 1962), R (Ratio between the longest and the shortest arms of the chromosomes, Siljak-Yakovlev, 1986), S% (equals to length of the shortest chromosome dividede on length of the longest chromosome, Stebbins, 1971), Asymmetry index ($AsI\% = 100 \times \Sigma L / \Sigma TCL$; where l is long arms in chromosome set and TCL is total chromosome length in chromosome set, Arano and Saito, 1980) and Karyotype formulae: according to their arm ratios (long/short) designated by the position of the centromere: 1 (metacentric; M), 1-1.7 (metacentric; m), 1.7-3 (submetacentric; sm), 3-7 (subtelocentric; st), and 7-39 (telocentric; t)] (Levan *et al.,* 1964).

Table 1. Accessions of *H. bulbosum* (HB) collected from different places in Iran.

Accession no.	Region	Altitude (m)	Locality
HB2W	W	690	Ilam, Darehshahr, Shahre bastani
HB3W	W	642	Ilam, Darehshahr, Gharatmalgeh
HB6W	W	1509	Lorestan, Dorud,Siahkoleh
HB14W	W	1931	Lorestan, Khoramabad toward Borujerd, Zagheh
HB22W	W	1703	Ilam, 45 Km Islamabad-e-gharb toward Eivan
HB23W	W	1580	Kermanshah, 40 Km Eivan toward Islamabad-e-gharb
HB24W	W	1292	Ilam, Darehshahr toward Ilam, Mishkhas
HB30SW	SW	2100	Chaharmahal-va-Bakhtyari, Felard, Aboueshagh, Kahriz
HB73SW	SW	1690	Fars, Eghlid to Marvdasht, Dorudzan
HB76SW	SW	1702	Fars, Shiraz, Roknabad
HB77SW	SW	1975	Fars, Shiraz toward Kazeroon, Hoseinieh
HB79SW	SW	2051	Fars, Shiaz toward Kazerun, Dashte Arjan
HB81SW	SW	1050	Fars, Noorabad-e-Mamasany
HB84SW	SW	2050	Kohgiluie-va-Boyerahmad, Babameidan toward Yasooj
HB87SW	SW	1695	Kohgiluie-va- Boyerahmad, 25 KmYasooj toward Isfahan
HB90SW	SW	1752	Chaharmahal-va-Bakhtyari,Broojen toward Yasooj, Felard
HB91SW	SW	2240	Chaharmahal va Bakhtyari, Broojen toward Yasooj
HB95N	N	1640	Tehran, Boomehen
HB105NE	NE	1775	Golestan Azadshahr toward Shahrood, Khoshyeilagh
HB106NE	NE	700	Golestan, National Park of Golestan
HB109NE	NE	993	Khorasane Shomali, Bojnourd, Baba aman park
HB202W	W	1193	Ilam, Darehshahr toward Ilam, Pakal-e-Gerab
HB207W	W	1360	Kermanshah, Kermanshah toward Kamyaran, Vermenje
HB208W	W	1741	Kurdistan, Kamyaran toward Sanandaj, Morvarid
HB209W	W	1581	Kurdistan, Sanandaj
HB211W	W	1257	Kurdistan, 15 Km Sarvabad toward Sanandaj
HB212W	W	1222	Kurdistan, Sarvabad
HB213W	W	1249	Kurdestan, around of Zarivar lake
HB215W	W	1587	Kurdistan, Marivan toward Saghez, Sarshio
HB216W	W	1423	Azarbaijane Gharbi, Boukan, Kanitoomar
HB217NW	NW	1822	Azarbaijane Gharbi, Boukan,Mohabad, Gharehbolagh
HB221NW	NW	1537	Gilan, Astara, Heiran

Results and Discussion

All of the studied populations were tetraploid (2n=4x=28) and the results of the analyzed parameters of karyotype of *H. bulbosum* supported the autopolyploidy origin of the species with nearly symmetric karyotype combining four homologous or near homologous genomes that were in accordance with previous reports (Chin, 1941; Morrison, 1959; Xu and Snape, 1988). Karyotype was nearly symmetrical with chromosomes varying in mean total chromosome lengths from 5.22 (in HB90SW from Dasht-e Felard at Chaharmahal va Bakhtiari province) to 15.04 *µ*m (in B3W from Darrehshahr in Ilam province) (Table 2). The descriptions of karyotype formulae and their analyzed parameters results are shown in Tables 2 and 3, respectively.

Table 2. Karyotype analysis of the different populations of *H. bulbosum* species (n=chromosome number, TL=Total haploid chromatin length, MCL=Mean Chromosome Length, SE=Standard Error, TF%=Total Form percent, S%=Length of the shortest chromosome divided on length of the longest chromosome, R=ratio between the longest and the shortest arms of the chromosomes, AsI%=Asymetry index, *=Satellite.

Population	2n	TCL	MCL ± SE	TF%	S%	R	AsI%	Karyotype formulae
HB2W	28	63.68	9.1±1.117	45.1	68.56	1.23	54.89	2M + 5m*
HB3W	28	92.53	13.22±1.56	41.22	70.61	1.45	58.77	6m* + 1sm
HB6W	28	53.79	7.68 ±0.74	41.68	76.57	1.45	58.31	6m* + 1sm
HB14W	28	73.2	10.5±1.341	44.03	70.77	1.33	55.96	1M + 4m* +2sm
HB22W	28	76.84	10.1±1.288	44.15	73.92	1.28	55.84	2M + 5m*
HB23W	28	84.03	12.0 ±1.235	44.34	77.31	1.27	55.658	7m*
HB24W	28	67.6	9.66±0.933	42.42	76.88	1.39	57.573	1M* + 5m + 1sm
HB30SW	28	81.48	11.64±1.01	42.26	79.12	1.397	57.731	6m* + 1sm
HB73SW	28	55.97	7.1±1.261	43.79	59.3	1.297	56.208	1M + 5m* + 1sm
HB76SW	28	76.28	10.9±1.5	40.53	67.12	1.53	59.465	2M* + 4m + 1sm
HB77SW	28	54.38	7.77±1.055	41.28	65.29	1.467	58.716	1M* + 5m + 1sm
HB79SW	28	74.8	10.69±1.344	42.78	67.01	1.397	57.22	1M* + 5m + 1sm
HB81SW	28	59.26	8.47±0.908	39.7	73.52	1.655	60.3	5m* + 1sm + 1st
HB84SW	28	57	8.14±1.234	38.57	63.35	1.616	61.42	5m* + 2sm
HB87SW	28	57.79	8.26±0.94	41.18	71.48	1.56	58.81	5m* + 2sm
HB90SW	28	44.88	6.41±0.7	43.6	71.21	1.345	56.39	2M + 4m + 1sm
HB91SW	28	51.26	7.32±0.85	41.02	72.35	1.541	58.97	1M + 5m* + 1sm
HB95N	28	76.73	10.961±1.278	42.42	74.43	1.395	57.578	6m* + 1sm
HB105NE	28	55.7	7.96±1.04	40.68	68.6	1.491	59.317	6m* + 1sm
HB106NE	28	63.02	9.002±1.41	37.81	64.17	1.687	62.186	4m + 3sm*
HB109NE	28	59.77	8.538±1.147	40.48	68.65	1.543	59.511	6m* + 1sm
HB202W	28	53.08	7.582±0.79	39.44	70.97	1.57	60.55	1M + 3m + 3sm*
HB207W	28	70.06	10.01±1.03	40.1	74.32	1.53	59.006	1M* + 4m + 2sm
HB208W	28	85.41	12.201±1.55	40.86	71.03	1.493	59.138	1M + 5m* + 1sm
HB209W	28	48.86	6.98±0.69	41.17	76.41	1.533	58.821	1M + 4m* + 2sm
HB211W	28	59.86	8.55±1.74	38.79	51.3	1.6	61.209	5m* + 2sm
HB212W	28	53.45	7.64±0.8	42.37	75.57	1.467	57.623	5m* + 2sm
HB213W	28	85.61	12.23 ±1.27	42.42	73.33	1.438	57.575	1M + 5m* +1sm
HB215W	28	67.07	9.58 ±2.1	40.15	48.58	1.565	59.847	1M* + 4m + 2sm
HB216W	28	73.01	10.43 ±1.4	42.5	69.15	1.366	57.498	5m* + 2sm
HB217W	28	66.91	9.56 ±0.85	41.72	81.32	1.432	58.272	1M*+ 5m + 1sm
HB221W	28	62.61	8.94 ±.91	37.93	72.53	1.683	62.066	1M* + 3m + 3sm

The morphological characteristics of chromosomes are shown in Figure 1. As presented in Table 2, the metacentric (M and m) chromosomes dominated the observed karyotypes with 79.46% and the second frequency belongs to the submetacentrics (20.09%). Only one population (HB81SW from Noorabad –e Mamasany in Fars province) had a sub-telocentric (st) chromosome with karyotype formulae (5m* + 1sm + 1st). No telocentric chromosome was observed (see Table 2).

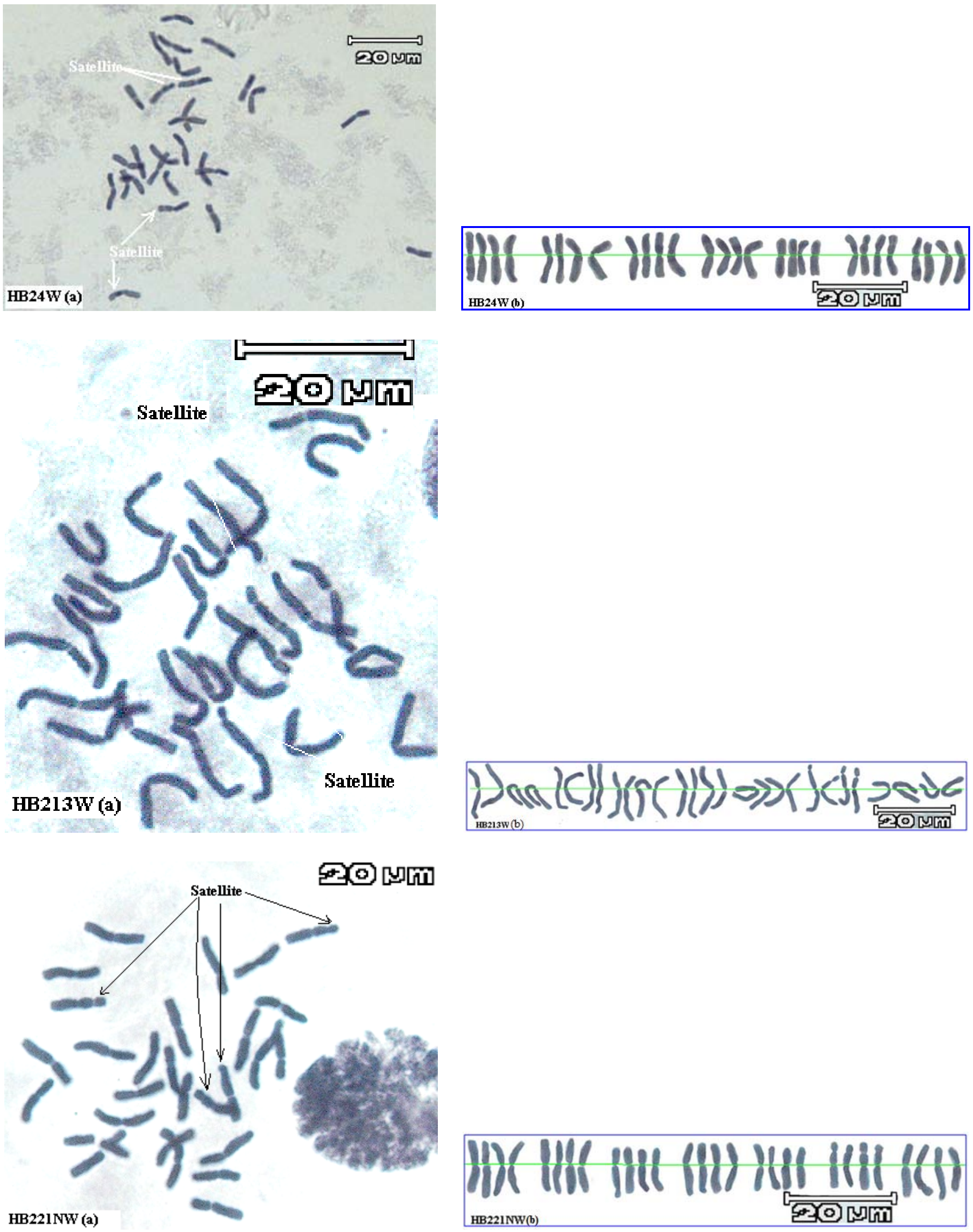

Figure 1: Somatic chromosomes (karyotype) of 32 Iranian *H. bulbosum* (HB) populations (2n=4x=28). Mitosis squash photograph for accessions: HB24W, HB213W and HB221NW with showing Satellited chromosoms are presented. Scale bar: 20 µm.

The populations HB106NE, HB201W and HB221W had 3 sub-metacentric, HB2W, HB22W (2M + 5m*) and HB23W (7m*) without sub-metacentric chromosome and other remaining populations (56.25%) had karyotype formulae of 6m+1sm including six metacentric and one sub-metacentric that were in accordance with Chin (1941), Linde-Laursen *et al*. (1990), Morrison (1959) and Vahidy and Jahan (1998) (Table 2). Nasirzadeh and Mirzaie Nadoushan (2005) have analyzed the karyotype parameters of *H. bulbosum* populations and have suggested that they originated from Fars province and showed that their karyotype formulae were 6m+1sm which was partly in agreement with the results of this study. Symeonidis and Lazaros (1985) reported that the karyotype of Greece populations of bulbous barely was 4m+2sm+1t. In this study, we have not found telocentric chromosome in tetraploids indicating that the karyotype of Iranian tetraploid bulbous barley is different from Greece populations. Our results showed that all populations have one metacentric or sub-metacentric satellited chromosome, except for HB90SW (from Dasht-e Felard in Chaharmahal va Bakhtiari province). Two populations (HB106NE from National Park of Golestan and HB202W from Ilam) had one submetacentric satellited chromosome with karyotype formulae (4M+3sm*). The presence of typical SM satellited chromosomes occurred more frequently among the studied populations of the *Hordeum bulbosum* (Rajhathy *et al.,* 1964; Vosa, 1976; Coucoli and Symeonidis, 1980; Chin, 1941; Linde-Laursen *et al.*, 1990; Morrison, 1959). As noted by Heneen (1977) and the different origin of the materials should be a logical explanation for the observed differences since SAT chromosomes in the Triticeae are well known to evident morphological variation the of shape and the indices among different populations or varieties of one species. The karyotype formulae polymorphism in homologous chromosomes of *H. bulbosum* could be correlated with their out-breeding nature. No B chromosome was observed among the materials studied.

The highest TL variation was found in HB215W population [SE (standard error) of MCL=2.1μm], and the lowest chromosome length variation was scored in HB209W population (SE of MCL=0.69 μm) (Table 2). The ratio between the longest and the shortest arms (R) ranged from 1.23 HB3W accession to 1.69 in HB106NE accession (Table 2). Asymmetry Index (AsI%) ranged from 54.89 in HB2W population to 62.19 in HB106NE population (Table 2). The degree of karyotype asymmetry as indicated by TF% values ranged from 37.1% (HB106NE and HB221NW accessions) to 45.1% (HB2W) (Table 2). As the TF% values were near to 50%, we can conclude that type of chromosomes were metacentric to submetacentric. Also the mean of S% (Stebbins 1971) indicating symmetry index was from 48.58% (HB215W) to 81.32 (HB217W) with mean of 70.1% indicating nearly symmetrical karyotype for *H. bulbosum*.

Based on the results of this study (the factors studied and the resulted asymmetry indices) HB221NW proved to have the most asymmetric karyotype (with the formulae of 1M* + 3m + 3sm) among the populations studied. Regarding the asymmetry indices observed in HB221NW it could be suggested that the karyotype asymmetry in this population was mainly affected by the place of the centromers rather than length of the chromosomes. HB2W with the least chromosomal arm ratio variability, showed the most symmetric karyotype (with the formulae of 2M + 5m*). Regarding all the analyzed factors, a high similarity were found between HB2W, HB14W, HB22W and HB23W (see Table 2).

The karyotype asymmetry can be a fine appearance of the general morphology of karyotype in plants (Romero Zarco, 1986). As Sharma (1990) has mentioned, symmetrical karyotypes are more primitive than asymmetrical ones and longer chromosomes than shorter ones; median centromers with chromosome arms of equal length are more primitive than chromosomes with arms of unequal length. From the chromosome length point of view, the longest chromosomes were found in HB3W that could be considered as most primitive population. We observed that the most asymmetric karyotypes within northeast populations (e.g. Golestan) and populations of the west of Iran had the most symmetric karyotypes. Therefore considering the above notions and the results of this study, it could be assumed that the oldest populations are in the slopes of Zagros Mountains (west of Iran) and the youngest ones occurred in the northeast of the country (Figure 2).

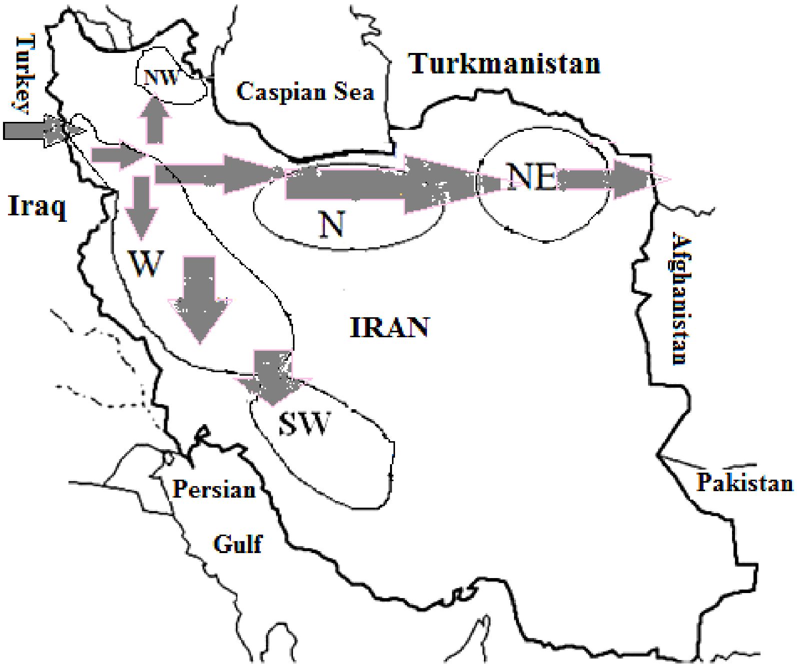

Figure 2: Distribution of collected accessions of *Hordeum bulbosum* (W=west, SW=southwest, N=north, NE=northeast, NW=northwest). Arrows indicate the distributions direction of *H. bulbosum* in Iran.

