Journal of Taxonomy and Biosystematics has been ranked as a scientific-research journal based on the document number 3/11/955 issued by the Evaluation Committee of Scientific Journals of Research and Technology Ministry in September, 2009; also it has been registered with International Standard Serial Number (ISSN): 2008-8906 by National Library and Archives of Islamic Republic of Iran.

The complete text of this Journal is available at the following sites:

http://uijs.ui.ac.ir/tbj
http://www.magiran.com
http://www.SID.ir
http://www.ISC.gov.ir

Publication and Lithography: University of Isfahan Publications
Publisher: University of Isfahan
Price: 20000 Rials
Number of copies: 1000 Copies
Published in: Winter 2012
Referees to this issue (3rd Year, No. 8, Autumn 2011)

We express our deep gratitude to the following faculty members of the universities and of educational-research Institutes who have co-operated in evaluation and assessment of the articles of this issue of Journal of Taxonomy and Biosystematics (TBJ):

**Dr. Farideh Attar**  
University of Tehran

**Dr. Lili Ghaem Maghami**  
University of Isfahan

**Dr. Farrokh Ghahremaninejad**  
Tarbiat Moallem University

**Dr. Seyed Zabihollah Hosseini**  
University of Yasouj

**Dr. Nastaran Jalilian**  
Kermanshah Agricultural and natural Resources Research Center

**Dr. Shahrokh Kazempour Osaloo**  
Tarbiat Modares University of Tehran

**Dr. Navaz Kharazian**  
University of Shahrkord

**Dr. Hamid Mir Mohammad Sadeghi**  
Isfahan University of Medical Sciences

**Dr. Masoud Ranjbar**  
Bu-Ali sina University

**Dr. Hojjatolah Saeidi**  
University of Isfahan

**Dr. Badraldin Ebrahim Sayed-Tabatabaei**  
Isfahan University of Technology
Contents

- Reporting *Potentilla botschantzeviana* Adylov (Syn: *Potentilla butkovii* var. *botschantzeviana* (Adylov) Soják) (Rosaceae) as a new record species for the flora of Iran
  Marzieh Beygom Faghir, Farideh Attar and Jiri Soják

- A taxonomic revision of the genus *Astragalus* L. (Fabaceae) in Zanjan province, Iran and describing a new species
  Ali Bagherti, Ali Asghar Maassoumi and Farrokh Ghahremaninejad

- Cytological study of *Hordeum bulbosum* L. in Iran
  Hamed Khodayari and Hojjatollah Saeidi

- *Matricaria* L. (Anthemideae, Asteraceae) in Iran: a chemotaxonomic study based on flavonoids
  Majid Sharifi-Tehrani and Nasrollah Ghasemi

- Evaluation of genetic diversity among Iranian pomegranate (*Punica granatum* L.) cultivars, using ISSR and RAPD markers
  Majid Talebi Bedaf, Masoud Bahar, Bahram Sharifnabi and Ahad Yamchi

- Population data on D6S2879 and D6S2806 markers located at HLA-DRB1 region in the Iranians: Identifying the signatures of balancing and directional selection
  Mansoureh Tajadod, Sadeq Vallian Boroujeni and Zahra Fazeli Attar

- Foliar anatomy and micromorphology of *Festuca* L. and its taxonomic applications
  Fatemeh Zarinkamar and Nasrin Eslami Jouyandeh
Reporting *Potentilla botschantzeviana* Adylov
(Syn: *Potentilla butkovii* var. *botschantzeviana* (Adylov) Soják)
(Rosaceae) as a new record species for the flora of Iran

Marzieh Beygom Faghir 1*, Farideh Attar 2 and Jiri Soják 3

1 Department of Biology, Faculty of Sciences, University of Guilan, Rasht, Iran
2 Central Herbarium of Tehran University, School of Biology, University College of Science, P.O. Box: 14155-6455, Tehran, Iran.
3 National Museum in Prague, Department of Botany, Cirkusova 1740 193 00 Praha 9 Horní Pocernice, Czech Republic

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).

* Corresponding Author: marziehfaghir@yahoo.com
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) Sojak (Figure 1)

Perennial; caudex sturdy, multicapital, 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*
Reporting *Potentilla botschantzeviana* Adylov (Syn: *Potentilla butkovii* var. *botschantzeviana* …

Figures 2-6: 1. *P. botschantzeviana*; 2. Radical and cauline leaves; 3. Flowers; 4. Internal view of flowers showing stamens; 5. External view calyx and calyces; 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).

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 Sojak, 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. Butkovi Botsch*. and *P. rvazica* Juz. ex. Botsch. were referred in to section *Lipskyanae* Czevtajbva (Sojak, 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 Sojak (2009) *Potentilla botschantzeviana* is referred to as *P. butkovi var. botschantzeviana* (Adylov) Sojak. This species is the closest relative to *P. butkovi* Botsch (Syn: *P. butkovi* Botsch var. *butkovi*) 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. butkovi* Botsch var. *butkovi* (Sojak, 2004 and 2009).

References


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

Ali Bagheri ¹ Ali Asghar Maassoumi ²* and Farrokh Ghahremaninejad ¹

¹ Department of Biology, Faculty of Sciences, Tarbiat Moallem University, Tehran, Iran
² Research Institute of Forests and Rangelands, Tehran, Iran

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

*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, 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 previously 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. microcephaus* Willd. subsp. *microcephaus* (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. eriosphaerus*, *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, SC, 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 annual 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.
Acknowledgment

We are grateful to Dr. Mohammadreza Rahiminejad Ranjbar (University of Isfahan) for editing the English text.

Reference


Cytological study of *Hordeum bulbosum* L. in Iran

Hamed Khodayari and Hojjatolah Saeidi *

Department of Biology, Faculty of Sciences, University of Isfahan, Isfahan, Iran

**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...
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 divided on length of the longest chromosome, Stebbins, 1971), Asymmetry index (AsI% = 100 × ΣL/ΣTCL; 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).
Cytological study of *Hordeum bulbosum* L. in Iran

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

<table>
<thead>
<tr>
<th>Accession no.</th>
<th>Region</th>
<th>Altitude (m)</th>
<th>Locality</th>
</tr>
</thead>
<tbody>
<tr>
<td>HB2W</td>
<td>W</td>
<td>690</td>
<td>Ilam, Darehshahr, Shahre bastani</td>
</tr>
<tr>
<td>HB3W</td>
<td>W</td>
<td>642</td>
<td>Ilam, Darehshahr, Gharatmalgeh</td>
</tr>
<tr>
<td>HB6W</td>
<td>W</td>
<td>1509</td>
<td>Lorestan, Dorud, Siahkoleh</td>
</tr>
<tr>
<td>HB14W</td>
<td>W</td>
<td>1703</td>
<td>Lorestan, Khoramabad toward Borujerd, Zagheh</td>
</tr>
<tr>
<td>HB22W</td>
<td>W</td>
<td>1305</td>
<td>Ilam, 45 Km Islamabad-e-gharb toward Eivan</td>
</tr>
<tr>
<td>HB23W</td>
<td>W</td>
<td>1580</td>
<td>Kermanshah, 40 Km Eivan toward Islamabad-e-gharb</td>
</tr>
<tr>
<td>HB24W</td>
<td>W</td>
<td>1292</td>
<td>Ilam, Darehshahr toward Ilam, Mishkhas</td>
</tr>
<tr>
<td>HB30SW</td>
<td>SW</td>
<td>2100</td>
<td>Chaharmahal-va-Bakhtyari, Felard, Aboueshagh, Kahriz</td>
</tr>
<tr>
<td>HB73SW</td>
<td>SW</td>
<td>1690</td>
<td>Fars, Eghlid to Marvdasht, Dorudzan</td>
</tr>
<tr>
<td>HB76SW</td>
<td>SW</td>
<td>1702</td>
<td>Fars, Shiraz, Roknabad</td>
</tr>
<tr>
<td>HB77SW</td>
<td>SW</td>
<td>1975</td>
<td>Fars, Shiraz toward Kazeroun, Hoseinch</td>
</tr>
<tr>
<td>HB79SW</td>
<td>SW</td>
<td>2051</td>
<td>Fars, Shiaz toward Kazerun, Dashe Arjan</td>
</tr>
<tr>
<td>HB81SW</td>
<td>SW</td>
<td>1050</td>
<td>Fars, Noorabad-e-Mamasany</td>
</tr>
<tr>
<td>HB84SW</td>
<td>SW</td>
<td>2050</td>
<td>Kohgilui-va-Boyerahmad, Babameidan toward Yasooj</td>
</tr>
<tr>
<td>HB87SW</td>
<td>SW</td>
<td>1695</td>
<td>Kohgilui-va-Boyerahmad, 25 Km Yasooj toward Isfahan</td>
</tr>
<tr>
<td>HB90SW</td>
<td>SW</td>
<td>1752</td>
<td>Chaharmahal-va-Bakhtyari, Broojen toward Yasooj, Felard</td>
</tr>
<tr>
<td>HB91SW</td>
<td>SW</td>
<td>2240</td>
<td>Chaharmahal va Bakhtyari, Broojen toward Yasooj</td>
</tr>
<tr>
<td>HB95N</td>
<td>N</td>
<td>1640</td>
<td>Tehran, Boomehen</td>
</tr>
<tr>
<td>HB105NE</td>
<td>NE</td>
<td>1775</td>
<td>Golestan Azadshahr toward Shahrood, Khoshyeilagh</td>
</tr>
<tr>
<td>HB106NE</td>
<td>NE</td>
<td>700</td>
<td>Golestan, National Park of Golestan</td>
</tr>
<tr>
<td>HB109NE</td>
<td>NE</td>
<td>993</td>
<td>Khorasane Shomali, Bojnourd, Baba aman park</td>
</tr>
<tr>
<td>HB202W</td>
<td>W</td>
<td>1193</td>
<td>Ilam, Darehshahr toward Ilam, Pakal-e-Gerab</td>
</tr>
<tr>
<td>HB207W</td>
<td>W</td>
<td>1360</td>
<td>Kermanshah, Kermanshah toward Kamyaran, Vermenje</td>
</tr>
<tr>
<td>HB208W</td>
<td>W</td>
<td>1741</td>
<td>Kurdistan, Kamyaran toward Sanandaj, Morvarid</td>
</tr>
<tr>
<td>HB209W</td>
<td>W</td>
<td>1581</td>
<td>Kurdistan, Sanandaj</td>
</tr>
<tr>
<td>HB211W</td>
<td>W</td>
<td>1257</td>
<td>Kurdistan, 15 Km Sarvabad toward Sanandaj</td>
</tr>
<tr>
<td>HB212W</td>
<td>W</td>
<td>1222</td>
<td>Kurdistan, Sarvabad</td>
</tr>
<tr>
<td>HB213W</td>
<td>W</td>
<td>1249</td>
<td>Kurdistan, around of Zarivar lake</td>
</tr>
<tr>
<td>HB215W</td>
<td>W</td>
<td>1587</td>
<td>Kurdistan, Marivan toward Saghez, Sarshio</td>
</tr>
<tr>
<td>HB216W</td>
<td>W</td>
<td>1423</td>
<td>Azarbaijane Gharbi, Boukan, Kanitoomar</td>
</tr>
<tr>
<td>HB217NW</td>
<td>NW</td>
<td>1822</td>
<td>Azarbaijane Gharbi, Boukan, Mohabad, Gharehbolagh</td>
</tr>
<tr>
<td>HB221NW</td>
<td>NW</td>
<td>1537</td>
<td>Gilan, Astara, Heiran</td>
</tr>
</tbody>
</table>

