Inter-simple sequence repeats (ISSR) and morphological diversity in Onosma L. (Boraginaceae) species in Iran

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Morphological characteristics as well as inter-simple sequence repeats (ISSR) molecular markers were used to study the species relationship in Onosma species (Boraginaceae) in Iran. Principal component analysis (PCA) showed that morphological characters like caule leaf size and shape, bract shape, corolla shape, nutlet length, venation and corolla teeth size and anther length are the most variable morphological characters among Onosma species studied. Out of 17 ISSR primers used, 6 primers produced 41 polymorphic and reproducible bands. Few specific ISSR bands were obtained for the species studied which show genetic material change during species diversification. Such bands may be used in the species identification too. Morphological and molecular trees obtained partly agreed with each other. In both trees, Onosma dasytrichum and Onosma microcarpum are placed close to each other, Onosma procerum and Onosma pachypodum show affinity to each other and Onosma araraticum, Onosma bodeanum, Onosma bistonensis, Onosma stenosiphon and Onosma bulbotrichum are placed close to each other while, Onosma rostellatum stands far from the other species. The combined morphological and ISSR tree obtained separated the members of three sections of Onosma, Podnosma and Protonosma from each other. Moreover, almost a good separation of different subsections in the section Onosma was observed in the combined tree.

Key words: Inter-simple sequence repeats, morphometry, Onosma.

INTRODUCTION

Onosma is a genus with about 150 species occurring in dry, clifffy and sunny habitats, distributed mainly in Eurasia and Mediterranean regions, having its center of distribution and maximum concentration of species in Iran (Ball, 1972; Willis, 1973).

The genus Onosma contains biennial or perennial, hispid herbs, with flowers in terminal cymes, calyx accrescent, stamens inserted at the middle of the corolla and generally 4 nutlets flat at the base (Binzet et al., 2010). It contains about 60 species in Flora Iranica region (Riedl, 1968), with 39 species growing in Iran (Khatamzaz, 2002; Attar and Jouharchi, 2006; Attar, 2007).

Molecular studies in the genus Onosma are very limited, mainly confined to amplified fragment length polymorphism (AFLP) study of genetic diversity in populations of Onosma echioides L. (Mengoni et al., 2006). Therefore, the present study considers numerical analysis of morphological and inter simple sequence repeats (ISSR) characteristics in 29 Onosma species growing in Iran and tries to show the species relationship based on these features.
### Table 1. Species studied and their respective sections and subsections divisions.