This suggestion is in accordance with the conclusion reported by Bothmer, *et al.* (1995) namely the *H. bulbosum* (4x) has originated from Greece and then distributed eastwards. Based on these results it can also be concluded that the Western populations (e.g. HB2W, HB14W, HB22W, HB23W and HB90SW) generally possessed the highest chromosomal length and the highest mean TCL (9.87 μm) and the most symmetric karyotypes are the oldest populations and the northeast populations with mean TCL of 8.5 μm are the youngest populations of *H. bulbosum* in Iran (Figure 2).

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Matricaria L. (Anthemideae, Asteraceae) in Iran: a chemotaxonomic study based on flavonoids

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Abstract

Matricaria L. belongs to the tribe Anthemideae and the subtribe Matricineae (Asteraceae) and comprises 7 species of which 2 species grow wild in Iran. This study was aimed to characterize the Iranian materials of *Matricaria* using profiles of flavonoid spots and determination of skeletons of major flavonoids in each species. Twelve bulked population samples from *Matricaria aurea* and *M. recutita* were examined. Presence -absence data from two dimensional maps (2DM) of their flavonoid spots were processed using Cluster and PCA analyses. Differences at species level in flavonoid skeleton properties were investigated and a taxonomic review of close taxa was provided.

Key words: 2D-TLC, Asteraceae, Flavonoid skleton, Iran, *Matricaria*

Introduction

Matricaria L. is classified in subtribe Matricineae (Anthemideae (Cass.), Asteraceae (Dumortier)). This genus is a closely related taxon to *Tripleurospermum* Sch. Bip. and morphologically resembles to some Anthemidae's such as *Anthemis* L., *Microcephala* Pobed. and *Tanacetum* L.; a group of genera that have long been a matter of controversy, both taxonomically and nomenclaturally (Jeffrey, 1979; Xifreda, 1985; Applequist, 2002; Oberprieler and Vogt, 2006). *Matricaria* comprises seven species worldwide: *M. recutita* L. (type species of the genus), *M. aurea* (Loefl.) Sch. Bip., *M. matricarioides* (Less.) Porter ex Britton, *M. occidentalis* Greene, *M. macrotis* Rech. f., *M. tzvelevii* Pobed., and *M. songaria* Bunge (Bremer and Humphries, 1993). *Matricaria songarica* was later transferred to genus *Microcephala* (Bremer *et al.*, 1996). Furthermore, the recognized *Matricaria macrotis* based on the absence of receptacular scales (pales) on its heads was transferred to *Anthemis* under the legitimate name *A. macrotis* (Rech. f.) Oberpr. & Vogt (Oberprieler and Vogt, 2006). Sequencing the nr DNA internal transcribed spacer (ITS) region and some other morphological characters like indumentums, achene shape and anatomy support this transfer (Oberprieler and Vogt, 2006). Geographically, *M. matricarioides* and *M. occidentalis* mainly occur in North America and Western North America, respectively. The old world species of

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the genus grow as: *M. macrotis* (Turkey) *M. tzvelevii* (Crimea) and *M. songarica* (Kazakhstan, Mongolia and Sinkiang in China), *M. recutita* (Eurasia and Mediterranean) and *M. aurea* (Southwest-Central Asia). *M. macrotis* was considered as a basionym of Anthemis macrotis (Rech.f.) Oberpr. & Vogt (Oberprieler and Vogt, 2006). The two latter grow as sympatric species along Zagros mountain chain (Podlech *et al.*, 1986). *M. aurea* is distinguished from its co-generic traditionally well known medicinal species i.e., *M. recutita* by the absence of the white radial ligulate florets. The latter species has been confused with its closely related taxa, particularly *Microcephala lamellata* (Bunge) Pobed. and *Tripleurospermum* spp. Occasional taxonomic revisions show that more detailed understanding of taxa within Anthemideae is a requisite for a better classification.

Flavonoids are choice chemical characters in chemotaxonomic and biosystematic studies (Stace, 1989). Although principal chemical constituents of *Matricaria recutita* have already been reported (*e.g.* Mulinacci et al., 2000); it should be clarified whether all those already reported compounds have been well extracted, scored and analyzed in a given chemotaxonomic study. It would become most critical when comparing two chemotaxonomic studies respecting a given taxon or even a closely related taxa.

Chemical characterization of chamomile extracts have been studied for long. . Zekovic *et al*., (1994) used chromatographic methods for qualitative and quantitative analysis of non-volatile and volatile compounds of *Matricaria chamomilla* L. (synonym for *M. recutita*). In this study, apigenin (flavonoid) series, had been determined using HPTLC and HPLC (Zekovic et al., 1994). These variable chemical compounds have not been used for characterization of variation between natural populations so far (up to our knowledge), nor have been characterized for congeneric non-medicinal species in *Matricaria*.

This study was mainly aimed at (i) reassessment of the taxonomic status of *Matricaria* and its allies in Iran, (ii) comparison of the two Iranian species of *Matricaria* using their flavonoid skeleton properties, and (iii) the use of flavonoid two-dimensional maps of selected populations in Iran for a multivariate analysis and numerically classifying them.

Materials and Methods

Plant material

In this study, a total of twelve population samples including seven populations from *Matricaria recutita* and five from *M. aurea* were specifically collected and examined for flavonoid analysis (Table 1). A voucher specimen from each population was deposited in Herbarium of the University of Isfahan, Iran. For taxonomic purposes and geographical distributions, the *Matricaria* specimens of other herbaria including herbarium of the University of Tehran (HTU) and Research Institute of Forest and Rangelands, Iran (TARI) were examined. Taxonomic identifications were based on Tutin *et al.* (1964); Zohary (1966); Podlech *et al.* (1986) and Grierson (1975).

Methods

Flavonoid extraction and 2D-TLC: Total flavonoids were extracted following Gornall and Bohm (1980). A two-dimensional TLC map of total flavonoids for each population was performed using 20 x 20 cm glass plates coated with Polyamide DC6, 0.35 mm. Solvent systems were adopted from Wagner *et al.* (1996). Each TLC plate was run once in an aqueous solvent system (Water: 70, Ethanol: 20, n-Butanol: 10) and then in an organic solvent system (1, 2-diChloroEthan: 50, Methanol: 25, Butanone: 21, Water: 4). Plates were examined under UV_{254} nm before and after spraying by Diphenyl Boric Acid-2-Amino

Ethyl Ester (NP). Flavonoid spots were scored and entered in a data matrix for multivariate analysis using NTSYS-pc ver. 2.11 (Rohlf, 2000). Cluster analysis of specimens (a normal analysis) was performed using Dice similarity coefficient (Dice, 1945) including in SIMQUAL (NTSYS-pc software).

Flavonoid skeletons determination: Total flavonoids were extracted from the bulk samples and separation was performed using column chromatography (column of sephadex LH20, h: 38 cm, r: 1.5 cm). The solvent system used for column chromatography was 20, 40, 60, 80 and 100% methanol (100 ml each) and fractions were collected in 50 ml volumes. Fractions were concentrated, then examined for flavonoid composition and further purification using preparative TLCs. The UV absorption spectrum of each purified component was determined using Carl-Zeiss-Tech Specord-S10 spectrophotometer in wavelength range 200-500 nm. UV absorption spectra of methanol extracts and their shifts after addition of shift-reagents AlCl3/HCl and NaOAc/H3BO3 were recorded for each purified flavonoid constituent. All spectra were interpreted according to Markham (1982).

Table 1: Details of the *Matricaricaria* accessions used in this study

Species	**Smaple Code**	**Locality**	**Alt. (m)**
M. aurea			
	Au01	Khuzestan: Between Sarkhun and Katula, Do-ab	810
	Au02	Khuzestan: Dehdez	410
	Au03	Fars: Gachsaran to Shiraz, After Brim bridge	-
	Au04	Fars: 25 km to Kazeroon from Dalaki	900
	Au05	Fars: Around Takht-e Jamshid	1570
M. recutita			
	Re01	Khuzestan: Dehdez	410
	Re02	Khuzestan: 40 km to Izeh from Dehdez	610
	Re03	Khuzestan: 25 km to Izeh from Dehdez	900
	Re04	Khuzestan: 5 km from Izeh to Baghmalek	800
	Re05	Fars: Gachsaran to Shiraz, after Brim bridge	-
	Re06	Fars: 25 km to Kazeroon from Dalaki	900
	Re07	Fars: Around Ghaemieh Town	860

Results and Discussion

Taxonomy

Matricaria chamomilla (scentless mayweed), *M. recutita* (chamomile), and *M. maritima* (sea mayweed) were first described by Linnaeus (1753); although, scientific names for chamomile and scentless mayweed were later considered as synonyms to *M. suaveolens* and *M. inodora* respectively by the author (Linnaus 1753). Sea mayweed was also considered as *M. inodora* var. *maritima* (Hansen and Christensen, 2009). These changes caused the first taxonomic and nomenclatural confusions in this genus and its allies as well.

Using *Chamomilla* instead of *Matricaria* in Flora Europaea led to some taxonomic confusions and misidentifications on a number of herbarium specimens; e. g., a herbarium sheet can be determined as *M. chamomilla* based on Flora of Turkey and *Chamomilla recutita* using Flora Europea’s key, interestingly both are a synonym to *Matricaria recutita*.

Treatment of genera *Matricaria*, *Tripleurospermum* and *Chamomilla* in Flora Europaea (Tutin *et al.*, 1964) were incorrect. In fact, descriptions of *Chamomilla* (and the four species under this name) in Tutin *et al.* (1964) belonging to the accepted name *Matricaria* L.; *Chamomilla* S. F. Gray, was treated as a synonym to *Matricaria* L. by Jeffrey (1979). The description of *Matricaria* L. in Tutin *et al.* (1964) coincided with that of *Tripleurospermum* Sch. Bip., while the name *Tripleurospermum* was considered as a synonym to *Matricaria*. These incorrect treatments caused some misidentifications when a number of *Tripleurospermum* specimens were identified as *Matricaria* spp. e. g., at HTU.

Despite two records from *M. aurea* from Northern Iran (Podlech *et al.*, 1986), the specimens were neither collected during our field trips, nor determined among the specimens collected from northern Iran at TARI and HTU. Since geographical distribution of the species is inconsistent with that report, it defied rather clear identification.

Tripleurospermum which is most confused with *Matricaria* differs in having two resin glands at the apex of the abaxial face of achenes and three prominent ribs on adaxial face. *Matricaria* differs from resembling genus *Microcephala* in which fruits are provided with scales, hairs, and a distinct crown-like pappus (Bremer *et al.*, 1996). However, marginal achenes of *Matricaria recutita* may sometimes be coronate, so that if only marginal achenes of *M. recutita* are used in determination, it could be incorrectly identified as *Microcephala lamellata*. Species of *Anthemis*, which are morphologically resembling *Matricaria* spp., differ by having chaffy bracts on the receptacle (which are absent in *Matricaria* spp.).

Recent reports of *M. discoidea* from Iran is uncertain; the taxonomic position of this species is stressed as being rather a member of *Achillea* clade (Oberprieler and Vogt, 2006) and may segregate it.

The two species of the genus *Matricaria* in Iran should be determined using two corresponding identification keys (Zohary, 1966; Podlech *et al.*, 1986). A modified brief key to genus *Matricaria* and its allies in tribe Anthemideae is as follow:

1- Achenes heteromorph, marginal achenes 3-winged, interior achenes 2-winged or with longitudinal middle veins.. *Chrisanthemum*
1- Achenes not heteromorph, not as above.
2- Receptacle bare
3- Achenes with two resin glands at the top of the abaxial face...................... *Tripleurospermum*
3- Achenes without two resin glands at the top of the abaxial face.
4- Achenes distinctly coronate.. *Microcephala*
4- Achenes ecoronate, only achenes of marginal ligulate florets (if present) sometimes coronate... *Matricaria*
2- Receptacle with chaffy bracts
5- Achenes compressed, laterally winged... *Anacyclus*
5- Achenes not winged as above
6- Capituls with ligulate florets in margins, disk florets in center
7- Middle nerve of chaffy bracts excurrent.. *Anthemis*
7- Middle nerve of chaffy bracts non excurrent... *Achillea*
6- Capituls without ligulate florets
8- Capituls in compound cymes.. *Handelia*
8- Capituls single in branches.. *Anthemis*

Chemodiversity

Patterns of flavonoid spots in *M. aurea* and *M. recutita* specimens are shown in Figure 1 (A-C). Co-migrating spots were considered identical for populations of the same species, but may not be identical between the two species (Stace, 1989). Therefore, patterns of flavonoid spots were recorded separately for each species. The Pattern of flavonoid spots in *M. recutita* was also found to be different for stems and capitula (Figure 1- B, C). Not all spots were present in all specimens. Spot data for each specimen are presented in tables 2, 3. Flavonoid spots of stems and capitula in *M. recutita* were not the same. Capitula spot profiles offered more data than stems which were used for subsequent cluster analysis of spot data in *M. recutita* using NTSYS-pc. Resulting dendrograms and PCA diagrams are presented in Figure 2 (A-D). Overall topology of both dendrograms (*M. aurea* and *M. recutita*; Figure 2- A, B) showed that specimens were well separated by the data matrix, and the grouping of the specimens did not suffer chaining.

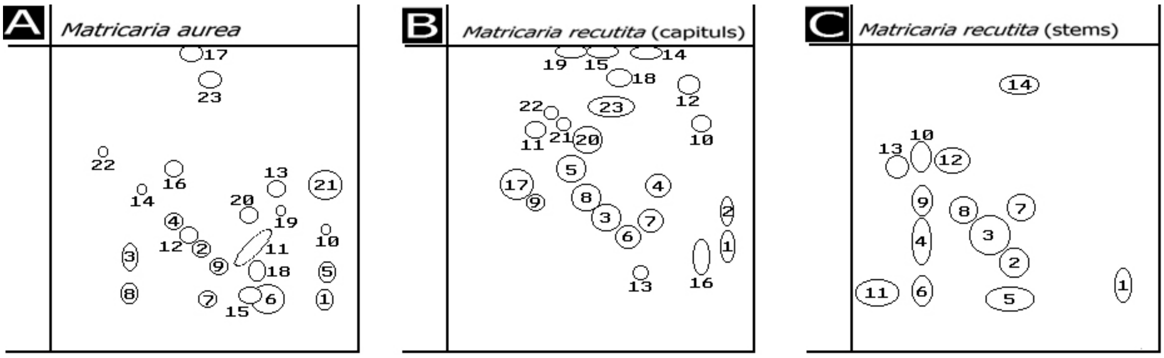

Figure 1: Flavonoid spots in 2D-TLC of *M. aurea* (A: all parts of plant) and *M. recutita* (B: capitula and C: stems) populations. Not all spots were present in all 2D-TLC chromatograms (See tables 2, 3). Spots are numbered according to an overall (combined) map.

Populations in this study were from two regions: West and South of Zagros (Table 1). Cluster analysis of flavonoid spot profiles separated populations of each species according to their geographical location. Re05, Re06, Re07 were clustered together; while Re01, Re02, Re03, Re04 made the second cluster which contained subclusters Re01+Re02 (Populations from Dehdez) and Re03+Re04 (Populations from Izeh). Populations belonging to *M. aurea* were also well clustered. The only misplaced population was Au02M which was an outlyer in a clade containing South Zagros Populations. Au02M was from West of Zagros (Dehdez) and could be interpreted as an outlayer because Au02H (same population, but only the capitula) was groped with other samples from West of Zagros.

Table 2: Flavonoid spots in populations of *M. aurea* (H: heads, M: all parts of plant). Spots 1-5 are yellow, spots 6-14 are orange, spots 15-17 are dark, and spots 18-23 are blue. Dark green spots were absent in spot profile of *M. aurea* populations.

	Spot color	Au01, H	Au02, H	Au01, M	Au02, M	Au03, M	Au04, M	Au05, M
1	Y1	1	1	1	1	0	0	0
2	Y2	1	1	1	1	1	1	1
3	Y3	1	1	1	1	1	1	1
4	Y4	1	1	1	0	1	1	1
5	Y5	0	0	1	0	0	0	0
6	O1	0	0	1	0	0	0	1
7	O2	1	1	1	0	1	1	1
8	O3	0	0	1	0	1	1	1
9	O4	1	0	1	0	1	1	1
10	O5	1	0	0	0	0	0	0
11	O6	1	1	0	0	0	1	1
12	O7	1	1	1	1	1	1	1
13	O8	1	0	1	0	0	0	2
14	O9	1	1	0	0	0	0	0
15	D1	0	0	0	1	1	1	0
16	D2	0	1	0	0	1	0	0
17	D3	0	1	0	1	0	1	1
18	B1	0	0	0	0	0	1	0
19	B2	0	0	0	0	1	0	0
20	B3	0	0	0	0	1	0	0
21	B4	0	0	1	0	0	0	1
22	B5	0	0	0	0	0	0	1
23	B6	1	1	1	1	1	1	1

Table 3: Flavonoid spots in populations of *M. recutita* (H: heads). Spots 1-5 were yellow, spots 6-12 were orange, spots 13-15 were dark, spots 16-19 were blue, and spots 20-23 were dark green, under UV 254nm.

	Spot color	Re01, H	Re02, H	Re03, H	Re04, H	Re05, H	Re06, H	Re07, H
1	Y1	0	1	1	1	0	0	1
2	Y2	0	0	0	0	0	0	1
3	Y3	1	1	1	1	1	1	1
4	Y4	1	1	0	1	1	1	0
5	Y5	1	1	1	1	1	1	1
6	O1	0	1	1	1	1	1	1
7	O2	1	1	1	1	1	1	1
8	O3	1	1	1	1	1	1	1
9	O4	0	1	1	0	0	0	0
10	O5	0	0	0	0	0	1	1
11	O6	0	0	0	1	0	0	0
12	O7	0	0	0	0	0	1	1
13	D1	0	0	0	0	1	0	0
14	D2	1	0	0	0	0	0	0
15	D3	1	1	1	0	1	1	1
16	B1	1	1	1	1	1	1	1
17	B2	0	1	1	1	0	0	0
18	B3	1	1	1	1	1	1	1
19	B4	0	0	0	0	0	2	0
20	G1	1	1	1	1	1	1	1
21	G2	0	1	0	0	0	0	0
22	G3	1	1	1	1	1	1	1
23	G4	1	1	1	1	1	1	1

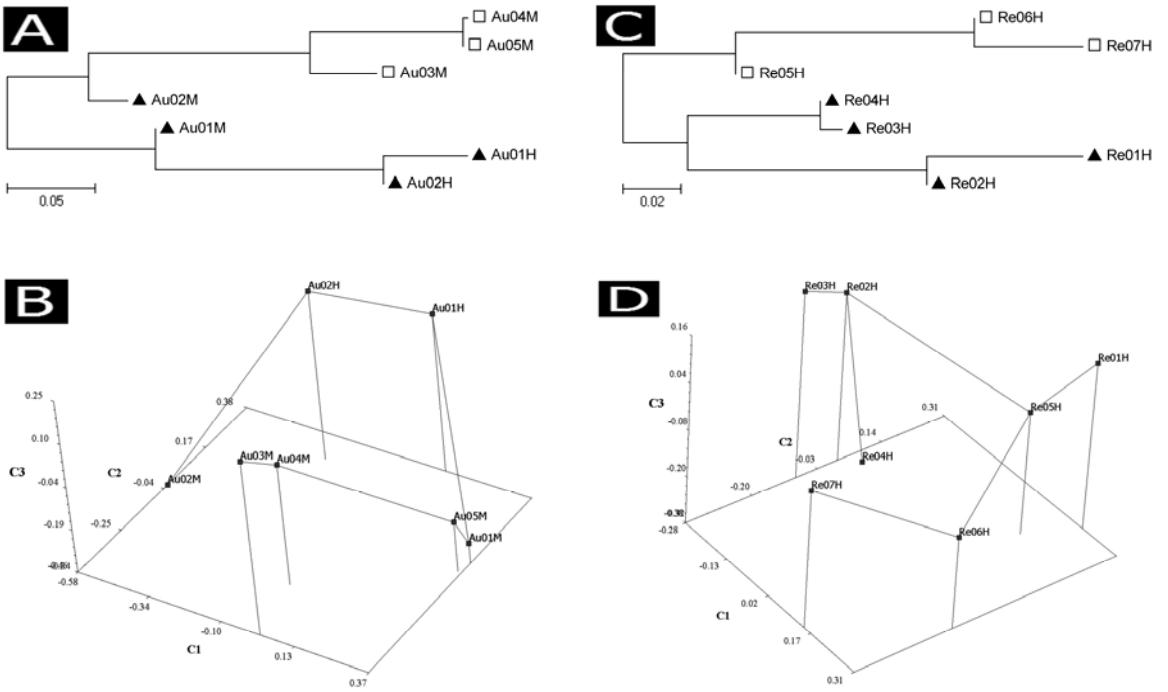

Figure 2: Resutls of multivariate analysis. A, C: Results (dendrograms) of cluster analyses of flavonoid spot profiles of *Matricaria aurea* (5 populations) and *M. recutita* (7 populations). Note that population of each species are analysed separately. Populations located in West of Zagros are black triangles; South of Zagros's are open squares. Scale bars under each dendrogram are relative distances. B, D: Results of Principal Coordinate Analysis (PCO) of flavonoid spot profiles of *M. aurea* and *M. recutita*. A Miminum lenght Spaning Tree is overimposed on each PCO graph which clarifies relationships between populations (see text).