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 autoploidy 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 Bakhtyari province) to 15.04 μm (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.

<table>
<thead>
<tr>
<th>Population</th>
<th>2n</th>
<th>TCL</th>
<th>MCL ± SE</th>
<th>TF%</th>
<th>S%</th>
<th>R</th>
<th>AsI%</th>
<th>Karyotype formulae</th>
</tr>
</thead>
<tbody>
<tr>
<td>HB2W</td>
<td>28</td>
<td>63.68</td>
<td>9.1±1.177</td>
<td>45.1</td>
<td>68.56</td>
<td>1.23</td>
<td>54.89</td>
<td>2M + 5m*</td>
</tr>
<tr>
<td>HB3W</td>
<td>28</td>
<td>92.53</td>
<td>13.22±1.56</td>
<td>41.22</td>
<td>70.61</td>
<td>1.45</td>
<td>58.77</td>
<td>6m* + 1sm</td>
</tr>
<tr>
<td>HB6W</td>
<td>28</td>
<td>53.79</td>
<td>7.68 ±0.74</td>
<td>41.68</td>
<td>76.57</td>
<td>1.45</td>
<td>58.31</td>
<td>6m* + 1sm</td>
</tr>
<tr>
<td>HB14W</td>
<td>28</td>
<td>73.2</td>
<td>10.5±1.341</td>
<td>44.03</td>
<td>70.77</td>
<td>1.33</td>
<td>55.96</td>
<td>1M + 4m* +2sm</td>
</tr>
<tr>
<td>HB22W</td>
<td>28</td>
<td>76.84</td>
<td>10.1±1.288</td>
<td>44.15</td>
<td>73.92</td>
<td>1.28</td>
<td>55.84</td>
<td>2M + 5m*</td>
</tr>
<tr>
<td>HB23W</td>
<td>28</td>
<td>84.03</td>
<td>12.0±1.235</td>
<td>44.34</td>
<td>77.31</td>
<td>1.27</td>
<td>55.658</td>
<td>7m*</td>
</tr>
<tr>
<td>HB24W</td>
<td>28</td>
<td>67.6</td>
<td>9.66±0.933</td>
<td>42.42</td>
<td>76.88</td>
<td>1.39</td>
<td>57.573</td>
<td>1M* + 5m + 1sm</td>
</tr>
<tr>
<td>HB30SW</td>
<td>28</td>
<td>81.48</td>
<td>11.64±1.01</td>
<td>42.26</td>
<td>79.12</td>
<td>1.397</td>
<td>57.731</td>
<td>6m* + 1sm</td>
</tr>
<tr>
<td>HB73SW</td>
<td>28</td>
<td>55.97</td>
<td>7.1±1.261</td>
<td>43.79</td>
<td>59.3</td>
<td>1.297</td>
<td>56.208</td>
<td>1M + 5m* + 1sm</td>
</tr>
<tr>
<td>HB76SW</td>
<td>28</td>
<td>76.28</td>
<td>10.9±1.5</td>
<td>40.53</td>
<td>67.12</td>
<td>1.53</td>
<td>59.465</td>
<td>2M* + 4m + 1sm</td>
</tr>
<tr>
<td>HB77SW</td>
<td>28</td>
<td>54.38</td>
<td>7.77±1.055</td>
<td>41.28</td>
<td>65.29</td>
<td>1.467</td>
<td>58.716</td>
<td>1M + 5m + 1sm</td>
</tr>
<tr>
<td>HB79SW</td>
<td>28</td>
<td>74.8</td>
<td>10.69±1.344</td>
<td>42.78</td>
<td>67.01</td>
<td>1.397</td>
<td>57.22</td>
<td>1M* + 5m + 1sm</td>
</tr>
<tr>
<td>HB81SW</td>
<td>28</td>
<td>59.26</td>
<td>8.47±0.908</td>
<td>39.7</td>
<td>73.52</td>
<td>1.655</td>
<td>60.3</td>
<td>5m* + 1sm + 1st</td>
</tr>
<tr>
<td>HB84SW</td>
<td>28</td>
<td>57</td>
<td>8.14±1.234</td>
<td>38.57</td>
<td>63.35</td>
<td>1.616</td>
<td>61.42</td>
<td>5m* + 2sm</td>
</tr>
<tr>
<td>HB87SW</td>
<td>28</td>
<td>57.79</td>
<td>8.26±0.94</td>
<td>41.18</td>
<td>71.48</td>
<td>1.56</td>
<td>58.81</td>
<td>5m* + 2sm</td>
</tr>
<tr>
<td>HB90SW</td>
<td>28</td>
<td>44.88</td>
<td>6.41±0.7</td>
<td>43.6</td>
<td>71.21</td>
<td>1.345</td>
<td>56.39</td>
<td>2M + 4m + 1sm</td>
</tr>
<tr>
<td>HB91SW</td>
<td>28</td>
<td>51.26</td>
<td>7.32±0.85</td>
<td>41.02</td>
<td>72.35</td>
<td>1.541</td>
<td>58.97</td>
<td>1M + 5m* + 1sm</td>
</tr>
<tr>
<td>HB95N</td>
<td>28</td>
<td>76.73</td>
<td>10.96±1.278</td>
<td>42.42</td>
<td>74.43</td>
<td>1.395</td>
<td>57.578</td>
<td>6m* + 1sm</td>
</tr>
<tr>
<td>HB105NE</td>
<td>28</td>
<td>55.7</td>
<td>7.96±1.04</td>
<td>40.68</td>
<td>68.6</td>
<td>1.491</td>
<td>59.317</td>
<td>6m* + 1sm</td>
</tr>
<tr>
<td>HB106NE</td>
<td>28</td>
<td>63.02</td>
<td>9.00±2.41</td>
<td>37.81</td>
<td>64.17</td>
<td>1.687</td>
<td>62.186</td>
<td>4m + 3sm*</td>
</tr>
<tr>
<td>HB109NE</td>
<td>28</td>
<td>59.77</td>
<td>8.53±1.147</td>
<td>40.48</td>
<td>68.65</td>
<td>1.543</td>
<td>59.511</td>
<td>6m* + 1sm</td>
</tr>
<tr>
<td>HB202W</td>
<td>28</td>
<td>53.08</td>
<td>7.58±0.79</td>
<td>39.44</td>
<td>70.97</td>
<td>1.57</td>
<td>60.55</td>
<td>1M + 3m + 3sm*</td>
</tr>
<tr>
<td>HB207W</td>
<td>28</td>
<td>70.06</td>
<td>10.01±1.03</td>
<td>40.1</td>
<td>74.32</td>
<td>1.53</td>
<td>59.006</td>
<td>1M* + 4m + 2sm</td>
</tr>
<tr>
<td>HB208W</td>
<td>28</td>
<td>85.41</td>
<td>12.20±1.55</td>
<td>40.86</td>
<td>71.03</td>
<td>1.493</td>
<td>59.138</td>
<td>1M + 5m + 1sm</td>
</tr>
<tr>
<td>HB209W</td>
<td>28</td>
<td>48.86</td>
<td>6.98±0.69</td>
<td>41.17</td>
<td>76.41</td>
<td>1.533</td>
<td>58.821</td>
<td>1M + 4m* + 2sm</td>
</tr>
<tr>
<td>HB211W</td>
<td>28</td>
<td>59.86</td>
<td>8.55±1.74</td>
<td>38.79</td>
<td>51.3</td>
<td>1.6</td>
<td>61.209</td>
<td>5m* + 2sm</td>
</tr>
<tr>
<td>HB212W</td>
<td>28</td>
<td>53.45</td>
<td>7.64±0.8</td>
<td>42.37</td>
<td>75.57</td>
<td>1.467</td>
<td>57.623</td>
<td>5m* + 2sm</td>
</tr>
<tr>
<td>HB213W</td>
<td>28</td>
<td>85.61</td>
<td>12.23±1.27</td>
<td>42.42</td>
<td>73.33</td>
<td>1.438</td>
<td>57.575</td>
<td>1M + 5m* + 1sm</td>
</tr>
<tr>
<td>HB215W</td>
<td>28</td>
<td>67.07</td>
<td>9.58±2.1</td>
<td>40.15</td>
<td>48.58</td>
<td>1.565</td>
<td>59.847</td>
<td>1M* + 4m + 2sm</td>
</tr>
<tr>
<td>HB216W</td>
<td>28</td>
<td>73.01</td>
<td>10.43±1.4</td>
<td>42.5</td>
<td>69.15</td>
<td>1.366</td>
<td>57.498</td>
<td>5m* + 2sm</td>
</tr>
<tr>
<td>HB217W</td>
<td>28</td>
<td>66.91</td>
<td>9.56±0.85</td>
<td>41.72</td>
<td>81.32</td>
<td>1.432</td>
<td>58.272</td>
<td>1M* + 5m + 1sm</td>
</tr>
<tr>
<td>HB221W</td>
<td>28</td>
<td>62.61</td>
<td>8.94±0.91</td>
<td>37.93</td>
<td>72.53</td>
<td>1.683</td>
<td>62.066</td>
<td>1M* + 3m + 3sm</td>
</tr>
</tbody>
</table>