<table>
<thead>
<tr>
<th>Species</th>
<th>Section</th>
<th>Subsection</th>
</tr>
</thead>
<tbody>
<tr>
<td>O. albo-roseum Fisch.&amp; C. A. Mey</td>
<td>Onosma</td>
<td>Asterotricha (Boiss) Gürke.</td>
</tr>
<tr>
<td>O. araraticum Riedl.</td>
<td>Onosma</td>
<td>Haplotricha (Boiss) Gürke.</td>
</tr>
<tr>
<td>O. armenum DC.</td>
<td>Onosma</td>
<td>Asterotricha</td>
</tr>
<tr>
<td>O. bistonenisis Attar.</td>
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<td>Asterotricha</td>
</tr>
<tr>
<td>O. bodeanum Boiss.</td>
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<td>Haplotricha</td>
</tr>
<tr>
<td>O. bulbotrichum DC.</td>
<td>Onosma</td>
<td>Haplotricha</td>
</tr>
<tr>
<td>O. chlorotrichum Boiss.</td>
<td>Onosma</td>
<td>Heterotricha Boiss.</td>
</tr>
<tr>
<td>O. cornutum Riedl.</td>
<td>Onosma</td>
<td>Haplotricha</td>
</tr>
<tr>
<td>O. dasytrichum Boiss.</td>
<td>Onosma</td>
<td>Asterotricha</td>
</tr>
<tr>
<td>O. demavendicum Riedl.</td>
<td>Onosma</td>
<td>Heterotricha Boiss.</td>
</tr>
<tr>
<td>O. dichoroanthum Boiss.</td>
<td>Onosma</td>
<td>Haplotricha</td>
</tr>
<tr>
<td>O. elwedicum Wettst.</td>
<td>Onosma</td>
<td>Heterotricha</td>
</tr>
<tr>
<td>O. intertextum Hub.</td>
<td>Onosma</td>
<td>Asterotricha</td>
</tr>
<tr>
<td>O. kilouyense Boiss.</td>
<td>Onosma</td>
<td>Heterotricha</td>
</tr>
<tr>
<td>O. kotschy Boiss.</td>
<td>Onosma</td>
<td>Haplotricha</td>
</tr>
<tr>
<td>O. longilobum Bge.</td>
<td>Onosma</td>
<td>Haplotricha</td>
</tr>
<tr>
<td>O. macrophyllum Borm.</td>
<td>Onosma</td>
<td>Heterotricha</td>
</tr>
<tr>
<td>O. microcarpum Steven ex DC.</td>
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<td>Haplotricha</td>
</tr>
<tr>
<td>O. olivieri Boiss.</td>
<td>Onosma</td>
<td>Heterotricha</td>
</tr>
<tr>
<td>O. pachypodum Boiss.</td>
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<td>Haplotricha</td>
</tr>
<tr>
<td>O. platyphylum Riedl.</td>
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<td>Haplotricha</td>
</tr>
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<td>O. procerum Boiss.</td>
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<td>Haplotricha</td>
</tr>
<tr>
<td>O. rasychaenium Boiss.</td>
<td>Onosma</td>
<td>Asterotricha</td>
</tr>
<tr>
<td>O. sabalanicum</td>
<td>Onosma</td>
<td>Haplotricha</td>
</tr>
<tr>
<td>O. sericeum Wild.</td>
<td>Onosma</td>
<td>Haplotricha</td>
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<tr>
<td>O. stenosiphon Boiss.</td>
<td>Onosma</td>
<td>Haplotricha</td>
</tr>
<tr>
<td>O. straussii (Riedl) Khatamsaz.</td>
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<td>Haplotricha</td>
</tr>
<tr>
<td>O. rostellatum Lehm.</td>
<td>Protonosma</td>
<td></td>
</tr>
<tr>
<td>O. orientale L.</td>
<td>Podnosma</td>
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</table>

ISSR markers show high level of repeatability and have been used as useful molecular markers in studying genetic diversity and species relationships (Pharmawati et al., 2004; Dogan et al., 2007). Morphometric analysis has also been used to clarify taxonomic status of Onosma species (Peruzzi and Passalacqua, 2008).

**MATERIALS AND METHODS**

**Plant materials**

27 Onosma species from two sections of Onosma and Protonosma were studied (Tables 1 and 2). The species studied have been placed in 3 subsections of Asterotricha (Boiss) Gürke, Haplotricha (Boiss) Gürke., and Heterotricha Boiss (Riedl, 1968; Khatamsaz, 2002; Attar and Jouharchi, 2006; Attar, 2007). Voucher specimens have been deposited in Herbarium of Shahid Beheshti University (HSBU) and Iran National Botanical Garden Herbarium (Iran).

**Morphometry**

In total, 36 morphological characters (quantitative and qualitative) were used for morphometry and coded accordingly (Table 3). Minimum of 10 randomly selected plants from each species from different populations were used for obtaining morphological data.

For multivariate analyses, the mean of quantitative characters were used, while qualitative characters were coded as binary/ multistate characters. Standardized variables (mean = 0, variance = 1) were used for multivariate statistical analyses (Podani, 2000). Principal components analysis (PCA) was performed to identify the most variable morphological characters among the species studied and PCA plot of the components obtained were used to get species groupings (Podani, 2000).

Unweighted paired group with arithmetic average (UPGMA) and Neighbor joining (NJ) clustering methods were performed for grouping of the species based on morphological characters by using PAUP vers. 4b (2000), while PCA analysis was performed by SPSS ver. 9 (1998) software.