A minimum length spanning tree is overimposed on PCA diagrams of *M. aurea* (Figure 2-B) and *M. recutita* (Figure 2-D). Au02M which was misplaced in cluster analysis is connected to Au02H and rest of West-Zagros populations. On the other hand, Re01H is connected to the rest of West-Zagros populations via Re05H (a South-Zagros Population). Population structure in accordance with geographical origin of samples in Zagros Mountain chain has been studied for grass species *Festuca arundinacea* using microsatellites (Sharifi-Tehrani *et al.*, 2009). Here, separation of *Matricaria* species populations across central Zagros region as revealed by flavonoids; supports for significance of Central Zagros region in effective separation of populations making genetic or chemical structure among them.

UV spectrophotometry

Most studies refered to Essential oil composition of *Matricaria* species of which *M. recutita* received more attention due to its importance as a known medicinal plant; however, *M. aurea* had also been studied for its oxygenated bisabolene compounds (Ahmed and Elela, 1999; Teixeira da Silva, 2004). We evaluated differences among *M. aurea* and *M. recutita* in flavonoid classes and partial details of the substitutions on the skeleton. Common structure of flavonoid skeleton is shown in Figure 3. UV spectrophotometry of purified flavonoids of each species, performed in order to compare flavonoid skeleton and substitutions properties of them in species level. Hydroxylation on carbon 3 (the heterocyclic ring) converts flavones to flavonols. This simple change required additional steps in flavonoids biosynthetic pathway and made the molecule physiologically more active.

M. recutita contained qualitatively more flavonoid compounds compared to *M. aurea*. To determine the class and structural properties of purified flavonoids, shift reagents were used. Those flavonoids with enough concentration to be detected and purified by column chromatography and TLC were considered.

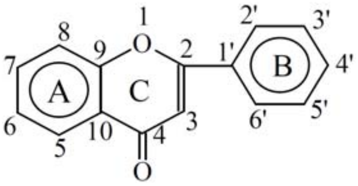

Figure 3: Common skeleton of flavonoids. Positions 2' and 6' on ring B (also 3' and 5') are identical (Markham, 1982).

Three flavonoid aglycons from *M. aurea* and nine from *M. recutita* were purified. Representative UV spectra of *M. aurea* and *M. recutita* are shown in Figure 4 and a summary of the properties of detected flavonoid constituents is presented in table 4. Three purified flavonoids extracted from *M. aurea* were of class flavones and shared the ortho-dihydroxyl system on ring B, which could be considered as a plesiomorphic chemical character shared by the two species. Two out of nine detected flavonoids in *M. recutita* belonged to class flavonols, one of which possessed two ortho-dihydroxyl systems on rings A and B.

Flavonoid skeleton 8 from *M. recutita* was a 3-hydroxy-flavone (apigenin) which was previously purified from capitula of *M. recutita* and characterized as a banzodiazepine receptor ligand with anxiolytic effects (Viola *et al.*, 1995).

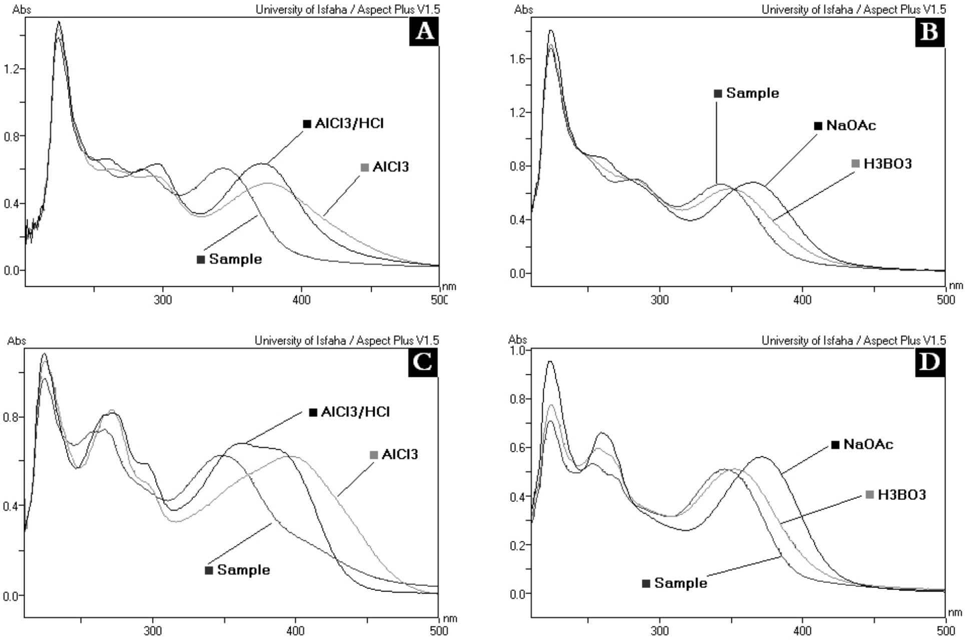

Figure 4: Representative spectra of UV absorption in 200-500 nm range. (A, B) UV spectra of one of the three flavones purified from *M. aurea*. Shifts to higher wavelengths in band (I) on spectrum (A) suggests 5-OH and 6-oxygenation. Shifts in band (I) on spectrum (B) suggests an ortho-di-hydroxyl system on ring B. (C, D) UV spectra of one of the nine flavonoids purified from M. recutita. Again, Shifts to higher wavelengths in band (I) on spectrum (C) suggests 5-OH and 6-oxygenation while shifts in band (I) on spectrum (D) suggests an ortho-di-hydroxyl system in positions 6, 7 or 7, 8 on ring A.

A qualitative comparison of flavonoids present in the two species showed that *M. aurea* may not be considered as a medicinal alternative for *M. recutita*; our results showed that it lacked (or had insufficient amount) physiologically active flavonoids: flavonols (Strack, 1997).

Conclusions

Matricaria recutita wasboth morphologically and chemically more complex than *M. aurea*, as revealed by flavonoid constituents. Heterogamous radiate capitula of *M. recutita* consisted of both white ligulate florets (rays) and pale-yellow central tubular disk florets. Capitula of *M. aurea* consisted of only disk florets. Disk florets were not the same in the two species; corolla tubes in disk florets were 4-lobed in *M. aurea*, while disk florets in *M. recutita* were 5-lobed.

Table 4: Flavonoid skletons from *M. aurea* and *M. recutita*, purified and determined in this study. Skeleton number corresponds to numbers in Figure 5

Skleton	Class	Skleton details	Species
1	Flavone	5-OH; oxygenation on carbon 6; ortho-dihydroxyl on ring B	*M. aurea*
2	Flavone	ortho-dihydroxyls on rings A and B	*M. aurea*
3	Flavone	ortho-dihydroxyl on ring B	*M. aurea*
4	Flavonol	5-OH; oxygenation on carbon 6; two ortho-dihydroxyls on rings A and B	*M. recutita*
5	Flavonol	5-OH; oxygenation on carbon 6; 7-OH	*M. recutita*
6	Flavone	5-OH; oxygenation on carbon 6	*M. recutita*
7	Flavone	ortho-dihydroxyl on ring A	*M. recutita*
8	Flavone	5-OH; ortho-dihydroxyl on ring A	*M. recutita*
9	Flavone	5-OH; ortho-dihydroxyl on ring A; oxygenation on carbon 6	*M. recutita*
10	Flavone	5-OH; prenyl group on carbon 6	*M. recutita*
11	Flavone	5-OH	*M. recutita*
12	Flavone	5-OH; two ortho-dihydroxyls on rings A and B	*M. recutita*

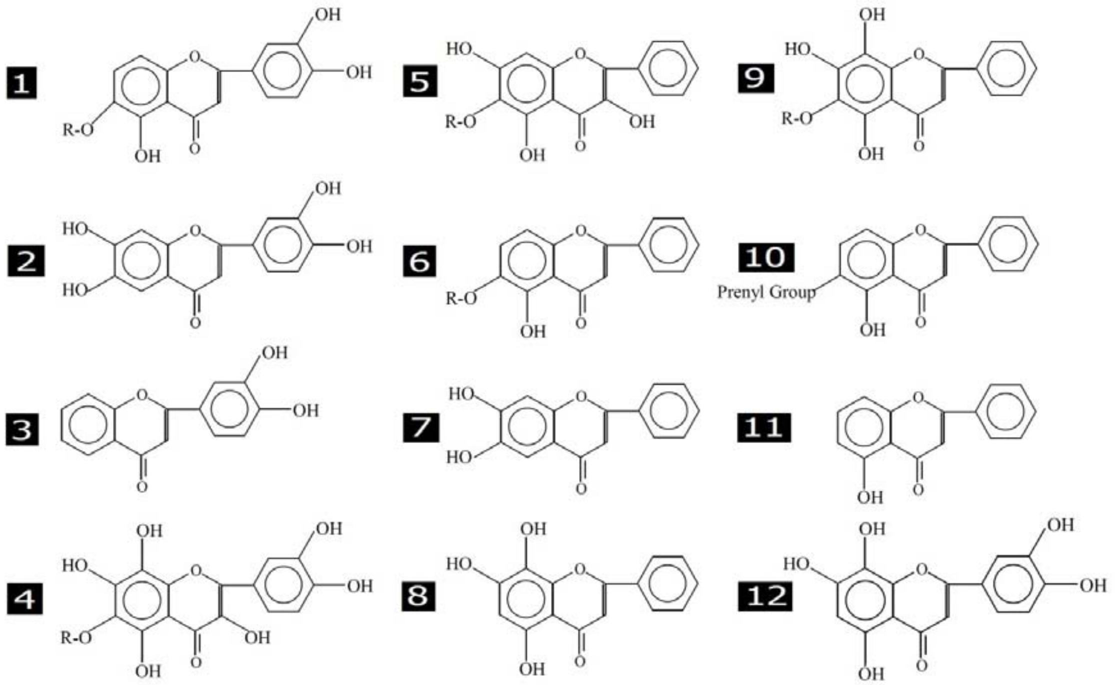

Figure 5: Flavonoid skletons from *M. aurea* and *M. recutita*, purified and determined in this study. Flavonoids 1-3 were separated from *M. aurea*; 4-12 from *M. recutita*. Skeleton numbers corespond numbers in Table 4.

From the biosynthetic aspects, flavones and flavonols were both derivatives of an intermediate class of flavonoids; namely flavanones which were directly resulted in flavones. Biosynthesis of flavonols from flavanones required construction of another extra intermediate class of flavonoids; namely flavanone-3-ols or (+)-Dihydroflavonols. Lack of ligulate florets in *M. aurea* in addition to lack of class flavonols could be interpreted as losses (synapomorphies).

Both discoid and radiate capitula are present in several close genera to *Matricaria*. However, it is unlikely that the ligulate flowers have evolved independently several times in those genera and species from ancestors without ligulate florets. This situation would be resolved by considering an ancestor with ligulate florets from which species with and without ligulate florets have been arisen via reversals in discoid (non-ligulate flower) capitula. It could be concluded that *M. recutita* is more primitive than *M. aurea* despite of being morphologically and phytochemically more complex. Taxonomic and nomenclatural problems within Anthmidae remain to be resloved by using different kinds of data from all genera in this tribe, through more detailed studies.

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Evaluation of genetic diversity among Iranian pomegranate (*Punica granatum* L.) cultivars, using ISSR and RAPD markers

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Abstract

Considering the high level of morphological diversity in Iranian pomegranate cultivars, comparison of genetic variation among 24 pomegranate cultivars was evaluated using random amplified polymorphic DNA (RAPD) and inter simple sequence repeat (ISSR) markers. RAPD primers amplified 131 DNA fragments among which 29 were polymorphic (22.14%) and ISSR markers produced 173 amplification products, out of which 64 were polymorphic (37%). Mean PIC (polymorphic information content) was 0.128 for RAPD and 0.163 for ISSR. The results suggested that the ISSR markers produced much better reproducible bands and were more efficient in grouping cultivars. Pairwise similarity index values ranged from 0.353 to 1.0 (RAPD), 0.291 to 0.930 (ISSR) and mean similarity index values of 0.604 and 0.674 for RAPD and ISSR, respectively. The analysis of molecular variance (AMOVA) for RAPD and ISSR data showed no significant differences among the geographical regions and juice acidity of the used cultivars ($P>0.05$) indicated that genetic and geographic distances were not correlated.

Key words: *Punica granatum*, genetic diversity, pomegranate, RAPD, ISSR

Introduction

Pomegranate (*Punica granatum* L.) belongs to Punicaceae family and is an important fruit tree of tropical and subtropical regions of the world which is valued highly for its delicious edible fruits. In addition, the tree is also cultivated for its pharmaceutical and ornamental usages (Levin, 1994). The pomegranate tree has a wide geographical distribution that spreads from Iran to the Himalayas in northern India, and has been cultivated since ancient times throughout the Mediterranean regions of Asia, Africa and Europe (Levin, 1994). Pomegranate may be classified according to the acidity of its fruit into sour, sour-sweet or sweet.

Development of highly reliable and discriminatory methods have become increasingly important to plant breeders for identifying cultivars and to those in the nursery industry who need sensitive tools to differentiate and identify cultivars for plant patent protection

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(Wunsch and Hormaza, 2002). In the past, cultivars were identified primarily based on horticultural, morphological and physiological descriptions. In most cases, the descriptions and measurements varied considerably due to environmental fluctuation and differences human judgment. Differences in DNA sequence among individuals could be detected by different methods. Almost all kind of DNA markers can be used for fingerprinting fruit tree species (Wunsch and Hormaza, 2002). The Random Amplified Polymorphic DNA (RAPD) technique (Williams *et al*., 1990) based on the Polymerase Chain Reaction (PCR) has been used to detect polymorphism in some species (Williams *et al.,* 1990). Thus, the RAPD technique can generate polymorphisms between very closely related genotypes. Since 1990, RAPD markers have been successfully used to identify cultivars and/or clones of various plant species (Belaj *et al.,* 2001; Besnard *et al.,* 2001; Claros *et al.,* 2001; Ozden-Tokatli *et al.,* 2010; Takeda *et al.,* 1998).

Also, Inter-simple sequence repeat (ISSR) amplification is a technique which can rapidly differentiate closely related individuals (Zietkiewicz *et al.,* 1994). ISSR markers involve PCR amplification of DNA using a single primer composed of a microsatellite sequence such as $(CA)_8$ anchored at the 3' or 5' end by 2-4 arbitrary, often degenerate nucleotides. The sequences of repeats and anchored nucleotides are randomly selected. Coupled with the separation of amplifications products on a polyacrylamide gel, ISSR amplification can reveal a higher number of fragments per primer than RAPD. ISSR markers have been used for cultivar identification and for genetic relationship studies in various plant species (Awasthi *et al.,* 2004; Martin and Sanchez-Yelamo, 2000; Weiguo *et al.,* 2007).

Although a wide range of morphological and physiological characters show variabilities in the pomegranate, molecular studies of the pomegranate have been restricted to examinations of RAPD (Dorgac *et al.,* 2008; Zamani *et al.,* 2007; Sarkhosh *et al.,* 2009), ISSR (Talebi Bedaf *et al.,* 2005), AFLP (Jbir *et al.,* 2008; Rahimi *et al.,* 2006) and SSR (Koohi-Dehkordi *et al.,* 2007; Ebrahimi *et al.,* 2010; Pirseyedi *et al.,* 2010) to investigate the population dynamics of economically important cultivars. To meet various breeding programs and to conserve the existing genetic resources of pomegranate, the objectives of this study were to assess the levels of polymorphisms detected by RAPD and ISSR markers, comparison of information content the marker systems and using them to identify 24 Iranian pomegranate cultivars.

Materials and Methods

Plant materials and DNA extraction

Twenty four cultivars of *P. granatum* were collected from Agricultural Research Center of Yazd province, Yazd, Iran. The selection of cultivars was based on the acidity of fruits and the morphological characteristics, such as color and shape of fruit (Table 1).

Total DNA was extracted from young leaves following the CTAB (Hexadecyltrimethylammonium bromide) method described by Murray and Thompson (1980) with modifications. The purified total DNA was quantified by agarose gel electrophoresis and its quality was verified by spectrophotometry. DNA samples were diluted to 25ng/µl and stored at -20 ºC.