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).
Cytological study of *Hordeum bulbosum* L. in Iran

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 Satellite chromosomes 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 Hordeum bulbosum (Rajhathy et al., 1964; Vosa, 1976; Coucoli and Symeonidis, 1980; Chin, 1941; Linde-Laursen et al., 1990; Morrison, 1959) 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 Hordeum 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).

References


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

Majid Sharifi-Tehrani *1 and Nasrollah Ghasemi 2

1 Department of Biology, Department of Sciences, University of Shahrekord, Shahrekord, Iran
2 Department of Pharmacognosy, Faculty of Pharmacy and Pharmaceutical Sciences, Isfahan University of Medical Sciences, Isfahan, Iran

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 skeleton, 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 Anthemideae’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. tzelelevii 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
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 taxon.

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 *Matricaria* accessions used in this study |
|----------------------|-----------------|-----------------|
| Species   | Sample 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 Brimm 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 Ghaemi 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*
2. Receptacle bare
3. Achenes with two resin glands at the top of the abaxial face...................... *Tripleurospermum*
4. Achenes distinctly coronate.................................................................................. *Microcephala*
5. Achenes ecoronate, only achenes of marginal ligulate florets (if present) sometimes coronate................................................................. *Matricaria*
6. Receptacle with chaffy bracts
7. Achenes compressed, laterally winged............................................................... *Anacyclus*
8. Achenes not winged as above
9. Capituls with ligulate florets in margins, disk florets in center
10. Middle nerve of chaffy bracts excurrent.......................................................... *Anthemis*
11. Middle nerve of chaffy bracts non excurrent.................................................. *Achillea*
12. Capituls without ligulate florets
13. Capituls in compound cymes.................................................................................... *Handelia*
14. 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,
Matricaria \textit{L.} (Anthemideae, Asteraceae) in Iran: a chemotaxonomic study based on flavonoids

and the grouping of the specimens did not suffer chaining.

Figure 1: Flavonoid spots in 2D-TLC of \textit{M. aurea} (A: all parts of plant) and \textit{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 \textit{M. aurea} were also well clustered. The only misplaced population was Au02M which was an outlier in a clade containing South Zagros Populations. Au02M was from West of Zagros (Dehdez) and could be interpreted as an outlier because Au02H (same population, but only the capitula) was grouped with other samples from West of Zagros.

Table 2: Flavonoid spots in populations of \textit{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 \textit{M. aurea} populations.

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

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

Figure 2: Results 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 Minimum length 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.

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 benzodiazepine receptor ligand with anxiolytic effects (Viola et al., 1995).
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* was both 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 skeletons from *M. aurea* and *M. recutita*, purified and determined in this study. Skeleton number corresponds to numbers in Figure 5

<table>
<thead>
<tr>
<th>Skeleton</th>
<th>Class</th>
<th>Skeleton details</th>
<th>Species</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Flavone</td>
<td>5-OH; oxygenation on carbon 6; ortho-di-hydroxyl on ring B</td>
<td><em>M. aurea</em></td>
</tr>
<tr>
<td>2</td>
<td>Flavone</td>
<td>ortho-di-hydroxyls on rings A and B</td>
<td><em>M. aurea</em></td>
</tr>
<tr>
<td>3</td>
<td>Flavone</td>
<td>ortho-di-hydroxyl on ring B</td>
<td><em>M. aurea</em></td>
</tr>
<tr>
<td>4</td>
<td>Flavonol</td>
<td>5-OH; oxygenation on carbon 6; two ortho-di-hydroxyls on rings A and B</td>
<td><em>M. recutita</em></td>
</tr>
<tr>
<td>5</td>
<td>Flavonol</td>
<td>5-OH; oxygenation on carbon 6; 7-OH</td>
<td><em>M. recutita</em></td>
</tr>
<tr>
<td>6</td>
<td>Flavone</td>
<td>5-OH; oxygenation on carbon 6</td>
<td><em>M. recutita</em></td>
</tr>
<tr>
<td>7</td>
<td>Flavone</td>
<td>ortho-di-hydroxyl on ring A</td>
<td><em>M. recutita</em></td>
</tr>
<tr>
<td>8</td>
<td>Flavone</td>
<td>5-OH; ortho-di-hydroxyl on ring A</td>
<td><em>M. recutita</em></td>
</tr>
<tr>
<td>9</td>
<td>Flavone</td>
<td>5-OH; ortho-di-hydroxyl on ring A; oxygenation on carbon 6</td>
<td><em>M. recutita</em></td>
</tr>
<tr>
<td>10</td>
<td>Flavone</td>
<td>5-OH; prenyl group on carbon 6</td>
<td><em>M. recutita</em></td>
</tr>
<tr>
<td>11</td>
<td>Flavone</td>
<td>5-OH</td>
<td><em>M. recutita</em></td>
</tr>
<tr>
<td>12</td>
<td>Flavone</td>
<td>5-OH; two ortho-di-hydroxyls on rings A and B</td>
<td><em>M. recutita</em></td>
</tr>
</tbody>
</table>
Figure 5: Flavonoid skeletons 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 correspond 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 resolved by using different kinds of data from all genera in this tribe, through more detailed studies.

**Acknowledgment**

The authors wish to thank the office of graduate studies of the University of Isfahan for their support. Special thanks to the directors of herbaria TARI and HTU for making the herbarium facilities available to this study. With thanks to Stephan J. Darbyshire for reading the early manuscript and making useful suggestions.