**ISSR assay**

Total genomic DNA was extracted from fresh leaves using the CTAB method by Murry and Tompson (1980) with the modification described by De la Rosa et al. (2002). Six ISSR primers used were (GA)_n, UBC810, UBC811, UBC834, UBC849 and CA7GT commercialized by the University of British Columbia (UBC).
Table 2. Species locality and their vouchers.

<table>
<thead>
<tr>
<th>Species</th>
<th>Locality</th>
<th>Collector</th>
<th>Voucher number</th>
</tr>
</thead>
<tbody>
<tr>
<td>O. albo-roseum</td>
<td>Kermanshah, Ghasre-Shrin to Gilanegharb</td>
<td>Sharif</td>
<td>IRAN-2684</td>
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<tr>
<td>O. araraticum</td>
<td>Zanjan, Belgheis Mt.</td>
<td>Mehrabian</td>
<td>HSBU-2010200</td>
</tr>
<tr>
<td>O. armenum</td>
<td>Azarbaidjan, Khoy to Ghotor</td>
<td>Sharif</td>
<td>IRAN-2693</td>
</tr>
<tr>
<td>O. bistonensis</td>
<td>Kermanshah, Biston Mt.</td>
<td>Mehrabian</td>
<td>HSBU-2010207</td>
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<tr>
<td>O. bodeanum</td>
<td>Eastern Azarbaidjan, Mishodagh Mt.</td>
<td>Mehrabian</td>
<td>HSBU-2010211</td>
</tr>
<tr>
<td>O. bulbotrichum</td>
<td>Kurdestan, Marivan to Saghez</td>
<td>Mehrabian</td>
<td>HSBU-2010203</td>
</tr>
<tr>
<td>O. cornutum</td>
<td>Eastern Azarbaidjan, Mishodagh Mt.</td>
<td>Mehrabian</td>
<td>HSBU-2010204</td>
</tr>
<tr>
<td>O. dasytrichum</td>
<td>Kermanshah, Javanrod to Paveh</td>
<td>Mehrabian</td>
<td>HSBU-2010216</td>
</tr>
<tr>
<td>O. demavendicum</td>
<td>Markazi, Arak</td>
<td>Mehrabian</td>
<td>HSBU-2010228</td>
</tr>
<tr>
<td>O. dichoroanthum</td>
<td>Khorasan Razavi, Mashhad</td>
<td>Sheikhkabari &amp; Ghorbani</td>
<td>HSBU-2010222</td>
</tr>
<tr>
<td>O. elwedicum</td>
<td>Tehran, Lashkarak</td>
<td>Mehrabian</td>
<td>HSBU-20110205</td>
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<td>O. kilouyense</td>
<td>Kermanshah, Gahvareh</td>
<td>Mehrabian</td>
<td>HSBU-2010209</td>
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<tr>
<td>O. kotschy</td>
<td>Lorestan, Aligodarz to Ghalikuh</td>
<td>Iranshahr</td>
<td>IRAN-2697</td>
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<tr>
<td>O. longilobum</td>
<td>Khorasan, Darehgaz, Allah-o-Akbar Mt.</td>
<td>Iranshahr &amp; Zargani</td>
<td>IRAN-2831</td>
</tr>
<tr>
<td>O. macrophyllum</td>
<td>Kermanshah, Gahvareh, Baba Shah Ahmad</td>
<td>Mehrabian</td>
<td>HSBU-2010226</td>
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<tr>
<td>O. microcarpum</td>
<td>Mt. Kurdestan, Dizli to Nodesheh</td>
<td>Mehrabian</td>
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<td>O. oliveri</td>
<td>Kurdestan, Nosoud to Marivan</td>
<td>Mehrabian</td>
<td>HSBU-2010202</td>
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<tr>
<td>O. orientale</td>
<td>Kohkilouye, Dogonbadan</td>
<td>Zairi</td>
<td>IRAN-2907</td>
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<tr>
<td>O. pachypodum</td>
<td>Tehran, Lashkarak</td>
<td>Mehrabian</td>
<td>HSBU-202024</td>
</tr>
<tr>
<td>O. platyphyllum</td>
<td>Lorestan, Oshtorankuh, Dsht-e-Takht</td>
<td>Iranshahr</td>
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<td>O. procerum</td>
<td>Esfahan, Semirom, Sivar Village</td>
<td>Mehrabian</td>
<td>HSBU-2010221</td>
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<tr>
<td>O. rasychaenum</td>
<td>Kurdestan, Sanandaj, Salavatabad,</td>
<td>Pahlevani &amp; Amini</td>
<td>IRAN-2919</td>
</tr>
<tr>
<td>O. rostellatum</td>
<td>Kurdestan, Bayangan, 4555ft</td>
<td>Mehrabian</td>
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<tr>
<td>O. sabalanicum</td>
<td>Ardebil, Meshkinshahr, Sabalan mountain</td>
<td>Mehrabian</td>
<td>HSBU-2010201</td>
</tr>
<tr>
<td>O. sericeum</td>
<td>Tehran, Lashkarak</td>
<td>Mehrabian</td>
<td>HSBU-2010227</td>
</tr>
<tr>
<td>O. stenosiphon</td>
<td>Kerman, Rafsjanan, Sarcheshmeh</td>
<td>Riedl &amp; Etemad</td>
<td>IRAN-2797</td>
</tr>
<tr>
<td>O. straussii</td>
<td>Markazi, Arak, Gavar village</td>
<td>Mehrabian</td>
<td>HSBU-2010212</td>
</tr>
</tbody>
</table>