Table 1: Iranian pomegranate genotypes included in the study

No.	Cultivar codes	Cultivar names	Acidity	Origin
1	PGpsy	Poust syah yazdi	Sweet	Yazd
2	PGgsh	Goroch shahvar	Sweet	Yazd
3	PGtl	Tab va larz	Sweet	Yazd
4	PGbl	Bihasteh ladiz	Sweet	Systan va Balouchestan
5	PGas	Asali sarvestan	Sweet	Fars
6	PGghr	Golabi hasteh riz	Sour-sweet	Systan va Balouchestan
7	PGna	Nabati ardakan	Sour-sweet	Yazd
8	PGdhv	Dokhtar hamoumi varamin	Sour-sweet	Tehran
9	PGggn	Galu gandeh neiriz	Sour-sweet	Fars
10	PGakh	Amaneh khatouni	Sour-sweet	Yazd
11	PGtg	Togh gardan	Sour-sweet	Yazd
12	PGda	Dom anbarouti	Sour	Khorasan
13	PGpa	Panjeh arous khafr	Sour	Fars
14	PGapgh	Ardestani poust ghermez	Sour	Esfahan
15	PGvns	Vahshi narak sarvestan	Sour	Fars
16	PGds	Dabbei sarjangal	Sour	Kerman
17	PGgf	Golnar fars	Ornamental	Fars
18	PGkgs	Kaleh gavi sangan	Sour-sweet	Systan va Balouchestan
19	PGsh	Shahvar shirin	Sweet	Yazd
20	PGhm	Hasibi mehriz	Sour-sweet	Yazd
21	PGbs	Bihasteh sangan	Sweet	Systan va Balouchestan
22	PGaps	Ardestani poust sefid	Sweet	Esfahan
23	PGtmz	Torsh mamuli zabol	Sour	Systan va Balouchestan
24	PGsb	Sabi bam	Sour	Kerman

RAPD assay

One hundred-four 10mer oligonucleotide primers among sets A, B, C, AC, AD, AE, P and S (Operon technologies, Inc, USA), AJ, MG and UBC (Roche Molecular Biochemicals, Germany) were used as single primers for DNA amplification and 13 of them were selected based on clear and reproducible banding patterns. The PCR was performed in a Genius (FGENO5TD) Thermal Cycler, in a 25µl volume containing of 2.5µl of 1X reaction buffer [100mM Tris-HCl, 15mM $MgCl_2$, 500mM KCl, pH 8.3 (20ºC)], 0.5mM $MgCl_2$, 200µM each of dNTPs (Roche, Germany), 0.4µM of 10mer primer, 0.75 units of *Taq* DNA polymerase (Roche, Germany) and 100ng of template DNA, overlaid with 25µl of sterile mineral oil. The amplification condition was: initial step of denaturation at 94ºC for 2 min followed by 40 cycles of denaturation at 92ºC for 1 min, primer annealing at 35ºC for 1 min and extension at 72ºC for 2 min, followed by an extended elongation step at 72ºC for 5 min. The amplification products were analysed on 1.2% MP agarose gel (Roche, Germany) in 1X TBE buffer running at 60 volts for three hours and stained in ethidium bromide (0.5mg/ml) and visualized under UV light and photographed. The DNA size marker used was 1Kb ladder (Life technologies).

ISSR assay

A total of 15 primers were tested to amplify DNA from which six primers with considerable polymorphism and reproducibility were selected for further analysis (Table 2). PCR were performed in 15 µl volume consisted of 1X PCR buffer, 2mM $MgCl_2$, 200 µM each of dNTPs, 1 µM primer, 1 U of *Taq* DNA polymerase (Roche, Germany), 2% formamide and 25 ng of template DNA. Each reaction mixture was overlaid with 25 µl of sterile mineral oil. Amplification was performed in a Genius (FGENO5TD) Thermal Cycler under the following conditions: 4 min at 94ºC for 1 cycle, followed by 30 s at 94ºC, 45 s at

52ºC, and 2 min at 72ºC for 30 cycle, and 5 min at 72ºC for a final extension. Amplification products were separated on 6% denaturing polyacrylamide gels containing 7 M Urea and 1X TBE buffer in Biometra sequencing gel (S2 model). Amplified DNA segments were detected using silver staining (Bassam *et al.,* 1991).

Data analysis

A marker index was calculated for the RAPD and ISSR markers to characterize the capacity of each primer to detect polymorphic loci among the cultivars. As such, the marker index was the sum of the polymorphism information content (PIC) values for all the selected markers produced by a particular primer. The PIC value was calculated using the formula $PIC=1-\Sigma pi^2$, where *Pi* is the frequency of the *i* allele (Smith *et al.,* 1997).

Only reproducible and clear bands in the replications were considered as potential polymorphic markers. The data obtained by scoring the presence (1) or absence (0) of amplified fragments from the RAPD, ISSR and also collective of marker profiles were subjected to the construction of a similarity matrix using Jaccard's (Jaccard, 1908) coefficients of similarity. The matrices were then used for a cluster analysis. Sequential agglomerative hierarchical nonoverlapping (SAHN) clustering was performed using the unweighted pair group method with arithmetic averages (UPGMA), and then, the results were summarized as dendrograms using NTSYSpc software 2.02 (Raholf, 1998).

The pertinency of the dendrograms to the original similarity matrix was calculated by computing the cophenetic values (rcoph) using the cophenetic (COPH) and matrix comparison (MXCOMP) modules of NTSYSpc.

Finally, the frequency of occurrence of each marker in each cultivar was computed, to render a matrix of 24 cultivars by RAPD and ISSR markers. These matrices were afterward subjected to principal component analysis (PCA).

Analysis of molecular variance (AMOVA) was performed to estimate variance components for RAPD and ISSR data and partitioning the variation into within and among local regions and acidity of cultivars, using Arlequin 3.1 software (Excoffier *et al.,* 2005).

Results

One hundred-four 10 mer RAPD primers were screened and among which 13 were chosen for their clear and reproducible band patterns (Table 2). The thirteen selected primers generated 131 RAPD fragments, an average of 10.08 bands per primer. The size of the amplified products ranged from 400 to 3,000 bp. The total number of polymorphic markers and percentage of polymorphism were 29 and 22.14%, respectively (Table 2). Primers OPAD02 and MG16 amplified maximum number of polymorphic bands. Primers OPAD02 and OPAE10 put out the highest level of distinguishable polymorphism. The PIC values, a reflection of the allele diversity and frequency among the cultivars, were not uniform with respect to for all the RAPD loci tested. The PIC values ranged from 0.012 (MG01) to 0.373 (OPAD02) with a mean of 0.128. The result showed that the minimum similarity (0.353) existed in the two local cultivars "Dom anbarouti" and "Poust syah yazdi" and the maximum similarity (1.00) occurred in the cultivars "Tab va larz" and "Bihasteh ladiz". The mean similarity index was 0.604. The data obtained from RAPD analysis of 24 pomegranate cultivars was subjected to UPGMA analysis. The cophenetic correlation coefficient (0.91) indicated little distortion between the original similarity values from the similarity matrix and the values used to construct the dendrogram. A cluster analysis was performed based on Jaccard's similarity coefficient matrices, calculated from the RAPD markers. At the similarity of 59%, twenty cultivars were categorized in one group and the four remaining cultivars were placed in the separate groups (Figure 1a).

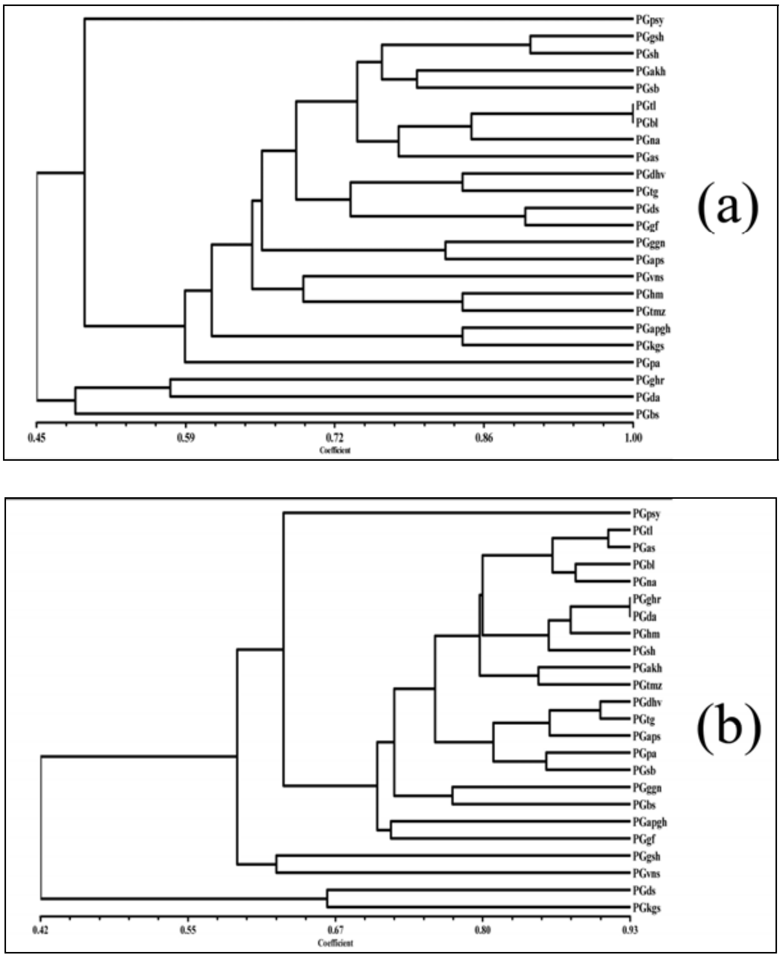

Figure 1: UPGMA dendrogram showing relations among Iranian pomegranate cultivars using RAPD (a) and ISSR (b) data and Jaccard's similarity coefficient.

Eighteen ISSR primers were initially tested using pomegranate DNA as single or combined. Three primers as single and three as combined were based on $(AG)_n$, $(GT)_n$, $(GA)_n$, $(AC)_n$, $(CT)_n$ or $(CA)_n$ repeats, each anchored by various nucleotides used in this study as polymorphic primers (Table 2). The six selected primers generated 173 fragments, an average of 28.83 bands per primer. The size of the amplified products ranged from 80 to 3,000 bp with the scoreable region being from 100 to 2,000 bp accordingly, and the total number of polymorphic markers and percentage of polymorphism were 64 and 36.99%, respectively (Table 2). In the case of the ISSR analysis, the mean PIC value was 0.163, and the lowest and highest PIC values were 0.099 (ISSR5 and ISSR6) and 0.257 (ISSR11), respectively. The similarity coefficients for 24 pomegranate cultivars based on the ISSR fragments ranged from 0.291 ("Dabbei sarjangal" and "Poust syah yazdi") to 0.930 ("Dom anbarouti" and "Golabi hasteh riz"). The mean similarity index was 0.674. Twenty four cultivars were clustered in five distinct groups at the similarity level of 65%, 19 of which were placed in one group (Figure 1b).

Table 2: RAPD and ISSR primers successfully used in this study and the number of total and polymorphic bands amplified in pomegranate cultivars.

Marker name	Primer name	Sequence (3'-5')	Total band number	Number of polymorphic bands	Polymorphic bands (%)	PIC value
RAPD	OPAC11	CCTGGGTCAG	12	3	25	0.188
	OPAD02	CTGAACCGCT	8	5	62.5	0.373
	OPAD04	GTAGGCCTCA	8	2	25	0.246
	OPAD13	GGTTCCTCTG	5	2	40	0.172
	OPAD15	TTTGCCCCGT	6	1	16.67	0.014
	OPAD16	AACGGGCGTC	10	2	20	0.110
	OPAE10	CTGAAGCGCA	11	2	18.18	0.081
	OPB10	CTGCTGGGAC	12	1	8.33	0.013
	OPP02	TCGGCACGCA	10	2	20	0.135
	OPP14	CCAGCCGAAC	12	2	16.67	0.096
	MG01	AGCGCCGACG	14	2	14.29	0.012
	MG11	AGGAGCTGCC	16	2	12.5	0.024
	MG16	GAAGAACCGC	7	3	42.86	0.200
	Total		131	29		
	Mean		10.08	2.23	22.14	0.128
ISSR	LK7	5'–CCA(CT)$_8$-3'	32	10	31.25	0.256
	ISSR5	5'–CCA(AG)$_8$T-3'	25	8	32	0.099
	ISSR6	5'–(GA)$_8$C-3'				
	ISSR10	5'–(GT)$_8$A-3'	38	11	28.94	0.106
	ISSR12	5'–(AG)$_8$YT-3'				
	ISSR11	5'–(AG)$_8$YT-3'	24	6	25	0.156
	ISSR12	5'–(GA)$_8$YT-3'				
	ISSR11	5'–(AG)$_8$YT-3'	26	15	57.69	0.257
	ISSR12	5'–(GA)$_8$YT-3'	28	14	50	0.103
	Total		173	64		
	Mean		28.83	10.67	36.99	0.163

The matrices for RAPD and ISSR markers were also compared using Mantel's test (Mantel, 1967) for matrix correspondence. The correlation between the matrices of cophenetic values relating to the dendrograms based on RAPD and ISSR data was very low (r=0.02).

The relationships among cultivars were initially defined by the first three principal vectors of the PCA, which together accounted for 65% (RAPD), 73% (ISSR) and 66% (RAPD+ISSR) of the total variation at the molecular level (data not shown).

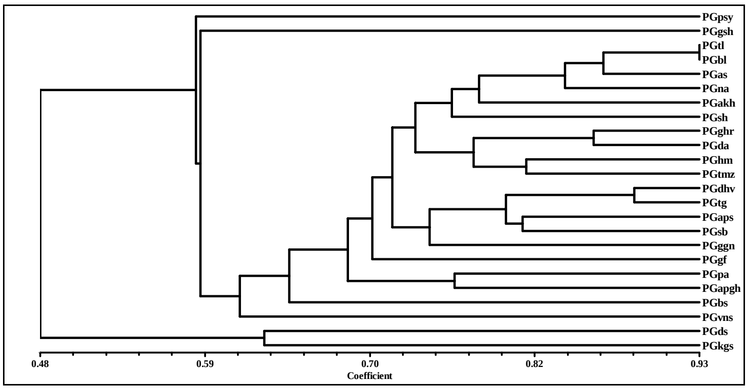

Figure 2: UPGMA dendrogram of 24 pomegranate cultivars based on RAPD and ISSR pulled data and Jaccard's similarity coefficient.

The similarity coefficients of 24 pomegranate cultivars based on 29 RAPD and 64 ISSR markers ranged from 0.338 ("Dabbei sarjangal" and "Poust syah yazdi") to 0.932 ("Bihasteh ladiz" and "Tab va larz"), and accordingly the mean similarity index value of the combined RAPD and ISSR was 0.655. A cluster analysis performed based on combination of the data for both markers, separated the cultivars into two distinct clusters. The first cluster included only two cultivars, whereas the second cluster was further divided into three subclusters. Among these subclusters, two local cultivars ("Poust syah yazdi" and "Goroch shahvar") were separated from other cultivars (Figure 2).

AMOVA for RAPD and ISSR data indicated that there were no significant differences among the geographical regions and juice acidity of the used cultivars ($P> 0.05$).

Discussion

Among markers, RAPD and ISSR are simple, which provide a quick screen for DNA polymorphism and very small amounts of DNA are required. In addition, information on template DNA sequence is not necessary. However, with respect to RAPD markers problems of reproducibility are reported (Muthusamy *et al.,* 2008). In order to assure reproducibility, optimization of PCR reaction and also its repetition is essential. In this study, each RAPD analysis was repeated in separate experiments at least twice, and only reproducible markers were considered. ISSR primers consist of 17-19 nucleotides and optimization of annealing temperature is important. The choice of annealing temperature for further ISSR analysis is based on the complexity and reproducibility of banding patterns. The primers that were based on $(AT)_n$ or $(TA)_n$ repeats amplified no products at all. Possibly, this indicates that the pomegranate genome lacks, or else has very few of these two microsatellites, although Wang *et al.,* 1994, reported that $(AT)_n$ was the most abundant microsatellite in plant nuclear genomes. Alternatively, lack of amplification products may be due to the self-complementary nature of $(AT)_n$ or $(TA)_n$ primers. In this study, when optimal conditions for PCR had been determined, reproducible patterns were obtained for both RAPD and ISSR assays.

In general, among the set of accessions investigated, the efficiency of a molecular marker technique depends on the amount of polymorphism it can detect. In our study, ISSR fingerprinting was more efficient than the RAPD assay; it detected 37% polymorphic DNA markers among the 24 cultivars analyzed, compared with 22.14% for RAPD fingerprinting. Similar results were obtained for several other plant species (Galvan *et al.,* 2003; Nkongolo *et al.,* 2005; Qian *et al.,* 2001; Raina *et al.,* 2001). However, Fang and Roose (1997) showed that RAPD had a higher level of variation in *Citrus* spp. than ISSR, and Metais *et al.,* (2000) demonstrated that the two techniques produced similar levels of polymorphism in *Phaseolus vulgaris*. The correlation between the matrices of cophenetic values for the dendrograms based on RAPD and ISSR data was also very low ($r=0.02$). It is probably due to the nature of different marker systems. RAPD markers cover the entire genome, revealing length polymorphisms in coding or noncoding and repeated or single-copy sequences (Williams *et al.,* 1990), whereas, the origin of the amplification products in ISSR is known to be from the sequences between the two microsatellite sites (Zietkiewicz *et al.,* 1994).

Three first principle eigen vectors of the PCA, which together accounted for RAPD, ISSR and combined data showed high total variation at the molecular level, indicating the suitability of the RAPD and ISSR approaches for genetic clustering.

Based on the pairwise analysis of the amplification products which were obtained with the 13 tested RAPD primers, all the tested pomegranate cultivars showed a very high similarity values. Different relationships were observed between various cultivars. "Bihasteh sangan", "Dom anbarouti" and "Golabi hasteh riz" were quite distinct from the rest of cultivars. "Tab va larz" and "Bihasteh ladiz" were clustered together and in the studied cultivars showed the highest average similarity value (similarity coefficient of 1.0) which indicates that although there are some morphological differences in fruit characteristics, these cultivars may probably be mutants of each other (Sarkhosh *et al.,* 2009). All the remaining cultivars showed very limited differences, but sufficient to distinguish the different cultivars.

Among the 24 cultivars analyzed with ISSR marker, four main groups were recognized by UPGMA based on Jaccard's similarity coefficient (Figure 1b). The first group contained "Poust syah yazdi", the second group included "Goroch shahvar" and "Vahshi narak sarvestan", the third group consisted of "Dabbei sarjangal" and "Kaleh gavi sangan", while all remaining cultivars formed the fourth group. "Vahshi narak sarvestan", "Dabbei sarjangal" and "Kaleh gavi sangan" were quite distinct from the rest of cultivars and were readily separated from other cultivars. These cultivars are wild and it seems that they differ from other cultivars, morphologically e.g. fruit size, fruit color, seed color and the taste. "Dom anbarouti" and "Golabi hasteh riz" grouped and showed the highest average similarity value among the studied cultivars. "Poust syah yazdi" cultivars that have a black bark as a distinguishable marker from the other cultivars, was separate in both RAPD and ISSR analysis. In total, ISSR analysis, was more efficient than RAPD analysis.

A close genetic similarity was found in some of the cultivars analyzed as shown by high values of similarity index. Also, the similarities detected with ISSRs are greater than the similarities measured according to RAPD data. Fernandez *et al.,* (2002) and Muthusamy *et al.,* (2008) have studied barley cultivars and rice bean (*Vigna umbellata*) landraces, respectively, and they also found higher similarity index by ISSRs than by RAPDs.