**References**


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

Majid Talebi Bedaf 1*, Masoud Bahar 1, Bahram Sharifnabi 1 and Ahad Yamchi 2

1 Department of Agricultural Biotechnology, College of Agriculture, Isfahan University of Technology, Isfahan, Iran, 84156-83111.
2 Gorgan University of Agricultural Sciences and Natural Resources, Gorgan, Iran

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: \textit{Punica granatum}, genetic diversity, pomegranate, RAPD, ISSR

Introduction

Pomegranate (\textit{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.
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 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)_n 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

<table>
<thead>
<tr>
<th>No.</th>
<th>Cultivar codes</th>
<th>Cultivar names</th>
<th>Acidity</th>
<th>Origin</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>PGpsy</td>
<td>Poust syah yazdi</td>
<td>Sweet</td>
<td>Yazd</td>
</tr>
<tr>
<td>2</td>
<td>PGgsh</td>
<td>Goroch shahvar</td>
<td>Sweet</td>
<td>Yazd</td>
</tr>
<tr>
<td>3</td>
<td>PGIl</td>
<td>Tab va larz</td>
<td>Sweet</td>
<td>Yazd</td>
</tr>
<tr>
<td>4</td>
<td>PGBl</td>
<td>Bihasteh ladiz</td>
<td>Sweet</td>
<td>Systan va Balouchestan</td>
</tr>
<tr>
<td>5</td>
<td>PGbl</td>
<td>Asali sarvestan</td>
<td>Sweet</td>
<td>Fars</td>
</tr>
<tr>
<td>6</td>
<td>PGGhr</td>
<td>Golabi hasteh riz</td>
<td>Sour-sweet</td>
<td>Systan va Balouchestan</td>
</tr>
<tr>
<td>7</td>
<td>PGna</td>
<td>Nabati ardakan</td>
<td>Sour-sweet</td>
<td>Yazd</td>
</tr>
<tr>
<td>8</td>
<td>PGdhr</td>
<td>Dokhtar hamoumi varamin</td>
<td>Sour-sweet</td>
<td>Tehran</td>
</tr>
<tr>
<td>9</td>
<td>PGGgn</td>
<td>Galu gandeh neiriz</td>
<td>Sour-sweet</td>
<td>Fars</td>
</tr>
<tr>
<td>10</td>
<td>PGakh</td>
<td>Amaneh khatouni</td>
<td>Sour-sweet</td>
<td>Yazd</td>
</tr>
<tr>
<td>11</td>
<td>PGTg</td>
<td>Togh gardan</td>
<td>Sour-sweet</td>
<td>Yazd</td>
</tr>
<tr>
<td>12</td>
<td>PGDa</td>
<td>Dom anbarouti</td>
<td>Sour</td>
<td>Khorasan</td>
</tr>
<tr>
<td>13</td>
<td>PGpa</td>
<td>Panjeh arous khafir</td>
<td>Sour</td>
<td>Fars</td>
</tr>
<tr>
<td>14</td>
<td>PGapgh</td>
<td>Ardestani poust ghermez</td>
<td>Sour</td>
<td>Esfahan</td>
</tr>
<tr>
<td>15</td>
<td>PGVns</td>
<td>Vaashi narak sarvestan</td>
<td>Sour</td>
<td>Fars</td>
</tr>
<tr>
<td>16</td>
<td>PGDs</td>
<td>Dabbei sarjangal</td>
<td>Sour</td>
<td>Kerman</td>
</tr>
<tr>
<td>17</td>
<td>PGgf</td>
<td>Golnar fars</td>
<td>Ornamental</td>
<td>Fars</td>
</tr>
<tr>
<td>18</td>
<td>PGKgs</td>
<td>Kaleh gavi sangan</td>
<td>Sour-sweet</td>
<td>Systan va Balouchestan</td>
</tr>
<tr>
<td>19</td>
<td>PGSh</td>
<td>Shahvar shirin</td>
<td>Sweet</td>
<td>Yazd</td>
</tr>
<tr>
<td>20</td>
<td>PGHm</td>
<td>Hasibi mehriz</td>
<td>Sour-sweet</td>
<td>Yazd</td>
</tr>
<tr>
<td>21</td>
<td>PGbs</td>
<td>Bihasteh sangan</td>
<td>Sweet</td>
<td>Systan va Balouchestan</td>
</tr>
<tr>
<td>22</td>
<td>PGapg</td>
<td>Ardestani poust sefid</td>
<td>Sweet</td>
<td>Esfahan</td>
</tr>
<tr>
<td>23</td>
<td>PGtmz</td>
<td>Torsh mamuli zabol</td>
<td>Sour</td>
<td>Systan va Balouchestan</td>
</tr>
<tr>
<td>24</td>
<td>PGSb</td>
<td>Sabi bam</td>
<td>Sour</td>
<td>Kerman</td>
</tr>
</tbody>
</table>

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 MgCl2, 500mM KCl, pH 8.3 (20°C)], 0.5mM MgCl2, 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 94°C for 2 min followed by 40 cycles of denaturation at 92°C for 1 min, primer annealing at 35°C for 1 min and extension at 72°C for 2 min, followed by an extended elongation step at 72°C 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 MgCl2, 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 94°C for 1 cycle, followed by 30 s at 94°C, 45 s at
52°C, and 2 min at 72°C for 30 cycle, and 5 min at 72°C 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 $\text{PIC} = 1 - \sum P_i^2$, where $P_i$ 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
Evaluation of genetic diversity among Iranian pomegranate (*Punica granatum* L.) cultivars... 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 ("Dabbeisarjangal" 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.

<table>
<thead>
<tr>
<th>Marker name</th>
<th>Primer name</th>
<th>Sequence (3'-5')</th>
<th>Total band number</th>
<th>Number of polymorphic bands</th>
<th>Polymorphic bands (%)</th>
<th>PIC value</th>
</tr>
</thead>
<tbody>
<tr>
<td>RAPD</td>
<td>OPAC11</td>
<td>CCTGGGTCAG</td>
<td>12</td>
<td>3</td>
<td>25</td>
<td>0.188</td>
</tr>
<tr>
<td></td>
<td>OPAD02</td>
<td>CTTACCGCCTG</td>
<td>8</td>
<td>5</td>
<td>62.5</td>
<td>0.373</td>
</tr>
<tr>
<td></td>
<td>OPAD04</td>
<td>GTAGGCCCTCA</td>
<td>8</td>
<td>2</td>
<td>25</td>
<td>0.246</td>
</tr>
<tr>
<td></td>
<td>OPAD13</td>
<td>GGTTCTCTCTG</td>
<td>5</td>
<td>2</td>
<td>40</td>
<td>0.172</td>
</tr>
<tr>
<td></td>
<td>OPAD15</td>
<td>TTTGCCGCCGT</td>
<td>6</td>
<td>1</td>
<td>16.67</td>
<td>0.014</td>
</tr>
<tr>
<td></td>
<td>OPAD16</td>
<td>ACGGGCCGTC</td>
<td>10</td>
<td>2</td>
<td>20</td>
<td>0.110</td>
</tr>
<tr>
<td></td>
<td>OPAE10</td>
<td>CTGAAGCGCA</td>
<td>11</td>
<td>2</td>
<td>18.18</td>
<td>0.081</td>
</tr>
<tr>
<td></td>
<td>OPB10</td>
<td>CTGCTGGGAC</td>
<td>12</td>
<td>1</td>
<td>8.33</td>
<td>0.013</td>
</tr>
<tr>
<td></td>
<td>OPP02</td>
<td>TCGGACACGCA</td>
<td>10</td>
<td>2</td>
<td>20</td>
<td>0.135</td>
</tr>
<tr>
<td></td>
<td>OPP14</td>
<td>CCAAGCGGACG</td>
<td>12</td>
<td>2</td>
<td>16.67</td>
<td>0.096</td>
</tr>
<tr>
<td></td>
<td>MG01</td>
<td>AGCCCGCGAG</td>
<td>14</td>
<td>2</td>
<td>14.29</td>
<td>0.012</td>
</tr>
<tr>
<td></td>
<td>MG11</td>
<td>AGGAGCTGCC</td>
<td>16</td>
<td>2</td>
<td>12.5</td>
<td>0.024</td>
</tr>
<tr>
<td></td>
<td>MG16</td>
<td>GAAAGAACCGC</td>
<td>7</td>
<td>3</td>
<td>42.86</td>
<td>0.200</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td></td>
<td>131</td>
<td>29</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td></td>
<td>10.08</td>
<td>2.23</td>
<td>22.14</td>
<td>0.128</td>
</tr>
<tr>
<td>ISSR</td>
<td>LK7</td>
<td>5'-CCA(CT)6-3'</td>
<td>32</td>
<td>10</td>
<td>31.25</td>
<td>0.256</td>
</tr>
<tr>
<td></td>
<td>ISSR5</td>
<td>5'-CCA(AG)6T-3'</td>
<td>25</td>
<td>8</td>
<td>32</td>
<td>0.099</td>
</tr>
<tr>
<td></td>
<td>ISSR6</td>
<td>5'-CAAGC-3'</td>
<td>32</td>
<td>8</td>
<td>32</td>
<td>0.099</td>
</tr>
<tr>
<td></td>
<td>ISSR10</td>
<td>5'-GTG6A-3'</td>
<td>38</td>
<td>11</td>
<td>28.94</td>
<td>0.106</td>
</tr>
<tr>
<td></td>
<td>ISSR12</td>
<td>5'-AG6YT-3'</td>
<td>24</td>
<td>6</td>
<td>25</td>
<td>0.156</td>
</tr>
<tr>
<td></td>
<td>ISSR11</td>
<td>5'-AG6YT-3'</td>
<td>26</td>
<td>15</td>
<td>57.69</td>
<td>0.257</td>
</tr>
<tr>
<td></td>
<td>ISSR12</td>
<td>5'-AG6YT-3'</td>
<td>28</td>
<td>14</td>
<td>50</td>
<td>0.103</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td></td>
<td>173</td>
<td>64</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td></td>
<td>28.83</td>
<td>10.67</td>
<td>36.99</td>
<td>0.163</td>
</tr>
</tbody>
</table>

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 sarjangular" and "Kaleh gavi sangan", while all remaining cultivars formed the fourth group. "Vahshi narak sarvestan", "Dabbei sarjangular" 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 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.