Polymerase chain reaction (PCR) reactions were performed in a 25 μl volume containing 10 mM Tris–HCl buffer at pH 8; 50 mM KCl; 1.5 mM MgCl\textsubscript{2}; 0.2 mM of each dNTP; 0.2 μM of a single primer; 20 ng genomic DNA and 3 unit of Taq DNA polymerase (Bioron, Germany). Amplifications reactions were performed in Techne thermocycler (Germany) with the following program: 5 min initial denaturation step 94°C, 30 s at 94°C; 1 min at 50°C, 1 min at 72°C. The reaction was completed by final extension step of 7 min at 72°C. Amplification products were visualized by running on 2% agarose gel, following ethidium bromide staining. Fragment size was estimated by using a 100 base pairs (bp) molecular size ladder (Fermentas, Germany).

ISSR bands obtained were treated as binary characters and coded accordingly (presence = 1, absence = 0). The average taxonomic distance and Manhatan distance were used as dissimilarity coefficient in cluster analysis of data (Podani, 2000). UPGMA and NJ clustering methods were performed for grouping of the species by using PAUP vers. 4b (2000), while PCA analysis was performed by SPSS ver. 9 (1998) software.

RESULTS

Morphometry

The PCA analysis performed to identify the most variable morphological characters among Onosma species studied, revealed that the first five PCA factors comprise about 60% of total variance (data not given). In the first factor, characters like caule leaf size, bract shape, corolla shape and nutlet length showed the highest positive correlation (r>0.60), while in the second factor with about 12% of total variance, morphological characters like venation and corolla teeth size, showed the highest negative correlation (r= -0.60). In the third and forth factors with about 7 and 6% of total variance respectively, characters like caule leaf shape and anther long had the highest positive correlation (r>0.69).

UPGMA and NJ clustering of morphological characters produced similar results and therefore UPGMA tree is discussed here. The first major cluster is formed by Onosma araraticum, Onosma armenum, Onosma rasychaenum, Onosma demavendicum and Onosma bodeanum. The second cluster is formed by Onosma bistonensis, Onosma orientale, Onosma sabalanicum and Onosma stenosiphon (Figure 1).

Onosma bulbotrichum, Onosma kotschy, Onosma platyphyllum, Onosma oliveri, Onosma elwedicum and Onosma kilouyense formed the third cluster, while
### Table 3. Morphological characters and their coding.