Observation of no significant difference among the geographical regions based on AMOVA for RAPD and ISSR data and the clustering pattern of cultivars revealed that there are no correlation between genetic diversity and geographic distances. In the study of RAPD profiles in Iranian pomegranates by Sarkhosh *et al.,* (2009) and of AFLP profiles in Tunisian pomegranates by Jbir *et al.,* (2008), the authors could not detect any correlation between provenance of the accessions and similarity or otherwise in the PCR profiles. This can be due to the exchange of plant materials across the regions during the history of pomegranate cultivation.

In conclusion, both RAPD and ISSR are methods useful for revealing molecular relationships among pomegranate cultivars and ISSR markers exhibited higher levels of polymorphisms than RAPD. Relationships among these pomegranate cultivars revealed by ISSR markers were not generally in agreement grouping showed by RAPD markers.

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Population data on D6S2879 and D6S2806 markers located at HLA-DRB1 region in the Iranians: Identifying the signatures of balancing and directional selection

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Abstract

In this study, the genetic diversity and neutrality test for the MHC microsatellite loci, D6S2879 and D6S2806, located within the HLA-DRB1 gene region, were investigated. The genotyping data from 73 unrelated individuals were analyzed for Shannon index, the effective allele number of the markers and neutrality test by use of PyPop and Popgene32 programs. The Shannon index for D6S2879 and D6S2806 markers in the studied population was 1.0372 and 0.8601, respectively. The Fnd value computed for D6S2879 and D6S2806 markers were also estimated -0.8449 and 0.9904, respectively. The results obtained from Ewens-Watterson test indicated that D6S2879 and D6S2806 markers were under balancing and directional selection in the Iranian populations, respectively. The data suggested the presence of a selection force on HLA-DRB1 gene region in the Iranian populations.

Key words: HLA-DRB1 gene, Gene diversity, Ewens-Watterson test, MHC microsatellite marker, Selection

Introduction

The major histocompatibility complex (MHC) shows high allelic diversity in many vertebrates and it plays a unique role in the immune system and autoimmunity (Parham and Ohta, 1996; Gaudieri *et al.*, 2000; Robinson *et al.*, 2000). HLA-DRB1 belongs to MHC class II and encodes the most prevalent beta subunit of HLA DR beta chain. In the study on patients with chronic pancreatitis (CP), the HLA-DRB1*0401 allele was introduced as a susceptibility factor for CP patients (Cavestro *et al.*, 2003). A genetic association between HLA-DRB1*15 status and the risk of developing keloid following injury was reported in a study on a group of Caucasoid patients (Brown *et al.*, 2008). Whereas a study on multiple sclerosis (MS) patients reported that the DRB1*0701, DRB1*04 sub-allele HLA-DRB1*0407 and HLA-DRB1*0901 may have been protective influence on MS susceptibility (Wu *et al.*, 2010). In fact, recent reports have indicated the association of particular alleles of HLA-DRB1 with resistance or susceptibility to different autoimmune

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and infectious diseases (Carrington *et al.*, 1999; de Groot *et al.*, 2002; Koo *et al.*, 2003; Migita *et al.*, 2006; Barnetche *et al.*, 2008).

In view of the high polymorphic content of the microsatellite markers present in this region, analysis for inferring the level of polymorphism and the impact of selection force on MHC region has been the main focus of many investigations (Ammer *et al.*, 1992; Ellegren *et al.*, 1993; Schwaiger *et al.*, 1993; Schwaiger *et al.*, 1994). According to previous studies, balancing selection, negative frequency- dependent selection and directional selection on MHC region seems to be evident. These selections could be used for interpretation of high allelic diversity on MHC region (Apanius *et al.*, 1997; Hedrick, 1999; Hedrick, 2002; Bernatchez and Landry, 2003; Sommer, 2005; Piertney and Oliver, 2006).

Characterization of HLA-DRB1 region indicated the presence of several polymorphic microsatellite alleles (Marsh *et al.*, 2002). Two MHC microsatellite markers, D6S2879 and D6S2806, were found in approximately 1 kbp downstream and 14 kbp upstream of HLA-DRB1 gene respectively (see http://www.ncbi.nlm.nih.gov/projects/gv/mhc/xslcgi.fcgi). In our recent study, characterization of D6S2879 and D6S2806 markers revealed a high variation in their allelic number (Vallian *et al.*, 2010). Analysis of deviations from Hardy-Weinberg equilibrium (HWE) demonstrated that D6S2806 was in equilibrium ($P>0.05$). However, D6S2879 locus showed a significant deviation from HWE ($P<0.05$) (Vallian *et al.*, 2010).

It has been suggested that all polymorphic markers were neutral and the changes of allele frequencies were rarely due to selection. A number of statistical tests of neutrality was devised that could be used to investigate neutral allele theory (Nielsen, 2001; Carlson *et al.*, 2005). To determine whether these two MHC microsatellite markers in HLA-DRB1 gene region were subjected to selection in the Iranian populations, we investigated genetic diversity and neutrality test for these markers.

Material and Methods

Isolation of genomic DNA and Genotyping

The genotyping data used in this study were obtained from our previous studies on 73 healthy unrelated individuals form the Iranian populations (Vallian and Moeini, 2006; Vallian and Lahmi, 2009; Vallian *et al.*, 2010). To investigate the genotypes, total genomic DNA was isolated from peripheral blood leukocytes and genotyped using PCR amplification with specific primers followed by sequencing using an ABI 737 sequencer (Perkin Elmer/ABI) as described (Vallian *et al.*, 2010).

Statistical Analysis

The genotype data of two MHC microsatellite markers, D6S2879 and D6S2806, were used to create input file. Slatkin (1994) implementation of the Ewens–Watterson homozygosity test of neutrality (Ewens, 1972; Watterson, 1977) was performed using PyPop (Lancaster *et al.*, 2003). The PyPop (Python for Population Genomics) is a computer program for performing population genetic analyses on genotype data. For each marker, the observed homozygosity (F), computed as the sum of the squared allele frequencies, the expected homozygosity and the normalized deviation of the homozygosity (Fnd), differences between the observed homozygosity and expected homozygosity, were estimated. The observed homozygosity (F) is computed on the basis of the actual data. In Ewens-Watterson test, the F value is compared to the expected homozygosity ($\hat{F}$) computed by simulation under neutrality/equilibrium expectations. If the difference between the observed and expected homozygosity were larger or smaller than zero in the studied population, it could be inferred that this polymorphism was under directional and balancing selection, respectively (Nielsen, 2001). The Fnd is the difference between the observed homozygosity and expected homozygosity, divided by the square root of the variance of the expected homozygosity obtained by simulations. The data pertaining to 10 non-MHC microsatellite markers were used for the purpose of comparing the selection effect on MHC and non-MHC microsatellite in the Isfahan population. The data related to allele frequency of these non-MHC microsatellite markers were previously described (Vallian and Moeini, 2006; Vallian and Lahmi, 2009).

Ewens-Watterson test was also performed by use of Popgene32 software version 1.31 (available at http://www.ualberta.ca/~fyeh/download.htm) on the basis of algorithm given by Manly, (1985). This program is designed for many different types of analyses on a variety of molecular marker types. The observed homozygosity (F) and limit (upper and lower) at 95% confidence for the test were calculated for two MHC microsatellite markers, D6S2879 and D6S2806, using the genotyping data. The observed number of alleles, the effective number of alleles and Shannon’s information index of the studied MHC microsatellite markers were also estimated using Popgene32. The allele frequency data of these 12 microsatellite markers, i.e. D6S2879, D6S2806, LPL, F13B, HUMvWA, HPRTB, HUMTPO, HUMTH01, HUMFES, D16S539, F13A01 and CSF1PO, were used to detect recent genetic bottlenecks in the Iranian populations. The bottleneck events were investigated using the homozygosity test implemented in BOTTLENECK 1.2.02 (Cornuet and Luikart, 1996). The homozygosity test was performed under the step-wise mutation model (SMM) and the two-phase mutation model (TPM). These two models were considered the most realistic mutation models for microsatellite markers (Ellegren, 2000). The sign test and Wilcoxon test were used to assess bottleneck in the studied population. The wilcoxon test provides relatively high power (Luikart and Cornuet, 1998) with as few as four polymorphic loci and any number of individuals (15-40 individuals and 10-15 polymorphic loci is recommend to achieve high power). The results of these statistical analyses could be used to determine evolutionary history of these microsatellite markers located on HLA-DRB1 gene region in the Iranian populations.

Results

Various measures of genetic diversity in terms of observed number of alleles, effective number of alleles and Shannon’s information index are presented in Table 1. As shown, D6S2879 marker was observed in 4 different sizes in the Iranian populations. These alleles were located between 284-338 base pair (Vallian *et al.,* 2010). The effective number of the alleles for D6S2879 marker was estimated to be 2.2701. For D6S2806 marker, the observed and effective number of alleles was 6 and 1.6547, respectively. The observed alleles for D6S2806 marker in the Iranian populations were spaced between 312-338 base pair (Vallian *et al.,* 2010). As shown in Table 1, Shannon’s information index of the D6S2879 and D6S2806 markers is 1.0372 and 0.8601, respectively. The neutrality of two MHC microsatellite markers was tested by use of Popgene32 software. As presented in Table 2, the F values (the observed homozygosity) for D6S2879 and D6S2806 were calculated 0.4405 and 0.6043, respectively. These obtained values lies inside the lower and upper limit of 95% confidence region of expected F value at both MHC microatellite markers. Table 3 shows the results of the homozygosity tests of neutrality for both D6S2879 and D6S2806 microsatellite markers of HLA-DRB1 gene region in the Iranian populations. The Fnd value obtained for D6S2879 and D6S2806 markers were estimated to be -0.8449 and 0.9904, respectively. The negative Fnd value of all studied non-MHC microsatellite markers is significantly higher than 1. The p value obtained for all these non-MHC microsatellite markers were estimated to be less than 0.05, consistent with negative value of Fnd calculated for these markers. The data obtained from genetic bottleneck analysis is presented in Table 4. Under sign test, the expected numbers of loci with heterozygosity excess were 7.12 (TPM) and 7.08 (SMM), which were substantially lower than the observed numbers of loci 11 (TPM) and 10 (SMM) with heterozygosity excess. The probability values of 0.01709 (TPM) and 0.02124 (SMM) under Wilcoxon test were significant ($P<0.05$) in the Iranian populations.

Table 1: The observed number of alleles (na), effective number of alleles (ne) and Shannon’s Information Index (I) for microsatellite markers, D6S2879 and D6S2806, at the HLA-DRB1 gene region in the Iranian populations.

Microsatellite marker	na	ne	I
D6S2879	4.0000	2.2701	1.0372
D6S2806	6.0000	1.6547	0.8601

Table 2: The Ewens-Watterson test for Neutrality at two microsatellite markers of HLA-DRB1 gene region by use of Popgene32 software in the Iranian populations.

MICROSATELLITE MARKER	K	F	L95	U95
D6S2879	4	0.4405	0.3167	0.9331
D6S2806	6	0.6043	0.2358	0.8087

k: the number of alleles; F: the sum of the squared allele frequencies; L95, U95: The 95% confidence interval upper and lower limit.

Table 3: The summarized results of Ewens-Watterson neutrality test applying to two MHC microsatellites and ten non-MHC microsatellites in the Iranian populations.

Marker		*F*	$\hat{F}$	Fnd	p-value of F
MHC microsatellite	D6S2879	0.4405	0.5931	-0.8449	0.2311
	D6S2806	0.6043	0.4523	0.9904	0.8251
non-MHC microsatellite	LPL	0.2001	0.5463	-1.9338	0.0000
	F13B	0.1925	0.4853	-1.7624	0.0008
	HUMvWA	0.1844	0.3865	-1.4042	0.0142
	HPRTB	0.2032	0.4368	-1.4930	0.0083
	HUMTPO	0.2125	0.4665	-1.5131	0.0093
	HUMTH01	0.2140	0.5142	-1.7051	0.0021
	HUMFES	0.2201	0.5142	-1.6706	0.0029
	D16S539	0.2118	0.4853	-1.6464	0.0028
	F13A01	0.2096	0.5466	-1.881	0.0001
	CSF1PO	0.1986	0.4368	-1.5227	0.0058

F: the observed homozygosity; $\hat{F}$: the expected homozygosity; Fnd: the normalized deviate of the homozygosity

Table 4: Bottleneck analysis of the Iranian populations using sign test and wilcoxon test under TPM and SMM.

Test	TPM		SMM	
	Expected	Observed	Expected	Observed
Sign Test: Number of loci with heterozygosity excess	7.12	11	7.08	10
Wilcoxon Test: Probability of heterozygosity excess	0.01709		0.02124	

Parameters for TPM: variance = 30.00, proportion of SMM= 70.00%, estimation based on 1000 replications.

Discussion

Two markers, D6S2879 and D6S2806, were presented in dbMHC web site as potential microsatellite markers in HLA-DRB1 gene region (Gourraud *et al.*, 2007). In this study, we used genotyping data of these two polymorphic markers and other previously studied markers in order to investigate the evolutionary history of the Iranian populations. According to Table 1, the highest number of alleles is observed for D6S2806 marker, but the highest effective number of alleles and the Shannon's information index are estimated for D6S2879 marker. Shannon index for D6S2879 marker is almost 1 and heterozygosity of this marker is high in the Iranian populations. It appears that genetic diversity of D6S2879 marker is higher than D6S2806 marker in the Iranian populations.

In Ewens-Watterson test of neutrality for these markers, F value (the observed homozygosity) lied inside the limit of 95% confidence region (Table 2). If the F value would have lied outside the lower and upper limit of 95% confidence region of expected F value, these markers were probability under genetic hitchhiking and associated with a selected allele at another gene. The results obtained from Ewens-Watterson test which were performed by use of Popgene32 software indicated that these two MHC microsatellite markers in HLA-DRB1 gene region were not under genetic hitchhiking. Therefore, selection operated on another locus could not influence allelic frequency and heterozygosity of these markers in the Iranian populations.

It has been reported that balancing selection could affect the evolution of a number of genes in the humans and plays an important role in maintenance variation responsible for long-term adaptation to the environment (Andrés *et al.,* 2009). In the present study, for each marker, the observed homozygosity (F), the expected homozygosity ($\hat{F}$) and the normalized deviate of the homozygosity (Fnd) by use of PyPop software were estimated. As shown in Table 3, the normalized deviate of the homozygosity (Fnd) for D6S2879 marker is negative. The observed homozygosity value is also lower than the expected homozygosity for this marker. Indeed, these results provided the first support for heterozygote advantage as a source of balancing selection at D6S2879 marker in the Iranian populations. It seemed that the alleles of this marker were actively maintained in the studied population, which could reflect the consequence of higher adaptive value of heterozygotes in comparison with homozygotes.

Positive value of Fnd and also $F > \hat{F}$ are evidence of directional selection at D6S2806 marker. Directional selection changes the frequency of an allele in a particular and constant direction. Under directional selection, the advantageous allele will increase in frequency and even might fix. Although, six alleles is observed for D6S2806 marker in the Iranian populations, the effective number of alleles is 1.6547 alleles. This data may suggest that directional selection could play an important role in decreasing the effective number of alleles for D6S2806 marker in the Iranian populations. The negative Fnd and that fact that the p-value was significantly less than 0.05 for all ten non-MHC microsatellites implied that these markers were under balancing selection in the Iranian populations (Table 3). Comparison of MHC microsatellites and non-MHC microsatellites indicated that the selection on the studied non-MHC microsatellites had more potent than two studied MHC microsatellites, i.e. D6S2879 and D6S2806 markers. These differences could be related to their physical position in MHC gene region. The studied non-MHC microsatellite markers are located closer to coding sequence than MHC microsatellite markers. Data obtained from bottleneck analysis of the Iranian populations indicated that the null hypothesis that the population was under mutation-drift equilibrium could not be supported. The results of sign test and wilcoxon test showed that the studied population have undergone mild bottleneck.

Moreover, in this study, we reported balancing selection at almost 11 microsatellite markers in the Iranian populations. In our previous study, the balancing selection was observed in two markers of *PAH* gene (Fazeli and Vallian, 2010). Most of the studied markers in the Iranian populations showed observed heterozygosity higher than 50% (Fazeli and Vallian, 2009; Vallian and Moeini, 2006; Vallian and Lahmi, 2009). The high heterozyosity of studied markers implied that balancing selection probably counteract genetic bottleneck in the Iranian populations. Two studied MHC microsattelite markers, D6S2806 and D6S2879, showed low effective number of alleles (Table 1). It is likely that low gene diversity of these two MHC microsattelite markers could be the result of genetic bottleneck in the Iranian populations.

The high frequency of HLA-DRB1*15 and DRB1*04 has been found in the Iranian MS patients and the frequency of HLA-DRB1*07 and *11 has been shown a high increase in the Iranian optic neuritis (ON) patients (Amirzargar *et al.*, 2005). HLA-DRB1*1501 has been found significantly more frequent among MS patients, although no association was observed with clinical manifestation in the Iranian MS patients (Ghabaee *et al.*,2009). A significant positive association with AML for the HLA-DRB1*11allele was also reported in two studies performed on the Iranian populations (Sarafnejad *et al.*, 2006; Khosravi *et al.*, 2007). In the study on the Iranian non-Jewish patients with the Pemphigus vulgaris (PV), the HLA-DRB1*04 and DRB1*1401 alleles was reported as two major PV susceptibility factors (Shams *et al.*, 2009). The HLA-DRB1*07 was also found as the predisposing allele in the Iranian patients with pulmonary tuberculosis (Amirzargar *et al.*, 2004). The HLA-DRB1*13 allele was identified as an important factor in the protection against persisting hepatitis B infection in the Iranian populations (Ramezani *et al.*, 2008). As stated in the pervious study, the HLA-DRB1 region was under influence of selection force. Our results were also confirmed the presence of selection in this region. The evidence of balancing and directional selection at HLA-DRB1 gene region in the Iranian populations was found in the performed study. The results obtained from other researches could facilitate the interpretation of the polymorphism observed in the studied markers. It is highly probable that heterozygous individuals in D6S2879 marker could be more susceptible to a larger array of pathogens and autoimmune diseases than homozygous individuals. For D6S2806 marker, only one of the alleles could provide the most suitable role in immune response. In fact, it seems that this allele is in the process of being gradually fixed in the Iranian populations. Finally, in view of the unique role of HLA-DRB1 at response to pathogens, and susceptibility to autoimmune diseases, these results could make a novel contribution to the understanding of both the evolutionary history and the genetic diversity of this gene in the Iranian populations.

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Foliar anatomy and micromorphology of *Festuca* L. and its taxonomic applications

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Abstract

In this study, leaf micromorphological structure of eight species of *Festuca* (*F. akhanii*, *F. elwendiana*, *F. heterophylla*, *F. sulcata*, *F. valesiaca*, *F. arundinacea*, *F. gigantean* and *F. drymeia*) and leaf anatomy structure of three species of *Festuca* (*F. arundinacea*, *F. gigantean* and *F. drymeia*) belonging to the four subgenera were examined with different repeations. About 40 quantitative and qualitative anatomical features of the leaves were statistically analyzed for several times from superficial view and on transversal section. These characters included observation of ribs and furrows in epidermis, the density of stomata and trichome, and the arrangement of vascular bundles. Sclerenchyma and bulliform cells were studied and their taxonomic value was verified in order to classify different species. The micromorphology data and anatomy characteristics of the species were used for multivariate analysis, which partly supported the taxonomic treatment of the genus *Festuca* in the flora of Iran. In order to group the species studied on the basis of similarity in their anatomical features as well as their micro morphological characteristics, different clustering methods of Between Groups, Single Linkage and WARD were observed. The first cluster composed of *F. sulcata*, *F. valesiaca*, *F. elwendiana*, *F. heterophylla* and *F. akhanii*. The species of *F. arundinacea*, *F. gigantea* and *F. drymeia* were positioned in clusters two.