Acknowledgments

We are grateful to Seyed Ziaaddin Tabatabaei-Ardakani from Agricultural Research Center of Yazd province for providing pomegranate cultivars and all of the colleagues in biotechnology center of IUT.

References

Evaluation of genetic diversity among Iranian pomegranate (Punica granatum L.) cultivars ...


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

Mansoureh Tajadod 1, Sadeq Vallian Boroujeni *1 and Zahra Fazeli Attar 2

1 Department of Biology, Faculty of Sciences, University of Isfahan, Isfahan, Iran
2 Department of Genetics, Faculty of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran

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...
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/xslegi.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 from 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 homozgyosity 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 homozgyosity (F), computed as the sum of the squared allele frequencies, the expected homozgyosity and the normalized deviation of the homozgyosity (Fnd), differences between the observed homozgyosity and expected homozgyosity, were estimated. The observed homozgyosity (F) is computed on the basis of the actual data. In Ewens-Watterson test, the F value is compared to the expected homozgyosity (\(\hat{F}\)) 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 microsatellite 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.

<table>
<thead>
<tr>
<th>Microsatellite marker</th>
<th>na</th>
<th>ne</th>
<th>I</th>
</tr>
</thead>
<tbody>
<tr>
<td>D6S2879</td>
<td>4.0000</td>
<td>2.2701</td>
<td>1.0372</td>
</tr>
<tr>
<td>D6S2806</td>
<td>6.0000</td>
<td>1.6547</td>
<td>0.8601</td>
</tr>
</tbody>
</table>

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.

<table>
<thead>
<tr>
<th>MICROSATellite MARKER</th>
<th>K</th>
<th>F</th>
<th>L95</th>
<th>U95</th>
</tr>
</thead>
<tbody>
<tr>
<td>D6S2879</td>
<td>4</td>
<td>0.4405</td>
<td>0.3167</td>
<td>0.9331</td>
</tr>
<tr>
<td>D6S2806</td>
<td>6</td>
<td>0.6043</td>
<td>0.2358</td>
<td>0.8087</td>
</tr>
</tbody>
</table>

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.

<table>
<thead>
<tr>
<th>Marker</th>
<th>F'</th>
<th>ñF'</th>
<th>Fnd</th>
<th>p-value of F</th>
</tr>
</thead>
<tbody>
<tr>
<td>MHC microsatellite</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>D6S2879</td>
<td>0.4405</td>
<td>0.5931</td>
<td>-0.8449</td>
<td>0.2311</td>
</tr>
<tr>
<td>D6S2806</td>
<td>0.6043</td>
<td>0.4523</td>
<td>0.9904</td>
<td>0.8251</td>
</tr>
<tr>
<td>LPL</td>
<td>0.2001</td>
<td>0.5463</td>
<td>-1.9338</td>
<td>0.0000</td>
</tr>
<tr>
<td>F13B</td>
<td>0.1925</td>
<td>0.4853</td>
<td>-1.7624</td>
<td>0.0008</td>
</tr>
<tr>
<td>HUMvWA</td>
<td>0.1844</td>
<td>0.3865</td>
<td>-1.4042</td>
<td>0.0142</td>
</tr>
<tr>
<td>HPRKB</td>
<td>0.2032</td>
<td>0.4368</td>
<td>-1.4930</td>
<td>0.0083</td>
</tr>
<tr>
<td>HUMTHPO</td>
<td>0.2125</td>
<td>0.4665</td>
<td>-1.5131</td>
<td>0.0093</td>
</tr>
<tr>
<td>HUMTH01</td>
<td>0.2140</td>
<td>0.5142</td>
<td>-1.7051</td>
<td>0.0021</td>
</tr>
<tr>
<td>HUMFES</td>
<td>0.2201</td>
<td>0.5142</td>
<td>-1.6706</td>
<td>0.0029</td>
</tr>
<tr>
<td>D16S539</td>
<td>0.2118</td>
<td>0.4853</td>
<td>-1.6464</td>
<td>0.0028</td>
</tr>
<tr>
<td>F13A01</td>
<td>0.2096</td>
<td>0.5466</td>
<td>-1.881</td>
<td>0.0001</td>
</tr>
<tr>
<td>CSF1PO</td>
<td>0.1986</td>
<td>0.4368</td>
<td>-1.5227</td>
<td>0.0058</td>
</tr>
</tbody>
</table>

F: the observed homozygosity; ñF: 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.

<table>
<thead>
<tr>
<th>Test</th>
<th>TPM</th>
<th>SMM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Expected</td>
<td>Observed</td>
</tr>
<tr>
<td>Sign Test: Number of loci with heterozygosity excess</td>
<td>7.12</td>
<td>11</td>
</tr>
<tr>
<td>Wilcoxon Test: Probability of heterozygosity excess</td>
<td>0.01709</td>
<td>0.02124</td>
</tr>
</tbody>
</table>

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 heterozygosity of studied markers implied that balancing selection probably counteract genetic bottleneck in the Iranian populations. Two studied MHC microsatellite markers, D6S2806 and D6S2879, showed low effective number of alleles (Table 1). It is likely that low gene diversity of these two MHC microsatellite 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 (Ghabaei et al., 2009). A significant positive association with AML for the HLA-DRB1*11 allele 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 previous 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.

References:
Population data on D6S2879 and D6S2806 markers located at HLA-DRB1 region in the Iranians …


Khosravi, F., Amirzargar, A., Sarafnejad, A., Nicknam, M. H., Alimoghdam, K., Dianat, S., Solgi, G. and


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

Fatemeh Zarinkamar * and Nasrin Eslami Jouyandeh

Department of Biology, Faculty of Biological Sciences, Tarbiat Modares University, Tehran, Iran

**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 repetitions. 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. gigantean* and *F. drymeia* were positioned in clusters two.

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

**Introduction**

The genus *Festuca* L. contains 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).

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; Borrell, 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. akhania Tzvelev 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>
<thead>
<tr>
<th>Species</th>
<th>Morphological studied (Morphology and anatomy)</th>
<th>Voucher specimen</th>
</tr>
</thead>
<tbody>
<tr>
<td>F. akhania</td>
<td>Morphology</td>
<td>Golestan, Golestan Forest, 1600 m, Bagheri (TARI, 90264)</td>
</tr>
<tr>
<td>F. arundinacea</td>
<td>Micromorphology and Anatomy</td>
<td>Yazd, 2700-2900 m, Mozaffarian (TARI, 77545)</td>
</tr>
<tr>
<td>F. drymeia</td>
<td>Morphology and Anatomy</td>
<td>Gorgan, Loo forest, Bagheri (TARI, 90267)</td>
</tr>
<tr>
<td>F. drymia</td>
<td>Morphology and Anatomy</td>
<td>Mazandaran, Noshahr,500 m (TARI, 33471)</td>
</tr>
<tr>
<td>F. elwendiana</td>
<td>Morphology</td>
<td>Hamedan, Alvand, 2300 m, Kelovande &amp; Baghere (TARI, 90265)</td>
</tr>
<tr>
<td>F. elwendiana</td>
<td>Morphology</td>
<td>Hamedan, Ganjnameh, 2100 m, Assadi &amp; Mozaffarian (TARI, 36729)</td>
</tr>
<tr>
<td>F. gigantea</td>
<td>Morphology and Anatomy</td>
<td>Mazandaran, Tonekabon,1400 m, Hamzehee &amp; Asree (TARI, 71073)</td>
</tr>
<tr>
<td>F. heterophylla</td>
<td>Morphology</td>
<td>Tehran,Lar,2420-2550 m, Wendelbo &amp; Assadi (TARI, 13348)</td>
</tr>
<tr>
<td>F. heterophylla</td>
<td>Morphology</td>
<td>Azarbaijan, 45 KM Sabalan, 2900 m, Mozaffarian (TARI, 9319)</td>
</tr>
<tr>
<td>F. sulcata</td>
<td>Morphology</td>
<td>Azarbaijan, Arasbaran, 2500 m, Assadi (TARI, 23910)</td>
</tr>
<tr>
<td>F. sulcata</td>
<td>Morphology</td>
<td>Azarbaijan, Arasbaran, Dooghrool, 2600-2800 m, Assadi &amp; Sardabi (TARI, 23997)</td>
</tr>
<tr>
<td>F. valesiaca</td>
<td>Morphology</td>
<td>Golestan, Yakhte kalan, 2100 m, Bagheri (TARI, 90262)</td>
</tr>
<tr>
<td>F. valesiaca</td>
<td>Morphology</td>
<td>Golestan, Golestan Forest, Sharlagh, 2200 m, Bagheri (TARI, 90263)</td>
</tr>
</tbody>
</table>
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. akhani*, 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., 1987).