Key words: Anatomy, *Festuca*, Iran, Micro morphology, Sclerenchyma

Introduction

The genus *Festuca* L. *c*ontains an estimated 450 species (Clayton and Renvoize, 1986) and is a large and ancient group and one of the main evolutionary lines in the tribe Poeae which occur in polar, temperate, and alpine regions of both hemispheres (Tzvelev, 1976).

More than a century, foliar anatomy has been used in the taxonomy of the Festuca. Hackel (1882) published the first monograph of the genus Festuca relating to Europea He illustrated characteristics of 37 taxa using block diagrams. Metcalfe (1960) gave diagnostic generic characters and detailed information on the anatomy of several species of *Festuca*. Tzvelev (1976) in U.S.S.R. and Alexeev (1982) of North America made considerable use of leaf cross section as a taxonomic tool. In flora European Markgraf-Dannenberg (1980)

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using leaf cross section characters such as the number of veins and the distribution of sclerenchyma succeeded in identifying the species in this genus. The available literature from the other parts of the world dealing with anatomical study of *Festuca* supported such an assumption (Holmen, 1964; Badoux 1971; Borrill, 1972; Frederickson, 1977; Connor, 1960; Howarth, 1924, 1925; Snait-Yeves, 1925; Aiken *et al*., 1984, 1985, 1995; Sawicki *et al*., 2001; Aryavand and Panahi, 2003; Namaganda and Lye, 2008, 2009; Zarinkamar, 2008). However, anatomical study of the genus *Festuca* is insufficient for the species growing wild in Iran.

The present study considers anatomy and micromorphology of eight *Festuca* species occurring in Iran with the aim of providing some basic anatomical data for the country, and its taxonomic applications.

Materials and Methods

Micromorphological studies were performed on eight populations of eight *Festuca* species, namely *F. sulcata*, *F. arundinacea* Schreb, *F. valesiaca* s.l. Schleich. ex Gaudin, *F. gigantea* (L.). Vill. *F. elwendiana* Markgr.-Dann., *F. heterophylla* Lam., *F. akhanii* Tsvelev and *F. drymeia* Mertens et Koch (Table 1). The specimens were diagnosed using Flora Iranica (Bor, 1970), Flora Orientalis (Boisser, 1875), Flora of Iran, Flora of Iraq (Bor, 1968, 1970), the illustrated Flora of Golestan National Park, Iran (Akhanii, 2005) and Grasses of the Soviet Union (Tzvelev, 1976). The voucher specimens are deposited in the Herbarium of TARI (Table 1).

Table 1: Voucher specimen of *Festuca* specimens. Abbreviations: TARI, Research Institute of Forests and Rangelands Herbarium.

Species	Morphological studied (Morphology and anatomy)	Voucher specimen
F. akhanii	Morphology	Golestan, Golestan Forest, 1600 m, Bagheri (TARI, 90264)
F. arundinaceae	Micromophology and Anatomy	Yazd, 2700-2900 m, Mozaffarian (TARI, 77545)
F. drymia	Morphology and Anatomy	Gorgan, Loo forest, Bagheri (TARI, 90267)
F. drymia	Morphology and Anatomy	Mazandaran, Noshahr,500 m (TARI, 33471)
F. elwendiana	Morphology	Hamedan, Alvand, 2300 m, Kelovande & Baghere (TARI, 90265)
F. elwendiana	Morphology	Hamedan, Ganjnameh, 2100 m, Assadi & Mozaffarian (TARI, 36729)
F. gigantea	Morphology and Anatomy	Mazandaran, Tonekabon,1400 m, Hamzehee & Asree (TARI, 71073)
F. heterophylla	Morphology	Tehran,Lar,2420-2550 m, Wendelbo &Assadi (TARI, 13348)
F. heterophylla	Morphology	Azarbaijan, 45 KM Sabalan, 2900 m, Mozaffarian (TARI, 9319)
F. sulcata	Morphology	Azarbaijan, Arasbaran, 2500 m, Assadi (TARI, 23910)
F. sulcata	Morphology	Azarbaijan, Arasbaran, Dooghrool, 2600-2800 m, Assadi & Sardabi (TARI, 23997)
F. valesiaca	Morphology	Golestan, Yakhte kalan, 2100 m, Bagheri (TARI, 90262)
F. valesiaca	Morphology	Golestan, Golestan Forest, Sharlagh, 2200 m, Bagheri (TARI, 90263)

Methods

Anatomical analyses of leaves were done on permanent slides, prepared by the standard method for light microscopy (Strittmatter, 1973). Cross-sections of the external leaves from vegetative shoots were cut on a Reichert sliding microtome and by hand cutting. Sections were prepared from the middle region between one quarter and one half of the total length of blade, cleared in sodium hypochlorite and stained with carmine-vest (1% w/v in 50% ethanol) and methyl green (1% w/v, aqueous) then mounted in gelatin.

Epidermal surface were studied with Scanning Electron Microscope (SEM) for which the samples was covered by gold.

All morpho-anatomical measurements were done and the data processed in the statistical package. For each of the quantitative characters, 65 leaf samples were obtained from different individuals belonging to each of the eight populations analyzed. 37 quantitative and 40 qualitative anatomical characters were statistically analyzed in superficial view and on transversal section.

Statistical Analyses

In order to group the species studied based on similarities in their anatomical features as well as micromorphological characteristics, different clustering methods of Between Groups, Single Linkage and WARD (minimum spherical cluster method) were used. To obtain value of characters, PCA (principle component analysis) was employed**.**

Results

Festuca L. is divided into two groups based on lamina width: fine-leaved and broad-leaved. In the fine-leaved, abaxial leaf surface was coated by silica and there were single prickles on veins and leaf margin. In abaxial surface of *F. akhanii*, trichomes were distributed with different orientations and some outgrowths (Figure 1, A and B). In *F. elwendiana*, trichomes had different sizes (Figure 1, C). In *F. heterophylla*, prickles were only seen on veins and leaf margin of abaxial surface (Figure 1, E and F). There were ribs and furrows on adaxial surface which stomata frequently presented in furrows (Figure 1, D and J). The ribs were pubescent (Figure 1, E).

In the broad-leaved species, external surface and stomata were covered by silica (Figure 2, A and C). Trichomes were present on leaf margins (Figure 2, F). In *F. drymeia* there were no ribs and furrows and stomata were distributed on both sides of leaf surfaces. In *F. gigantea* and *F. arundinacea* there were ribs and furrows and stomata were frequently present on furrows.

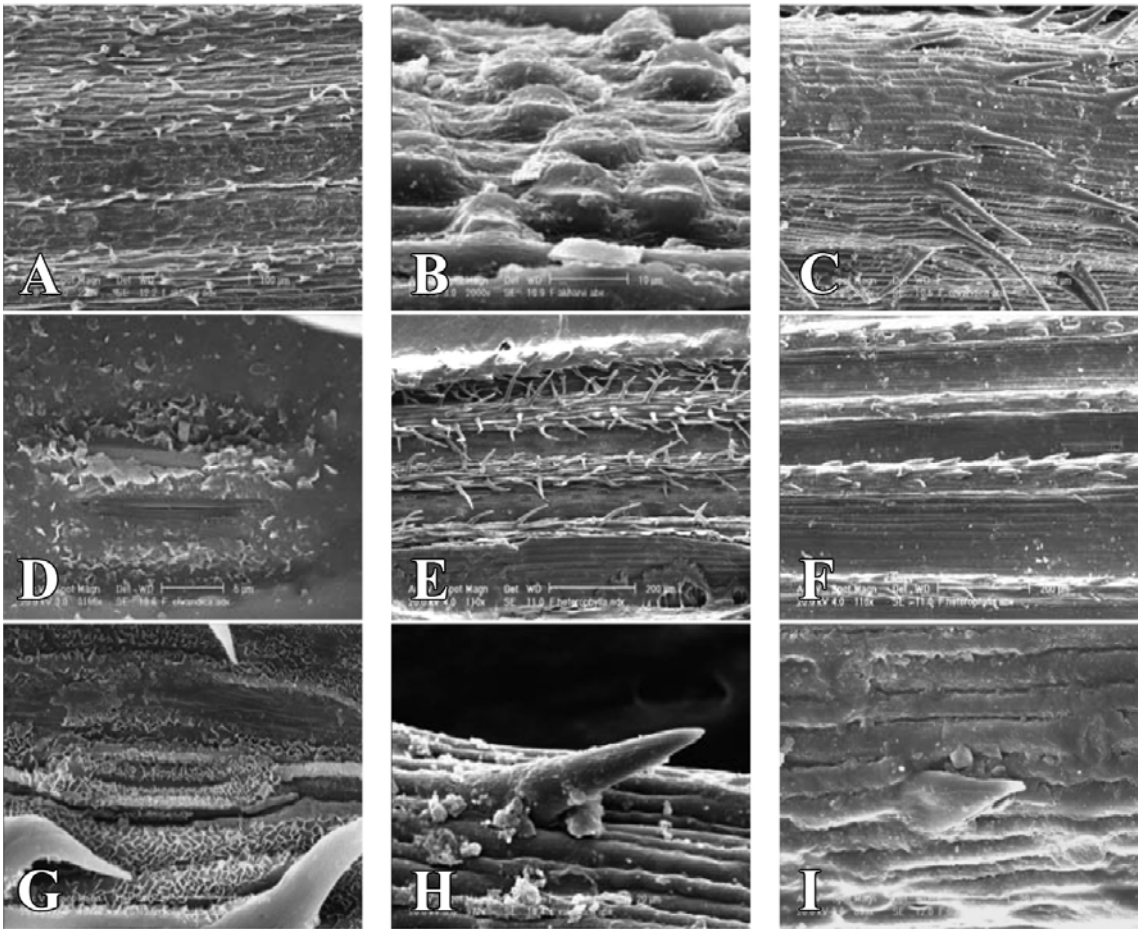

Figure 1: Superficial view of the fine-leaved species. A and B: *F. akhanii* (223X), (2000X); C and D: *F. elwendiana* (239X), (3155X); E and F: *F. heterophylla* (110X), (116X); J and H: *F. valesiaca* (1673X), (972X); I: *F. sulcata* (839X).

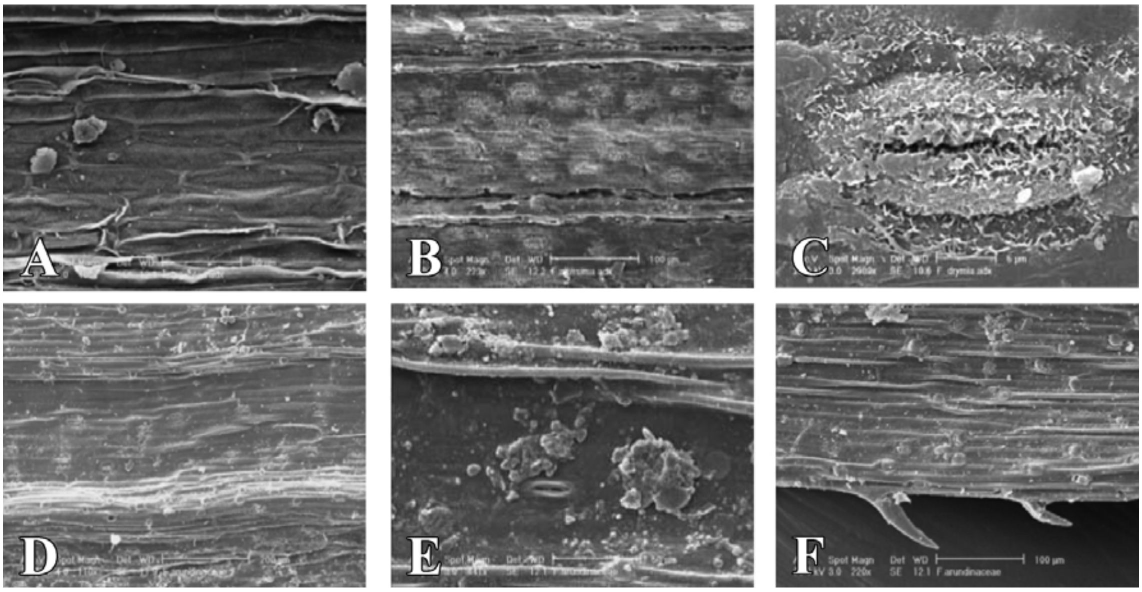

Figure 2: Superficial view of broad-leaved. A: *F. gigantea*; B and C: *F. drymeia;* D and F: *F. arundinacea.*

Leaf cross section: Fine-leaved of *Festuca* species were ≤ 2 mm wide because they were more or less tightly rolled or folded, often with leaf margin overlapping. A previous study on fine-leaved of *Festuca* species, demonstrated that some characters as number of ribs and furrows in adaxial surface, number of vascular bundles and number of sclerenchyma bundles could distinguished species (Eslami *et al*., 1387).

Broad-leaved species included *F. arundinacea*, *F. gigantea* and *F. drymeia*. Epidermal cells were different in size and type. Leaves were or appeared to be, ≤4 mm wide.

The characters are suitable for distinguishing the species as follows: Adaxial surface in *F. drymeia* is smooth (Figure 2, D, E and F), whereas in *F. arundinacea* (Figure 3, G, H and I) and *F. gigantea* (Figure 3, A, B and C) are observed as rounded rib over each vein. Bulliform cells occupy ¼ of the leaf thickness on the adaxial epidermal cells or at the base of the ribs (Figure 3, F). In the above-mentioned species, minute sclerenchyma tissues were observed opposite middle and as large vascular bundles. Sometimes sclerenchyma girders were extended from vascular bundles to both adaxial and abaxial epidermis (Figure 3, D, E, F, G, H and I).

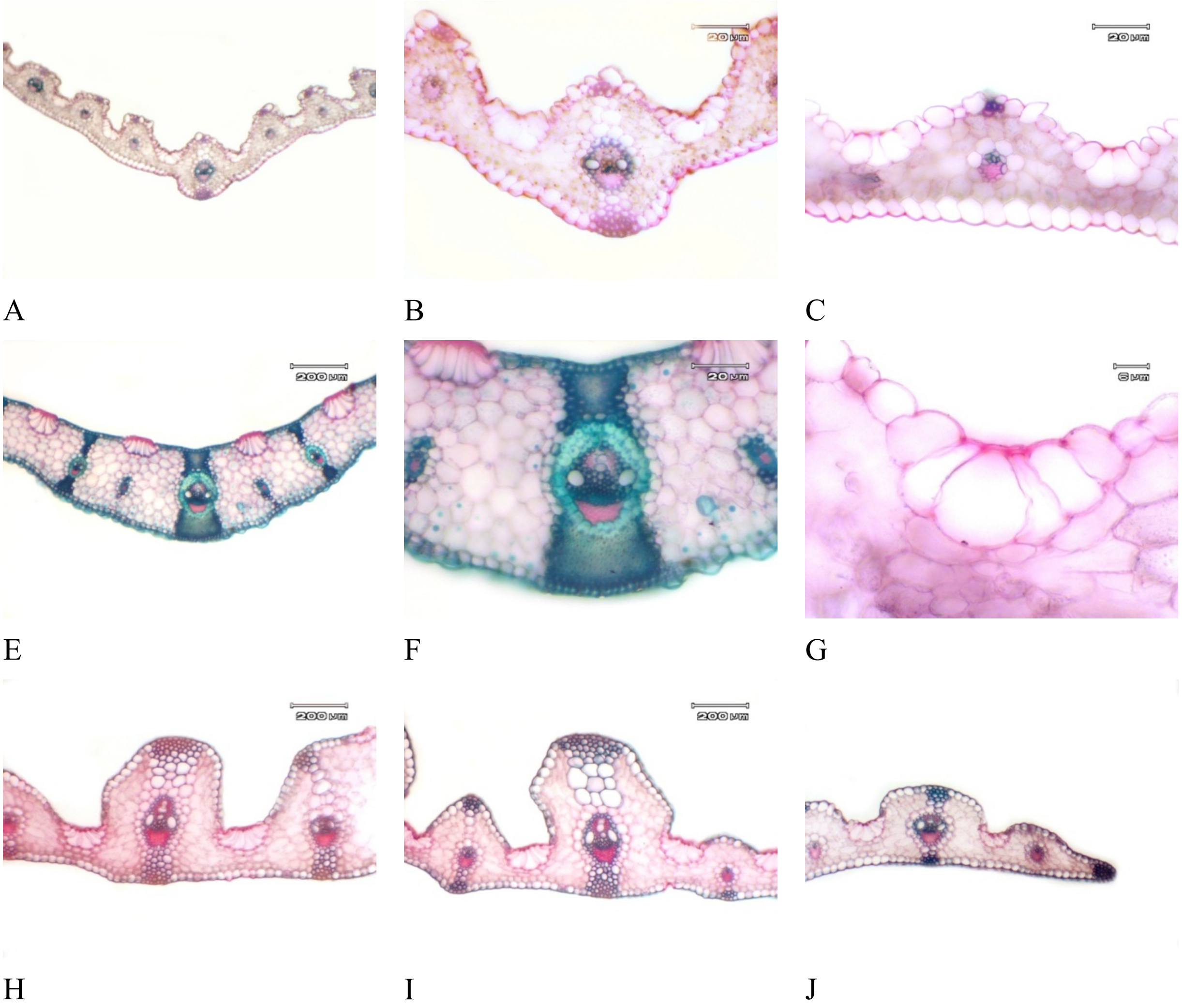

Figure 3: Transversal section of broad-leaved species. A and B: *C*, *F. gigantea;* D, E and F: *F. drymeia*, G, H and I: *F. arundinacea.*

Table 2: Useful anatomical characters for distinguish species. Relation the sclerenchyma tissue of Midrib and Adaxial epidermal: 1, attachment; 2, apart. Shape furrow: 1; V-shape between 45°; 2, V-shape between 90° and 180°. Epidermal cell shape: 1, oblong; 2, oblong- elliptic. Stomata type: 1, present, 2, absent.

	Taxon			
Characters	*F. drymia*	*F. drymia*	*F. gigantea*	*F. arundinaceae*
Relation the sclerenchyma tissue of midrib and adaxial epidermis	1	1	2	2
Number of large vascular bundle	3	3	2	1
Number of middle vascular bundle	6	6	2	3
Number of small vascular bundle	4	1	3	3
Shape furrow	0	0	1	2
Adaxial epidermal cell shape	2	2	1	2
Stomata type on adaxial surface	3	2	3	2
Buliform cells shape	1	1	4	4
Abaxial epidermal cell shape	1	1	3	2
Stomata type on abaxial surface	3	2	3	2
Number of adaxil furrow	0	0	13	13
Number of adaxil ribs	0	0	5	14
Number of buliform cells on adaxial surface	0	0	30	24
Number of sclerenchym bundle	26	23	8	16
Number of sclerenchym layer in tip leaf	6	4	2	7
Number of sclerenchym layer under midrib	10	6	3	7

Different clustering methods like Between Groups, Single Linkage and WARD produced similar results whereas Between Groups possessed the highest correlation. In analyses, two clusters were observed. The first cluster was composed of *F. sulcata*, *F. valesiaca*, *F. elwendiana*, *F. heterophylla* and *F. akhanii*. The species of *F. arundinacea*, *F. gigantea* and *F. drymeia* were positioned in cluster two (Figure 4).

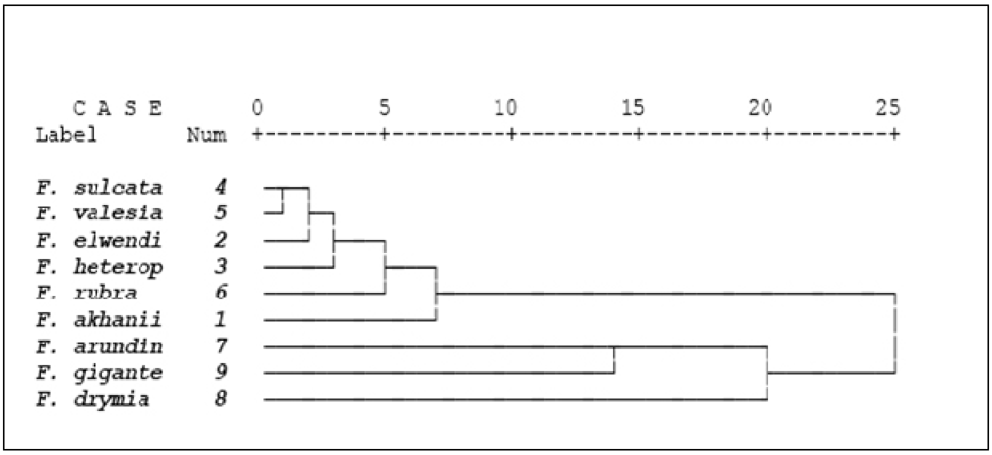

Figure 4: Ward method clustering of the *Festuca* species studied

The PCA data demonstrated the most important characters for anatomy as follow: width and thickness of leaf cross section, shape and number of veins and arrangement of vascular bundles. Moreover, the position and degree of scleranchyma development, number of ribs in adaxial surface, density of crystal, observation of stomata in abaxial surface, form of epidermal cells and number of bulliform cells could be also valuable for taxonomic application.

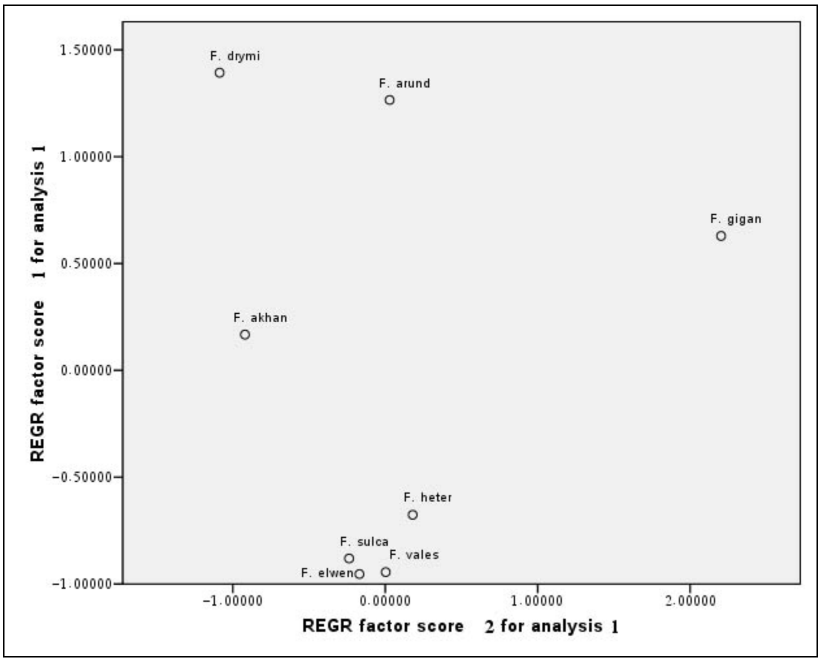

Figure 5: PCA plot of the *Festuca* species studied

The PCA plot suggested that the position of *F. akhanii* was between the fined and broad-leaved species of *Festuca* (Figure 5).

Discussion

The anatomical characters in cross section of leaf are important in identification of the taxa. Broad-leaved species such as *F. arundinacea* from the subgenus Schedorous, *F. drymeia* from the subgenus Drymanthele and F. gigantea from the subgenus Drymoneates have linear leaves with 2mm width and sheaths that divided into two groups based on ribs and furrows but in *F. drymeia* there are not ribs and furrows on both surface linear and have 2 mm width.

In general, bulliform cells are dispersed between veins on the adaxial surface. Papilla or prickle are scattered on both surfaces. There is one extension of sclerenchyma tissue from the abaxial to adaxial surface in midrib and the middle sized veins, this structure is actually absent in small veins. These species hasve no auricle in leaf sheath.

These furrows on the adaxial surface of *F. arundinacea* are distinguished from smooth adaxial surface of *F. gigantea*.

Stomata are present on both sides but more dense on adaxial furrows, and prickles are just present on the leaf margin. Another significant anatomical feature of *F. arundinacea* is the prensence of a big vascular bundle on both sides of midrib.

F. gigantea and *F. arundinacea* are morphologically distinguished from other broad-leaved species by the presence of auricle leaf sheath.

Fine-leaved species includes *F. elwendiana*, *F. valesiaca*, *F. sulcata*, *F. akhanii* and *F. heterophylla* from the subgenus *Festuca*.

The species of *F. akhanii* is distinguished by sclerenchyma strand, presence of furrows on both sides of the midrib and papilla, while other species of the subgenus Festuca has more than two furrows on adaxial surface and no sclerenchyma strand.

Another important and stable anatomical character is the number of sclerenchyma tissues on the adaxial surface which is useful character to distinguish other species of the subgenus Festuca. There are three groups of sclerenchyma tissues with different layers in *F. valesiaca*, *F. sulcata* and *F. elwendiana* on abaxial surface. The sclerenchyma tissues are completely separated in *F. valesiaca* and *F. sulcata* sometimes tends to continue in *F. elwendiana*. *F. elwendiana* is morphologically characterized by the presence of variable sized of trichomes on both leaf surfaces, pubescence sheathes and glabrous ovary. *F. sulcata* is morphologically and anatomically similar to *F. valesiaca* which is distinguished by group of four bulliform cells, complexes with wavy walls and furrows on adaxial surface near midrib.

There are five to seven sclerenchyma tissue groups with different layers in *F. heterophylla*. Sclerenchyma tissue forms discrete strands opposite to the large vascular bundles.

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فرم اشتراک مجله تاکسونومی و بیوسیستماتیک

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با واریز مبلغ ۸۰۰۰۰ ریال (هزینه پست و اشتراک) به حساب شماره ۲۱۷۷۲۴۰۲۳۸۰۰۲ بانک ملی، کد ۱۱۰۲۲۷، شعبه دانشگاه اصفهان، به نام درآمدهای اختصاصی دانشگاه اصفهان، متقاضی اشتراک یک‌ساله (چهار شماره) مجله تاکسونومی و بیوسیستماتیک هستم.
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آناتومی و ریز ریخت‌شناسی برگ جنس *Festuca* و کاربرد تاکسونومی آن

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چکیده

در این تحقیق، ساختار ریز ریخت‌شناسی برگ ۸ گونه از جنس *Festuca* با نام‌های *F. akhanii*، *F. arundinacea*، *F. drymeia*، *F. elwendiana*، *F. gigantea*، *F. heterophylla*، *F. sulcata*، *F. valesiaca* و ساختار تشریحی برگ در سه گونه *F. arundinacea*، *F. drymeia* و *F. gigantean* با تکرار های متفاوت مورد بررسی قرار گرفت. حدود ۴۰ صفت معنی‌دار کمّی و کیفی به منظور آنالیز آماری چند متغیره در نظر گرفته شد. برخی از این صفات شامل وجود یا عدم وجود فرورفتگی و برآمدگی در اپیدرم، تراکم روزنه‌ها و کُرک‌ها در سطح، آرایش دستجات آوندی و تعداد دستجات بافت‌های اسکلرانشیمی است. داده‌های حاصل از مطالعات ریزساختاری و تشریحی برگ‌ها مورد تجزیه و تحلیل آماری قرار گرفت. نتایج آماری حاصل از روش‌های دسته‌بندی متفاوت مانند Between Groups، Single Linkage و WARD مشابه بوده، دو خوشه جداگانه شامل گونه‌های ریز برگ (*F. valesiaca*، *F. sulcata*، *F. heterophylla*، *F. elwendiana* و *F. akhanii*) و پهن‌برگ (*F. drymeia*، *F. gigantean* و *F. arundinacea*) را نشان داد.

واژه‌های کلیدی: آناتومی، ایران، ریز ریخت‌شناسی، *Festuca*، اسکلرانشیم

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اطلاعات جمعیتی مارکرهای D6S2879 و D6S2806 واقع در ناحیه HLA-DRB1 در جمعیت ایرانی: شناسایی اثرهایی از انتخاب طبیعی و جهت‌دار

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چکیده

در این مطالعه، تنوع ژنتیکی و آزمون neutrality برای جایگاه‌های ژنی ریزماهواره MHC، D6S2879 و D6S2806 واقع در ناحیه ژنی HLA-DRB1، بررسی شدند. اطلاعات تعیین ژنوتیپ ۷۳ فرد غیر خویشاوند برای شاخص شانون، تعداد آلل مؤثر مارکرها و آزمون neutrality با استفاده از برنامه‌های PyPop و Popgene32 بررسی شدند. شاخص شانون برای مارکرهای D6S2879 و D6S2806 در جمعیت مطالعه شده به ترتیب ۱/۰۳۷۲ و ۰/۸۶۰۱ بود. ارزش Fnd محاسبه شده برای مارکرهای D6S2879 و D6S2806 نیز به ترتیب ۰/۸۴۴۹- و ۰/۹۹۰۴ تخمین زده شدند. نتایج به دست آمده از آزمون Ewens-Watterson بیانگر آن است که مارکرهای D6S2879 و D6S2806 در جمعیت‌های ایرانی به ترتیب تحت انتخاب طبیعی و جهت‌دار هستند. این اطلاعات پیشنهاد کننده حضور یک فشار انتخابی بر روی ناحیه ژنی HLA-DRB1 در جمعیت‌های ایرانی است.

واژه‌های کلیدی: ژن HLA-DRB1، تنوع ژنی، آزمون Ewens-Watterson، مارکر ریز ماهواره MHC، انتخاب

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ارزیابی تنوع ژنتیکی ارقام انار ایران (*Punica granatum* L.) با استفاده از نشانگرهای RAPD و ISSR

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چکیده

با توجه به تنوع بالای مورفولوژیکی در ارقام انار ایران، تنوع ژنتیکی ۲۴ رقم انار با استفاده از نشانگرهای RAPD و ISSR مورد بررسی قرار گرفت. آغازگرهای RAPD در مجموع ۱۳۱ قطعه DNA تکثیر نمودند که ۲۹ قطعه آنها (۲۲/۱۴ درصد) چندشکل بودند. آغازگرهای ISSR نیز از مجموع ۱۷۳ قطعه تکثیر شده، ۲۹ (۳۷ درصد) چندشکلی حاصل نمودند. میانگین محتوای اطلاعات چندشکل (PIC) برای آغازگرهای RAPD و ISSR به ترتیب ۰/۱۲۸ و ۰/۱۶۳ به دست آمد. نتایج نشان داد که نشانگرهای ISSR در مقایسه با نشانگرهای RAPD الگوی نواری تکرارپذیری ایجاد می‌کنند و برای گروه‌بندی ارقام انار مؤثرترند. ضریب شباهت بین ارقام از ۰/۳۵۳ تا یک (RAPD) و ۰/۲۹۱ تا ۰/۹۳۰ (ISSR) متغیر بود و میانگین آن در نشانگرهای RAPD و ISSR به ترتیب ۰/۶۰۴ و ۰/۶۴۷ گزارش شد. تجزیه واریانس مولکولی (AMOVA) در داده‌های RAPD و ISSR اختلاف معنی‌داری بین نواحی مختلف جغرافیایی و طعم میوه ارقام مورد مطالعه نشان نداد ($P>0/05$) که بیانگر عدم ارتباط تنوع جغرافیایی و تنوع ژنتیکی است.

واژه‌های کلیدی: *Punica granatum*، تنوع ژنتیکی، انار، RAPD، ISSR

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جنس *Matricaria* L. (Anthemideae, Asteraceae) در ایران: مطالعه کموتاکسونومیک بر اساس فلاونوئیدها

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چکیده

جنس *Matricaria* L. متعلق به قبیله Anthemideae و زیر قبیله Matricineae (از تیره Asteraceae) است و شامل ۷ گونه است که دو گونه از آن به طور طبیعی در ایران می‌روید. این مطالعه به منظور توصیف نمونه‌های جمع‌آوری شده از این جنس در ایران با استفاده از پروفایل لکه‌های فلاونوئیدی و تعیین ویژگی‌های اسکلت فلاونوئیدهای مطرح در هر یک از گونه‌های آن صورت گرفته است. ۱۲ نمونه جمعیتی بالک شده از دو گونه *M. aurea* و *M. recutita* مورد آزمایش قرار گرفتند. داده‌های حضور-غیاب حاصل از بررسی نقشه‌های دو بعدی لکه‌های فلاونوئیدی مربوط به همه نمونه‌ها ثبت و با استفاده از آنالیزهای خوشه‌بندی و اردیناسیون (PCA) مورد بررسی قرار گرفتند. در این مطالعه، ویژگی‌های مربوط به اسکلت‌های فلاونوئیدی در سطح گونه و تفاوت‌های آنها مورد بررسی قرار گرفته، مروری اجمالی بر موقعیت تاکسونومیک آرایه‌های نزدیک ارایه گردیده است.

واژه‌های کلیدی: اسکلت فلاونوئیدی، ایران، Asteraceae، کروماتوگرافی لایه نازک دو بعدی، *Matricaria*

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مطالعه سیتولوژیک *Hordeum bulbosum* L. در ایران

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چکیده

گونه *Hordeum bulbosum* همواره به عنوان یکی از منابع آللی مفید که می‌تواند در اصلاح غلات زراعی استفاده شود مد نظر است. در این تحقیق، کاریوتیپ ۳۲ نمونه جمعیتی این گونه جمع‌آوری شده از نقاط مختلف ایران مورد بررسی قرار گرفت. تقارن کایوتیپی جمعیت‌ها و ارتباط آن با مناطق جغرافیایی ارزیابی شد. تمامی جمعیت‌های مطالعه شده تتراپلوئید با کاریوتیپ، متقارن بودند و شاخص‌های بررسی شده نشان از اتوتتراپلوئید بودن این گونه دارد. مشاهدات نشان می‌دهد که جمعیت‌های شمال شرقی (گلستان) و شمال غربی (گردنه حیران) نامتقارن‌ترین و جمعیت‌های غربی، متقارن‌ترین کاریوتیپ را دارند. بر اساس این نتایج می‌توان گفت که قدیمی‌ترین جمعیت‌های این گونه در کوه‌های زاگرس و جوان‌ترین آنها در شمال شرق ایران قرار دارند. این گونه احتمالاً از غرب ایران وارد شده و به سمت شرق گسترش یافته است.

واژه‌های کلیدی: *Hordeum bulbosum* L.، ایران، تقارن کاریوتیپ، تتراپلوئید

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بازنگری تاکسونومیکی جنس گون *Astragalus* L. (Fabaceae) در استان زنجان و شرح یک گونه جدید

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[۲] مؤسسه تحقیقات جنگلها و مراتع کشور، تهران، ایران

چکیده

استان زنجان واقع در شمال غربی ایران، با توجه به موقعیت خاص جغرافیایی، آب هوایی و پستی بلندی خود شرایط مستعدی برای رویش جنس گون دارد. در طول سال‌های ۱۳۸۷-۱۳۸۹ جمع‌آوری وسیعی در خصوص این جنس در استان انجام گرفت. در نتیجه این مطالعات ۴۱ گونه به مجموع ۷۵ گونه قبلی گزارش شده از استان زنجان اضافه گردید، به علاوه تعداد ۹ گونه جدید برای دنیای گیاه‌شناسی معرفی شدند و در این مقاله گونه *Astragalus fausicola* Podlech ex Bagheri, Maassoumi & F.Ghahrem. از بخش *Incani* DC. به عنوان گونه جدید شرح داده و معرفی می‌شود.

واژه‌های کلیدی: گون، خانواده بقولات، گونه جدید، تاکسونومی، استان زنجان

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گزارش گونه *Potentilla botschantzeviana* Adylov
(Syn: *Potentilla butkovii* var. *botschantzeviana* (Adylov) Soják)
از تیره گل سرخ به عنوان گونه جدید برای فلور ایران

مرضیه‌بیگم فقیر [۱*]، فریده عطار [۲] و جیری سوجاک [۳]

[۱] گروه زیست‌شناسی، دانشکده علوم، دانشگاه گیلان، رشت، ایران
[۲] هرباریوم مرکزی، بخش گیاه‌شناسی، دانشکده زیست‌شناسی، پردیس علوم، دانشگاه تهران، تهران، ایران
[۳] گروه گیاه‌شناسی، موزه علوم طبیعی، پراگ، جمهوری چک

چکیده

گونه *Potentilla botschantzeviana* ((Syn: *P. butkovii* var. *botschantzeviana* (Adylov) Soják)) به عنوان گزارشی جدید از شمال ایران ارایه می‌شود. این گونه با دارا بودن دمبرگ‌هایی پوشیده از کُرک‌های راست-خوابیده یا گسترده؛ برگ‌هایی با تقسیم‌های عمیق و برگچه‌هایی با کُرک‌های تُنُک، افراشته –فر دار و نیز گل‌هایی به قطر ۱/۲ سانتی‌متر قابل شناسایی است. *P. botschantzeviana* دارای صفات ریخت‌شناسی (به‌ویژه خامه بلند در قاعده ضخیم) مشابهی با گونه‌های بخشه *Persicae* (Th. Wolf) Juz.، بزرگترین بخشه این جنس در ایران و شامل ۱۲ گونه بومی است. تصویر گیاه در مرحله گل‌دهی، تصاویر قطعات مختلف، تصویر کُرک سطح تحتانی برگ و نیز نقشه پراکنش این گونه ارایه می گردد.

واژه‌های کلیدی: *Potentilla*، فلور ایران، *Persicae* (Th. Wolf) Juz.