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*. 

---

*Foliar anatomy and micromorphology of Festuca L. and its taxonomic applications* 59
Table 2: Useful anatomical characters for distinguish species. Relation the sclerenchyma tissue of Midrib and Adaxial epidermis: 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.

<table>
<thead>
<tr>
<th>Taxon</th>
<th>F. drymiia</th>
<th>F. drymiia</th>
<th>F. gigantea</th>
<th>F. arundinaceae</th>
</tr>
</thead>
<tbody>
<tr>
<td>Relation the sclerenchyma tissue of midrib and adaxial epidermis</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Number of large vascular bundle</td>
<td>3</td>
<td>3</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Number of middle vascular bundle</td>
<td>6</td>
<td>6</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Number of small vascular bundle</td>
<td>4</td>
<td>1</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Shape furrow</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Adaxial epidermal cell shape</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Stomata type on adaxial surface</td>
<td>3</td>
<td>2</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>Buliform cells shape</td>
<td>1</td>
<td>1</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Abaxial epidermal cell shape</td>
<td>1</td>
<td>1</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>Stomata type on abaxial surface</td>
<td>3</td>
<td>2</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>Number of adaxial furrow</td>
<td>0</td>
<td>0</td>
<td>13</td>
<td>13</td>
</tr>
<tr>
<td>Number of adaxial ribs</td>
<td>0</td>
<td>0</td>
<td>5</td>
<td>14</td>
</tr>
<tr>
<td>Number of buliform cells on adaxial surface</td>
<td>0</td>
<td>0</td>
<td>30</td>
<td>24</td>
</tr>
<tr>
<td>Number of sclerenchyma bundle</td>
<td>26</td>
<td>23</td>
<td>8</td>
<td>16</td>
</tr>
<tr>
<td>Number of sclerenchyma layer in tip leaf</td>
<td>6</td>
<td>4</td>
<td>2</td>
<td>7</td>
</tr>
<tr>
<td>Number of sclerenchyma layer under midrib</td>
<td>10</td>
<td>6</td>
<td>3</td>
<td>7</td>
</tr>
</tbody>
</table>

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](image-url)

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

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 have 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.

References


فرم اشتراک مجله تاکسونومی و بیوسیستماتیک

نام و نام خانوادگی: .................................................. سمت: ..............................................................

با واریز مبلغ 80000 ریال (هزینه پست و اشتراک) به حساب شماره 2177240238002 بانک ملی، کد 110227، شعبه دانشگاه اصفهان، به نام مدیریت اختصاصی دانشگاه اصفهان، متقاضی اشتراک یک ساله (جهت شماره) مجله تاکسونومی و بیوسیستماتیک هستم. لطفاً مجله را از شماره ........... به نشانی زیر ارسال نمایید.

نشانی دقیق: ..............................................................

شماره تلفن: ..............................................................

نشرگاه: ..............................................................

نشانی پست الکترونیک: ..............................................................

مسئول پاسخگویی: ..............................................................

اصل فیش بانکی را به نشانی اصفهان- خیابان هزاری جربی- دانشگاه اصفهان- ساختمان کتابخانه مرکزی اداره چاپ، انتشارات و مجلات- دفتر مجله تاکسونومی و بیوسیستماتیک- کد پستی: 8174673441 ارسال فرمایید.
آناتومی و ریز ریختشناسی برگ جنس Festuca و کاربرد تاکسونومی آن

فاطمه زینب کمر و نسرین اسلامی جوینده

گروه علوم گیاهی، دانشکده علوم زیستی، دانشگاه تربیت مدرس، تهران، ایران

چکیده

در این تحقیق، ساختار ریز ریخت-شناسی برگ ۸ گونه از جنس Festuca با نام‌های F. arundinacea، F. akhanii و ساختار تشريحي برگ در F. valesiaca و F. sulcata، F. heterophylla، F. gigantea، F. elwendiana و F. drymeia سه گونه با تکرار های متوازن و بررسی قرار گرفت. حدود ۴۰۰ صفحه معنی‌دار کمی و کیفی به منظور آنالیز آماری و تجزیه و تحلیل روش‌ها و روش‌های موجود در نظر گرفته شد. برخی از این صفات شامل وجود یا عدم وجود فروپذیری و پرآمادگی در این دسته، راکم روزنه‌ها و گوگردگی در سطح آهیانی و تعداد استجارات بنا به استکلارشنمی است. داده‌های حاصل از مقایسه ریخت‌شناسی و تشريحي برگ، این موارد را تجزیه و تحلیل آماری قرار گرفت. نتایج آماری حاصل از روش‌های دسته‌بندی متوازن وان و Single Linkage Between Groups، F. elwendiana، F. heterophylla، F. sulcata و F. valesiaca مشابه بوده، دو خوشه جداگانه شامل گونه‌های ریز برگ (F. arundinacea و F. akhanii) و (F. gigantean داشتند.

واژه‌های کلیدی: آناتومی، ایران، ریز ریخت-شناسی، استکلارشنمی، Festuca

* zarinkamar@modares.ac.ir
اتلاعات جمعیتی مارکرهای D6S2806 و D6S2879 واقع در ناحیه HLA-DRB1 در جمعیت ایرانی: شناسایی اثرهایی از انتخاب طبیعی و جهت‌دار

چکیده

در این مطالعه، نوع زننده و آزمون neutrality برای ژن‌های زننده و آزمون MHC مورد بررسی قرار گرفت. اطلاعات زننده D6S2806 و D6S2879 واقع در ناحیه HLA-DRB1 بررسی شدند. اطلاعات تعمیم‌زدایی زننده 73 فرد غیر خویشاوند را شامل شانزه شانزه لگد است. شانزه Popgene32 و PyPop بررسی شدند. با استفاده از برنامه‌های neutrality، تعداد آتلی مارکرهای و آزمون MHC و شانزه D6S2806 و D6S2879 در جمعیت مطالعه شده به ترتیب 23/18/1000 بود. ارزش محاسبه شده برای مارکرهای D6S2806 و D6S2879 به ترتیب 8449/0 و 8990/0 تخمین زده شدند. نتایج به دست آمده از آزمون Fnd نشان داد که در این آزمون بین‌گرایی آن است که مارکرهای 73 و D6S2879 در جمعیت‌های ایرانی به ترتیب تحت انتخاب طبیعی و جهت‌دار هستند. این اطلاعات پیشنهاد کندن حضور یک فشار انتخابی بر روی ناحیه زننده HLA-DRB1 در جمعیت‌های ایرانی است.

واژه‌های کلیدی: ژن D6S2806، مارکر ریز ماهواره، HLA-DRB1، نتایج زننده، آزمون neutrality، آزمون

* svallian@biol.ui.ac.ir
ارزیابی تنوع زنده‌کی ارقام اتار ایران با استفاده از نشانگرهای ISSR و RAPD

مجد طالبی برف ۱، مسعود بهار ۱، بهرام شریف نیک ۱ و احمد یامچی ۲

گروه بیوتکنولوژی، دانشکده کشاورزی، دانشگاه صنعتی اصفهان، اصفهان، ایران

دانشگاه علوم کشاورزی و منابع طبیعی گرگان

چکیده

با توجه به نشانه‌پذیری در ارقام اتار ایران، تنوع زنده‌کی ۲۴ رهم اتار با استفاده از نشانگرهای ISSR و RAPD مورد بررسی قرار گرفت. آغازگرگاهی ۲۲ رهم اتار در مجموع ۱۳۱ فلله DNA را تشکیل داده که که ۲۹ فلله آنها در تکثیر نموده است. آغازگرگاهی ۲۹ فلله از مجموع ۱۲۳ فلله که در تکثیر شده، RAPD حاصل نموده، میانگین محتمل اطلاعات جداکلی (PI) برای آغازگرگاهی RAPD و ISSR به ترتیب ۰/۱۸ و ۰/۱۵ است. در مقیاسه با نشانگرهای RAPD، گروه نواری تکرارپذیری ابتدا می‌کند و برای گروهبنیاد ارقام اتار مؤثرترین. ضریب شیبین این ارقام از ۰/۳۵ تا ۰/۳۱ به ترتیب RAPD و ISSR محاسبه و میانگین آن در نشانگرهای RAPD و ISSR ندارد و این اختلاف معنی‌داری بین نواحی مخفف جغرافیایی و طعم میوه ارقام مورد مطالعه نشان نداده (P>۰/۰۵) که بیانگر عدم ارتباط تنوع جغرافیایی و تنوع زنده‌کی است.