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* marziehfaghir@yahoo.com

مجله علمی - پژوهشی تاکسونومی و بیوسیستماتیک
سال سوم - شماره هشتم - پاییز ۱۳۹۰
شماره استاندارد بین‌المللی: ۸۹۰۶-۲۰۰۸

فهرست

داوران علمی این شماره (سال سوم - شماره هشتم - پاییز ۱۳۹۰)

اعضای محترم هیأت علمی دانشگاه‌ها و مؤسسات آموزشی و پژوهشی کشور که در داوری و ارزیابی مقالات این شماره از مجله علمی- پژوهشی تاکسونومی و بیوسیستماتیک همکاری داشته‌اند، معرفی شده و از خدمات علمی آنها تقدیر می‌گردد:

دکتر نسترن جلیلیان	مرکز تحقیقات کشاورزی و منابع طبیعی کرمانشاه
دکتر سید ذبیح‌اله حسینی	دانشگاه یاسوج
دکتر نواز خرازیان	دانشگاه شهرکرد
دکتر مسعود رنجبر	دانشگاه بوعلی سینا
دکتر حجت‌اله سعیدی	دانشگاه اصفهان
دکتر بدرالدین ابراهیم سید طباطبایی	دانشگاه صنعتی اصفهان
دکتر فریده عطار	دانشگاه تهران
دکتر لی‌لی قائم مقامی	دانشگاه اصفهان
دکتر فرخ قهرمانی‌نژاد	دانشگاه تربیت معلم
دکتر شاهرخ کاظم‌پور اصالو	دانشگاه تربیت مدرس تهران
دکتر حمید میر محمد صادقی	دانشگاه علوم پزشکی و خدمات بهداشتی درمانی اصفهان

نمونه فارسی: رنگی‌پور، ا.، افشارزاده، س.، بلالی‌دهکردی، غ. ر. و صاحبی، ج. (۱۳۸۷) مطالعه جنس لویی در رودخانه زاینده‌رود. اولین همایش ملی زیست‌شناسی گیاهی، دانشگاه پیام نور، تالش.

نمونه انگلیسی:

- Mason-Gamer, R. J. and Helfgott, D. M. (2002) Molecular phylogenetic investigation of allopolyploid *Elymus* in North America. 4th International Triticeae Symposium, Prague, Czech Republic.

ب-۷) مرجع‌دهی به مقاله‌های کامل همایش‌ها (سمینارها، سمپوزیوم‌ها، کنگره‌ها، میتینگ‌ها و ...) (Proceedings):

به ترتیب شامل: نام نویسندگان، سال انتشار، عنوان مقاله، دوره و نام همایش، محل برگزاری، شهر، کشور.

نمونه فارسی: صفوی، و. و شریعتی، م. (۱۳۸٦) تأثیر الیسیتور متیل جاسمونات بر سنتز بتاکاروتن در جلبک سبز تک سلولی *Dunaliella salina*. مجموعه مقالات دومین همایش ملی زیست‌شناسی سلولی و ملکولی، کرمان، ایران.

نمونه انگلیسی:

- Mohsenzadeh, S. (1996) Study of nitrogen fertilizer time and amount on seed production and other characterizations of Sorgum. In: Proceeding of the 4th Iranian Congress of Agriculture and Plant Breeding, Isfahan, Iran.
- Shariati, M. and Lilley, R. McC. (1993) Triggering of glycerol synthesis in *Dunaliella tertiolecta* at constant osmotic pressure. 33rd Annual General Meeting of Australian Society of Plant Physiologist. Perth, Australia.

ب-۸) مرجع‌دهی به اینترنت: مرجع‌دهی به نشانی‌های اینترنتی تقریباً فاقد اعتبار بوده و پیشنهاد می‌شود استفاده نگردد. در مواقعی که ناگزیر از استفادهٔ محدود از آن باشد نام نویسنده، زمان چاپ و در انتها نیز زمان استخراج از اینترنت درج گردد.

نمونه:

- Rotblat, J. (2000) Fifty Pugwash conferences: a tribute to Eugene Rabinowitch. Retrieved from http://www.pugwash.org/reports/pac/pac256/otblat.htm. On: 22 June 2001.

پ) شکل‌ها و جدول‌ها: شکل‌ها و جدول‌ها به ترتیب ذکر شده درون متن قرار بگیرند، توضیحات شکل‌ها در پایین و توضیحات جدول‌ها در بالای آنها نوشته شود.

تذکر بسیار مهم: درستی نام علمی گونه‌های گیاهی از لحاظ صفت گونه‌ای و نام آتور در سایت اینترنتی www.ipni.org بررسی شود.

نحوه ارسال مقاله

مقالات به صورت فایل word نسخه ۲۰۰۳ (با نام و نشانی نویسندگان، بدون نام و نشانی نویسندگان، فرم کپی‌رایت) به پایگاه اختصاصی مجله http://uijs.ui.ac.ir/tbj ارسال گردد.

تماس با ما

نشانی پستی: اصفهان- خیابان هزار جریب- دانشگاه اصفهان- ساختمان کتابخانه مرکزی- طبقه دوم اداره چاپ، انتشارات و مجلات- دفتر مجله تاکسونومی و بیوسیستماتیک، کدپستی: ۸۱۷٤٦۷۳٤٤۱

شماره تماس: ۷۹۳٤۱٦٤ -۰۳۱۱، دورنگار: ۷۹۳۲۱۷۷ -۰۳۱۱

نشانی پست الکترونیک: tbj@ui.ac.ir.

ب-۱-۲) مقاله با دو نگارنده:

نمونه فارسی: ولی‌پور، ع. و حسینی، ا. (۱۳۷٦) بررسی پراکنش گیاهان مقاوم به شوری در ایران، مجلهٔ زیست‌شناسی ۳ (۵) : ۷۵ تا ۹۱.

نمونه مثالی انگلیسی:

- Baum, B. R. and Appels, R. (1992) Evolutionary change at the 5s DNA loci of species in the Triticaceae. Plant Systematics and Evolution 183: 195-208.

ب-۱-۳) مقاله با سه نگارنده و بیشتر:

نمونه فارسی: ولی‌پور، ع.، حسینی، ا. و امینی، ا. ر. (۱۳۷٦) بررسی پراکنش گیاهان مقاوم به شوری در ایران، مجلهٔ زیست‌شناسی ۳ : ۷۵ تا ۹۱.

نمونه انگلیسی:

- Jain, S. K., Qualset, C. O., Bhatt, G. M. and Wu, K. K. (1975) Geographical patterns of phenotypic diversity in a world collection of durum wheats. Crop Science 15: 404-700.

ب-۲) مرجع‌دهی به کتاب (Book): به ترتیب شامل: نام نویسنده یا نویسندگان، سال، عنوان کتاب، شمارهٔ Edition در صورت وجود، نام مؤسسهٔ انتشاراتی، نام اولین شهری که انتشار در آن انجام گرفته است.

نمونه فارسی: مظفریان، و. (۱۳۷۳) کورموفیت‌های ایران. جلد ٤، مرکز نشر دانشگاهی، تهران.

نمونه انگلیسی:

- Stace, C. A. (1989) Plant Taxonomy and Biosystematics. Edward Arnold, London.
- Rice, E. L. (1984) An Introduction to Microbiology. 2nd ed., Academic Press, New York.

مرجع‌دهی به ترجمه فارسی کتاب:

استیس، سی. اِی. (۱۳۷۵) تاکسونومی گیاهی و سیستماتیک زیستی، ترجمه خسروی، الف.، انتشارات دانشگاه شیراز، شیراز.

ب-۳) مرجع‌دهی به بخشی از کتاب (Chapter in Book) که هر بخش دارای نویسنده جداگانه باشد:

نمونه انگلیسی:

- Morrison, L. A. (1993) *Triticum-Aegilops* systemaics: taking an integrative approach. In: Biodiversity and Wheat Improvement (ed. Damania, A. B.) 59-66. John Willey & Sons, New York.
- Sears, E. R. (1956) The systematic, cytology and genetics of wheat. In: Handbuch der Pflazenzuchtung. (eds. Kapparet, H. and Rudorf, W.) 2: 164-187. Paul Parey, Berlin and Humburg.

ب-٤) مرجع‌دهی به پایان‌نامهٔ کارشناسی ارشد یا دکترا: نام نویسنده، سال، عنوان پایان‌نامه، مقطع تحصیلی، نام دانشگاه، نام شهر، نام کشور.

نمونه فارسی: حسین‌پور، م. (۱۳٦۵) تاکسونومی و بیوسیستماتیک جنس *Cardaria* L. در ایران. پایان‌نامه دکتری، دانشگاه اصفهان، اصفهان.

نمونه مثالی انگلیسی:

- Hassanpour, S. M. (2006) Study of Biosystematic of the genus *Rhamnus*. Ms.c. Thesis, University of Isfahan, Isfahan.

ب-۵) مرجع‌دهی به Patent:

- Suzuki, T., Ohishi, N. and Yagi, K. (2000) Methods of obtaining a composition *9-cis β-Carotene* in high purity. US Patent 6057484.

ب-٦) مرجع‌دهی به همایش‌ها (سمینارها، سمپوزیوم‌ها، کنگره‌ها، میتینگ‌ها و ...): به ترتیب شامل: نام نویسندگان، سال انتشار، عنوان مقاله، دوره و نام همایش، محل برگزاری، شهر، کشور.

عنوان: شامل کوتاه‌ترین عبارتی خواهد بود که بطور کلی گویای محتوای مقاله باشد، خط فارسی عنوان 16 B Lotus Bold و انگلیسی 14 Times New Roman Bold است.

نام و نشانی نگارندگان: مسؤولیت ترتیب نام نگارندگان بر عهدهٔ نویسندهٔ مسؤول خواهد بود. درج شماره مربوط به نشانی هر نگارنده بعد از نام نگارنده به صورت بالا نویس (Superscript) است؛ علاوه بر درج شماره مربوط، یک ستاره برای نام نویسندهٔ مسؤول (Corresponding Author) درج شود. نشانی‌ها به ترتیب و با خط 12 B Lotus Bold و 11 Times New Roman Bold در زیر نام نویسندگان ذکر می‌گردد. نشانی پست الکترونیک مسؤول مکاتبات با خط 10 B Lotus Bold و 10 Times New Roman Bold نوشته شود.

نمونه فارسی

معرفی گونه‌ای جدید در جنس *Centaurea* از ایران
علیرضا اسدی[۱*]، محمد کیانی[۲] و شهریار نظری [۲]
[۱*] دانشگاه اصفهان گروه زیست‌شناسی، [۲] مرکز تحقیقات زیستی
[*] asadi-a.r@ui.ac.ir

چکیده: خط 11 B Lotus و 10 Times New Roman شامل ۱۰۰ تا ۲۵۰ کلمه و بدون هر گونه کلمه اختصاری

کلمات کلیدی: حداکثر حاوی شش کلمه مرتب شده بر اساس حروف الفبا

مقدمه، مشاهدات، مواد و روش‌ها، نتایج، بحث و نتیجه‌گیری، قدردانی و منابع: 13 B Lotus، 11 Times New Roman Abstract و Key words: 12 Times New Roman

عنوان جدول در بالای جدول و عنوان نمودار و شکل در زیر آنها با خط 11 B Lotus و 9 Times New Roman نوشته شود.

نمونه: شکل ۱- ، شکل ۲- ، جدول ۱- ، جدول ۲-

نحوهٔ مرجع‌دهی:

الف) مرجع‌دهی در متن (References in text): در متن به صورت نام نویسنده و یا نویسندگان (بدون نام کوچک) و سال انتشار نوشته شود.

نمونه فارسی: یک نویسنده: (بهارلو، ۱۳۸۸)، **دو نویسنده:** (قاسم‌زاده و اشتری، ۱۳۶۵)، **سه نویسنده و بیشتر:** (شریعت‌مدار و همکاران، ۱۳۷۶)

نمونه انگلیسی: یک نویسنده: (Davis, 1985)، **دو نویسنده:** (Dagan and Zohary, 1970)، **سه نویسنده و بیشتر:** (Johnson *et al.*, 2000)

کلمه *et al.*، بایستی به صورت مورب باشد (این کلمه لاتین است.)

ب) مرجع‌دهی در بخش منابع (References list): فهرست منابع بایستی به ترتیب حروف الفبا مرتب شده ابتدا منابع فارسی و سپس منابع خارجی آورده شود.

ب-۱) مرجع‌دهی به مقاله (Paper): به ترتیب شامل: نام نویسنده یا نویسندگان، سال، عنوان، نام کامل مجله، شمارهٔ مجلد، شمارهٔ صفحات.

ب-۱-۱) مقاله با یک نگارنده

نمونه فارسی: بحرائی، ص. (۱۳۷۵) بررسی گوناگونی ژنتیکی در گونه‌های وحشی (*T. urartu* and *T. boeoticum*) با استفاده از الکتروفورز پروتئین بذر. مجلهٔ بذر و نهال ۲: ۱ تا ۹.

نمونه انگلیسی:

- Noda, K. (1981) C-banding technique for Wheat chromosomes. Wheat Information Service 52(8): 29-31.

معرفی مجله تاکسونومی و بیوسیستماتیک

مجله تاکسونومی و بیوسیستماتیک به صورت فصلنامه و هر سه ماه یکبار توسط دانشگاهِ اصفهان منتشر می‌شود. هدف از انتشار این مجله معرفی آخرین یافته‌های علمی استادان و پژوهشگران در زمینهٔ تاکسونومی و بیوسیستماتیک، به ویژه با تأکید بر خزانهٔ وراثتی جانداران (یوکاریوت‌ها و پروکاریوت‌ها) در ایران می‌باشد.

مجلهٔ علمی - پژوهشی تاکسونومی و بیوسیستماتیک در زمینه‌های معرفی تاکسون‌های جدید، مرور نامگذاری تاکسون‌ها، طبقه‌بندی تاکسون‌ها، معرفی روش‌های جدیدِ ایجاد و تحلیل داده‌ها، ژناکولوژی، ژنتیک جمعیت‌ها و تنوع وراثتی، تنوعِ زیستی و فیلوژنی تاکسون‌ها، مقاله‌های اصیل پژوهشی را به صورت مقاله کامل (Full Paper) و مقاله کوتاه (Short Paper) پس از داوری دقیق به چاپ می‌رساند.

پیش از ارسال مقاله، روش تدوین و نگارش مقاله خود را به دقت با مطالب زیر مطابقت فرمایید.

نکات قابل توجه

۱- در مقاله، قواعد دستور زبان فارسی و رسا بودن جملات مورد توجه ویژه قرار گیرد.

۲- مقالاتی که برای چاپ در این مجله ارسال می‌گردد نباید قبلاً چاپ شده باشد (مگر در شکل خلاصه در گردهمایی‌ها) همچنین نباید به طور همزمان برای چاپ به مجلات دیگر ارایه شده باشد.

۳- مسؤولیت مطالب مندرج در مقاله بر عهده نویسنده یا نویسندگان مقاله است.

٤- مجله در قبول، رد و اصلاح مقاله‌ها آزاد است.

۵- استفاده از مندرجات مجله با ذکر مآخذ آزاد است.

٦- مقاله‌های دریافتی توسط هیأت تحریریه با همکاری متخصصان امر داوری می‌گردد و در صورت تصویب با رعایت نوبت به چاپ می‌رسد. تصمیم نهایی برای چاپ مقاله توسط هیأت تحریریه صورت می‌گیرد.

نحوهٔ تدوین مقاله

۱- مقاله بایستی به زبان فارسی تهیه شود (به استثناء مقاله‌های پژوهشگران خارجی که باید به زبان انگلیسی باشد) و هر مقاله باید یک چکیده به زبان انگلیسی داشته باشد؛ این شرط تا زمانی که زبان مجله تغییر نکرده است پا برجا خواهد بود.

۲- هر مقاله علمی - پژوهشی بایستی به ترتیب دارای قسمت‌های: عنوان، مشخصات مؤلف یا مؤلفان و نشانی دقیق همراه با شماره تلفن و نشانی پست الکترونیک فرستنده (مسؤول مکاتبات)، چکیده فارسی، واژه‌های کلیدی، مقدمه، مواد و روش‌ها، نتایج، بحث، جمع‌بندی، قدردانی، منابع، Abstract و Key words باشد.

۳- تایپ مقاله با نرم‌افزار Microsoft Office Word 2003، به صورت یک رو، در کاغذ A4، با حاشیه‌های متن ۳ سانتی‌متر و به صورت یک ستونی و با فاصله خطوط ۱ سانتی‌متر (single) انجام شود.

٤- مقاله نباید از ۱۵ صفحه چاپ شده در مجله (حدود ٦ هزار کلمه) تجاوز کند.

۵- از درج پاورقی برای بیان توضیحات انگلیسی و فارسی و بالعکس خودداری شود و در صورت نیاز در درون پرانتز و در متن مقاله آورده شود.

٦- شکل‌ها، نمودارها و جدول‌ها شماره‌گذاری شده و به همراه زیرنویس آنها در متن مقاله آورده شود؛ در نرم‌افزار Word، فرمت شکل‌ها در بخش Text Wrapping، به صورت In line with text انتخاب شود؛ از ارسال شکل‌های گروه‌بندی شده (Group) اکیداً خودداری شود؛ نمودارها به صورت دو بعدی و سیاه و سفید طراحی شوند و الزاماً از حالت سه بعدی خارج شوند.

تاکسونومی و بیوسیستماتیک

سال سوم - شماره هشتم - پاییز ١٣٩٠

شماره استاندارد بین‌المللی: ٢٠٠٨-٨٩٠٦

علمی- پژوهشی

صاحب امتیاز: معاونت تحقیقات و فناوری دانشگاه اصفهان

سردبیر: دکتر محمدرضا رحیمی‌نژاد رنجبر دانشگاه اصفهان

مدیر اجرایی: فریبا هادیان (کارشناس ارشد)

ویراستار انگلیسی علمی - تخصصی: فریدون پرویزیان

صفحه‌آرا: فریبا هادیان

صفحه‌آرای تخصصی: فریبا هادیان

ناشر: انتشارات دانشگاه اصفهان

نشانی پستی

اصفهان- خیابان هزار جریب - دانشگاه اصفهان- ساختمان کتابخانه مرکزی- معاونت پژوهشی و فناوری- طبقه دوم
اداره چاپ، انتشارات و مجلات- دفتر مجله تاکسونومی و بیوسیستماتیک- کد پستی: ٨١٧٤٦٧٣٤٤١
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مجلهٔ تاکسونومی و بیوسیستماتیک بر اساس ابلاغیه شماره ۳/۱۱/۹۵۵ مورخ ۱۳۸۸/۰۶/۳۱ کمیسیون بررسی نشریات علمی وزارت علوم تحقیقات و فناوری، دارای درجه **علمی – پژوهشی** و شماره استاندارد بین‌المللی **۲۰۰۸-۸۹۰۶** از سازمان اسناد و کتابخانه ملی جمهوری اسلامی ایران می‌باشد.

متن کامل مجله در پایگاه‌های اطلاع‌رسانی زیر نمایه می‌شود:

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چاپ و لیتوگرافی: انتشارات دانشگاه اصفهان

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تاکسونومی و بیوسیستماتیک

علمی-پژوهشی

سال سوم - شماره هشتم - پاییز ۱۳۹۰