ISSR، RAPD، اندازه‌های کلیدی: تنوع زنده‌کی، اتار Punica granatum

* mtalebi@cc.iut.ac.ir
جلس در ایران: (Anthemideae, Asteraceae) Matricaria L.

مطالعه کموناشیکی سوم سال بیوشیمیکی و تاکسونومیک ۱۳۹۰

مجد شریفی‌نیا و تصدیق قاسمی

گروه زیست‌شناسی دانشکده علوم دانشگاه شهرکرد ایران

دانشکده داروسازی و گیاهان دارویی دانشگاه علوم پزشکی و خدمات بهداشتی درمانی اصفهان ایران

چکیده

گونه است که دو گونه از آن به طور طبیعی در ایران می‌روند. این مطالعه به منظور توصیف نمونه‌های جمع‌آوری شده از این جنس در ایران با استفاده از بروفاونتورهای فلورونیدی و تعیین ویژگی‌های اسکلت فلورونیدهای مطرح M. recutita و M. aurea در هر یک از گونه‌های آن صورت گرفته است. ۱۲ نمونه جمع‌یافته بالکل شده از دو گونه مورد آزمایش قرار گرفته‌اند. داده‌های حضور-غیاب حاصل از بررسی نقاط مارک مورد ساختار گونه‌ها به همه نمونه‌ها ثابت و با استفاده از آنالیزهای خطی و آیدنتیسیون (PCA) مورد بررسی قرار گرفته‌اند. در این مطالعه ویژگی‌های مربوط به اسکلت‌های فلورونیدهای مطرح گونه و تفاوت‌های آنها مورد بررسی قرار گرفته‌اند.

مروری اجمالی بر مویعیت ناشی از کروماتوگرافی نازک و دو بعدی، وازده‌های کلیدی: اسکلت فلورونیدهای ایران، گروه‌های فلورونیدهای است. (Asteraceae) گروه‌های فلورونیدهای نازک دو بعدی، Matricaria L.

* sharifi_m@sci.sku.ac.ir
مطالعه سیستولوژیک در ایران Hordeum bulbosum L.

حامد خداری و حجت‌الله سعیدی *

گروه زیست‌شناسی، دانشکده علوم، دانشگاه اصفهان، اصفهان، ایران

چکیده
همواره به عنوان یکی از منابع آلمی مفید که می‌تواند در اصلاح غلات زراعی استفاده
شود مورد نظر است. در این تحقیق، کارپوئیب 22 نمونه جمعیتی این گونه جمع آوری شده از نقاط مختلف ایران مورد
بررسی قرار گرفت. تفاوت کارپوئیب جمعیت‌ها و ارتباط آن با مناطق جغرافیایی اروپایی شد. تمامی جمعیت‌های
مطالعه نشان داده شد که نمونه‌های آن در ارتباط با کارپوئیب 22 گروه دارد. مشاهدات نشان داد که
جمعیت‌های غربی، منطقه‌های کارپوئیب و دارند. بر اساس این نتایج می‌توان گفت که قبیل‌های گروه‌های
این گونه در کوههای زاگرس و جوان تیرین آنها در شمال شرق ایران قرار دارند. این گونه احتمالاً از غرب ایران
وارد شده و به سمت شرق گسترش یافته است.

واژه‌کلیدی: Hordeum bulbosum L.

* ho.saeidi@sci.ui.ac.ir
با زنگری تاکسونومیکی گون جنس گون در استان زنجان

و شرح یک گون جدید

ج. علی بهرامی\(^1\)، ت. ع. اکبر‌مالکی نژاد\(^2\)

\(^1\) گروه زیست‌شناسی، دانشکده علوم، تربیت معلم تهران، تهران، ایران

\(^2\) مؤسسه تحقیقات گیاه‌شناسی و مرتع‌کشی، تهران، ایران

چکیده

استان زنجان واقع در شمال غربی ایران، با توجه به موقعیت ناحیه جغرافیایی آب‌های و پهنه‌ای خود شرایط مستندی برای روش جنس گون دارد. در طول سال‌های 1387-1389 جمعیت آوری و سبیعی در حضور این جنس در استان انجام گرفت. در نتیجه این مطالعات 41 گونه به مجموع 75 گونه اصلی گزارش شده از استان زنجان اضافه گردید، به علاوه تعداد 9 گونه جدید برای دنبای گیاه‌شناسی معرفی شدند و در این مقاله گونه‌ای به عنوان گونه Incani DC. Astragalus fausicola Podlech ex Bagheri, Maassoumi & F.Ghahrem. جدید شرح داده و معرفی می‌شد.

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

* maassoumi@rifr-ac.ir
گزارش گونه Potentilla botschantzeviana Adylov
(Syn: Potentilla butkovii var. botschantzeviana (Adylov) Soják)

از تیره گل سرخ به عنوان گونه جدید برای فلور ایران

مرضی‌بیگم فقیر ۱، فریده عطار ۲ و جوادی چوکا ۳

گروه زست‌شناسی، دانشکده علوم، دانشگاه گيلان، رشت، ایران ۱
هبهاریوم مرکزی، بخش گیاه‌شناسی، دانشکده زیست‌شناسی، پردیس علوم، دانشگاه تهران، تهران، ایران ۲
گروه گیاه‌شناسی، مرکز علوم طبیعی، پارک، جمهوری چک ۳

چکیده

گونه Potentilla botschantzeviana ((Syn: P. butkovii var. botschantzeviana (Adylov) Soják)) به عنوان گزارش جدیدی از شمال ایران ارائه می‌شود. این گونه با دارا بودن دم‌برگ‌های پوشیده از گرک‌کهای راست-خوابیده با گسترده‌ای بر گه‌های تغییری و برگ‌هایی با گرک‌کهای تغییری، افرشته‌های متفاوتی و نیز گل‌هایی با قطر ۱/۲ سانتی‌متری، جایی بی‌شناختی در P. botschantzeviana قرار دارد. به واسطه ضخیمی متشابه با گونه‌های پیشین در این گونه بزرگ‌گری خاصی در ایران و شامل ۱۲ گونه بومی است. تصویر گیاه در مرحله گل‌دهی، تصاویر قطعات مختلفی، تصویر گرک سطح تحتانی برگ و نیز نقشه پراکنش این گونه ارائه می‌گردد.

Persicae (Th. Wolf) Juz. و زده‌های گل‌پذیر: Potentilla فلور ایران,

* marziehfaghir@yahoo.com
فهرست

1. گزارش گونه (Syn: Potentilla butkovii var. Potentilla botschantzeviana Adylov) گونه botschantzeviana (Adylov) Sojak از تیره گل سرخ به عنوان گونه جدید برای فلور ایران محسوب می‌شود.

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

3. مطالعه سیتوپاتولوژیک Hordeum bulbosum L. در ایران.

4. جنس Matricaria L. (Anthemideae, Asteraceae) در ایران: مطالعه کمونتاکسونومیک بر اساس فلاونوئیدها. مجید شریفی نهانی و نصرالله قاسمی.

5. ارزیابی توزیع زنتیکی ارقام انداز ایران (Punica granatum L.) با استفاده از نشانگرهای ISSR و RAPD.

6. اطلاعات جمعیت مارکرهای HLA-DRB1 و D6S2806 واقع در تالیه D6S2879 و D6S2806 ایرانی: شناسایی الگوها از انتخاب طبیعی و جهتدار مصرفه تجدید. صادق ولیان بروجنی و زهرا فاضلی عطار.

7. آناوتو می و ریز ریخت شناسی برگ جنس Festuca و کاربرد تاکسونومی آن. فاطمه زرین کم و نسرین اسلامی جویانه.
داوران علمی این شماره (سال سوم - شماره هشتم - پاییز ۱۳۹۰)

اعضای محترم یاپات علمی دانشگاه‌ها و مؤسسات آموزشی و پژوهشی کشور که در داوری و ارژیابی مقالات این شماره از مجله علمی-پژوهشی تاکسونومی و پیوستمکاتی همکاره دانش‌آموز محترم بوده‌اند، معرفی شده و از خدمات علمی آنها تقدير می‌گردد:

- دکتر نسترن جليلیان
- دکتر سید ذبیح‌الله حسینی
- دکتر سید نازع خراسانی
- دکتر سهیل مسعود رنجر
- دکتر حجت‌الله سعیدی
- دکتر بدرالدین ابراهیم میرطاطبایی
- دکتر فردید عطار
- دکتر لیا قاچام مقامی
- دکتر مریم فرح‌پور نمازی
- دکتر سهیل نادری ابادانی
- دکتر حمید میر مهدی صادقی

دانشگاه‌های تحقیقات کشاورزی و منابع طبیعی کرمانشاه

- دانشگاه پاسیفیک
- دانشگاه شهرکرد
- دانشگاه بعلی سمنان
- دانشگاه اصفهان
- دانشگاه صنعتی اصفهان
- دانشگاه تهران
- دانشگاه اصفهان
- دانشگاه تربیت معلم
- دانشگاه تربیت مدیره تهران
- دانشگاه علوم پزشکی و خدمات بهداشتی درمانی اصفهان
شنومنه فارسی: زنگی بور، ا.، افشارزاده، س.، بلاطی هدکردو، غ. و صاحبی، ج. (۱۳۸۷) مطالعه جنس لویی در رودخانه زاینده‌رود. اولین همایش ملی زیست‌شناسی گیاهی، دانشگاه پام‌نور، تالش.

شنومنه انگلیسی:


ب-1-2 مقاله با دورگاه:
نمونه فارسی: نگارنده دو بیشتر و نگارنده دو مقاله:
(1376) بررسی پراکنش گیاهان مقاوم به شوئی در ایران، مجله زیست‌شناسی 3 89:
(5) 57
نمونه متایی انگلیسی:

ب-1-3 مقاله با دورگاه و پیشتر:
نمونه فارسی: نگارنده دو بیشتر و نگارنده دو مقاله:
(1376) بررسی پراکنش گیاهان مقاوم به شوئی در ایران، مجله زیست‌شناسی 3 87
نمونه انگلیسی:

ب-2 مرجع دیگه به کتاب (Book) به ترتیب شمار: نام نویسنده، نام نویسنده، نام کتاب، شماره
در صورت وجود، نام مؤسسه انتشارات، نام اولین شهی که انتشار در آن انجام گرفته است.
نمونه فارسی: مظفریان، و. (1373) کورموفیت‌های ایران. جلد 4، مرکز نشر دانشگاهی، تهران.
نمونه انگلیسی:

ب-3 مرجع دیگه به بخشی از کتاب (Chapter in Book) که حیرت‌داری نویسنده، جدایگان باشد:
نمونه انگلیسی:

ب-4 مرجع دیگه به یاپان‌نامه کارشناسی ارشد با دکتر: نام نویسنده، نام، عنوان یاپان‌نامه، مقطع تحصیلی، نام دانشگاه، نام، شهر، نام کشور.
نمونه فارسی: حسنی، پور، م. (1365) ناکونوتومو و بیوسیستماتیک جنس Cardaria L. در ایران. یاپان‌نامه دکتری، دانشگاه اصفهان، اصفهان.
نمونه انگلیسی:

ب-5 مرجع دیگه به Patent:

ب-6 مرجع دیگه به همایش‌ها (سیمینارها، سمینارها، کنگردها، میتینگ‌ها و ...) به ترتیب شامل: نام نویسنده، سال انتشار، عنوان مقاوله، دوره، نام همایش، محل برگزاری، شهر، کشور.
عنوان: شامل کوتاهترین عباراتی خواهد بود که بطور کلی گویای محتواهی مقاله باشد. خط فارسی عنوان 14 Times New Roman Bold و انگلیسی Bold است.

نام و نشانی نویسنده: سوئیفت نام نویسنده بعده نویسنده مسئول خواهد بود. درج شماره مربوط به نشانی هر نویسنده بعد از نام نویسنده به صورت بالا نوشته و درج سوئیفت نام نویسنده به صورت پایین نوشته می‌شود.

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

مقدمه، مشاهده، مواد و روش‌ها، نتایج، بحث و نتیجه‌گیری، فقدان‌دادن و منابع.

12 B Lotus 11 Times New Roman Bold

9 Times New Roman Abstract

Key words و References in text

2-1) مراجع دیگر به صورت مورب باشد (این کلمه لاتین است).

و سال انتشار نوشته شود.

نمونه فارسی: یک نویسنده برای مربوط شده بر اساس حروف الفبا معرفی می‌شود.

(References list)

(References in text)

نمونه انگلیسی: یک نویسنده برای مربوط شده بر اساس حروف الفبا معرفی می‌شود.

(Johnson et al., 2000)

کلمه با بررسی به صورت موبی باشد (این کلمه لاتین است).

ب) مراجع دیگر به صورت دوره انتشار (References list) بیشترین منابع فارسی و سپس منابع خارجی آورده شود.

ب-1) مراجع دیگر به صورت (Paper) به ترتیب شامل: نام نویسنده، نام مقاله، شماره مجله، جلد، شماره صحافه.

ب-1-1) مقاله با نام نگارش:

ب(T. urartu and T. boeoticum) نمونه انگلیسی: برجستی ص. (1375) دو بررسی گوناگونی زننده در گونه‌ها و حیاتی استفاده از الکترودیور پرتویی بذر مسجل به تنهال 1 نا 8.

نمونه انگلیسی:

مجله تاکسنومی و بیوسیستمیک

مجله تاکسنومی و بیوسیستمیک به صورت فصلی و هر سه ماه یکبار توسط دانشگاه اصفهان منتشر می‌شود. هدف از انتشار این مجله عرضه این اطلاعات علمی استاندارد و پژوهشگران در زمینه تاکسنومی و بیوسیستمیک، به ویژه با تأکید بر خریده و روزنامه‌نگاران (پوکارپوترا و پرکارپوترا) در ایران می‌باشد.

مجله علمی - پژوهش تاکسنومی و بیوسیستمیک در زمینه‌های معرفی تاکسنومی جدید، مورور نام‌گذاری تاکسنومی‌ها، طبقه‌بندی تاکسنومی‌ها، معرفی روشهای جدید ایجاد و تحلیل داده‌ها، زنگ‌آوری، تسکین جمعیت‌ها و نوع وراثی، توجه زیستی و فیلوژنی تاکسنومی‌ها، مقاله‌های اصلی 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.
تاریخچه انتشارات علمی، پژوهشی و بیوستمتاتیک
سال سوم - شماره هشتم - پاییز ۱۳۹۰
شماره استاندارد بین المللی:۲۰۰۸-۸۹۰۶
علی-پژوهشی

صاحب امتیاز: معاونت تحقیقات و فناوری دانشگاه اصفهان
سردرب: دکتر محمدرضا رحیمی نژاد رنجر

اعضای هیأت تحریریه

دکتر حمید اجتهادی
دکتر علی اکبر اسفندیاری
دکتر جمشید درویش
دکتر حمید رجبی
دکتر محمدرضا رحیمی نژاد رنجر
دکتر بدرالدین ابراهیم سید طباطبایی
دکتر مهدی عباسی
دکتر حسن فتح پور
دکتر علی اصغر مصویی
دکتر ابراهیم نیکی
دکتر صادق ولایی پروچی

مدیر اجرایی: فریبا هادیان (کارشناس ارشد)

ویراستار انگلیسی علمی - تخصصی: فریدون پرویزیان

صفحه آرای: فریبا هادیان

صفحه ناشر: تابعیت دانشگاه اصفهان

نشانی پستی

دانشگاه اصفهان-خیابان هزار جنب-دانشگاه اصفهان-سابک ورودی کتابخانه مرکزی-معاونت پژوهشی و فناوری-پستی:۸۹۱۶۳۴۴۱

http://uijs.ui.ac.ir/tbj
TBJ@ui.ac.ir

نشانی پست الکترونیک: TBJ@ui.ac.ir
 مجله‌ی تاکسونومی و بیوستاتیک - شماره ابلاطی - شماره 31/06/1388

پایگاه اختصاصی مجله:
http://uijs.ui.ac.ir/tbj

بانک اطلاعات نشریات کشور:
http://www.magiran.com

پایگاه اینترنتی جهاد دانشگاهی:
http://www.SID.ir

پایگاه استادی علوم جهان اسلام:
http://www.ISC.gov.ir

چاپ و لیتوگرافی: انتشارات دانشگاه اصفهان

ناشر: دانشگاه اصفهان

قیمت: ۲۰۰۰۰ ریال

تیراژ: ۱۰۰۰ نسخه

انتشار: زمستان ۱۳۹۰
کتاب سوم
سید حسین نوروزی
علی شهیدی

سال قوم - شماره هفتم - تیر ۱۳۹۴