<table>
<thead>
<tr>
<th>Morphological character</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anther exertion</td>
<td>Absent</td>
<td>Present</td>
<td>High</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Corolla trichome</td>
<td>Absent</td>
<td>Low</td>
<td>25-15</td>
<td>35-25</td>
<td>45-35</td>
<td>55-45</td>
<td>≥55 cm</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Height</td>
<td>≥15 cm</td>
<td>25-15</td>
<td>35-25</td>
<td>45-35</td>
<td>55-45</td>
<td>≥55 cm</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Anther-connection</td>
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<td>Present</td>
<td>1/3</td>
<td>1/2</td>
<td></td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>Trichome kind</td>
<td>Simple</td>
<td>Branched basal</td>
<td>Stellate</td>
<td></td>
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<td>Nectar trichome</td>
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<td>Present</td>
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<td></td>
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</tr>
<tr>
<td>Corolla color</td>
<td>Yellow</td>
<td>Purple</td>
<td>White</td>
<td>Yellow-pink</td>
<td>White-blue</td>
<td>Yellow-blue</td>
<td>Red</td>
<td>Red-purple-yellow</td>
<td>Red-blue</td>
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<tr>
<td>Corolla lobe</td>
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<td>Present</td>
<td></td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>Corolla teeth size</td>
<td>0.5-1</td>
<td>1.1-1.5</td>
<td>1.6-2</td>
<td>2.1-2.5</td>
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<td>10.1-15</td>
<td>15.1-20</td>
<td>20.1-25</td>
<td>25.1-30</td>
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<td>Bract size</td>
<td>1-5</td>
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<tr>
<td>Bract shape</td>
<td>Lanceolate</td>
<td>Wide lanceolate</td>
<td>Large</td>
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<tr>
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<td>2.1-3</td>
<td>3.1-4</td>
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<td>5.1-6</td>
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<td>7.1-8</td>
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<td>20.1-30</td>
<td>30.1-40</td>
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<td></td>
<td></td>
<td></td>
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<tr>
<td>Nutlet curve</td>
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<td>Present</td>
<td>Reticulate</td>
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<td>Venation</td>
<td>Absent</td>
<td>Present</td>
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<td></td>
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<td></td>
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<tr>
<td>Trichome color</td>
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<td>Present</td>
<td></td>
<td></td>
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<td></td>
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<td>Campanulate</td>
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<tr>
<td>Basal leaf shape</td>
<td>Spathulate</td>
<td>Linear-spathulate</td>
<td>Obovate</td>
<td>Oblong-Spathulate</td>
<td>Lanceolate-Spathulate</td>
<td>Lanceolate-Oblong</td>
<td>Lanceolate</td>
<td>Linear-lanceolate</td>
<td>Linear-lanceolate</td>
<td>Ovate-Lanceolate</td>
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<tr>
<td>Caule leaf shape</td>
<td>Spathulate</td>
<td>Linear spathulate</td>
<td>Obovate</td>
<td>Oblong-Spathulate</td>
<td>Lanceolate-Spathulate</td>
<td>Lanceolate-Oblong</td>
<td>Lanceolate</td>
<td>Linear-lanceolate</td>
<td>Linear-lanceolate</td>
<td>Ovate-Lanceolate</td>
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<tr>
<td>Trichome orientation</td>
<td>Vertical</td>
<td>Repent</td>
<td></td>
<td></td>
<td></td>
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<td></td>
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</tr>
<tr>
<td>Inflorescent dense</td>
<td>Scant</td>
<td>Medium</td>
<td>Dense</td>
<td></td>
<td></td>
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<tr>
<td>Style exertion</td>
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<td>Present</td>
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<tr>
<td>Style length</td>
<td>5-10</td>
<td>11-15</td>
<td>16-20</td>
<td>21-25</td>
<td>26-30</td>
<td></td>
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<tr>
<td>Anther length</td>
<td>5-7</td>
<td>7-9</td>
<td>10≤</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Nutlet length</td>
<td>2-3</td>
<td>3-4</td>
<td>4-5</td>
<td>5-6</td>
<td>6-7</td>
<td>7-8</td>
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<tr>
<td>Nutlet wide</td>
<td>2-3</td>
<td>3-4</td>
<td>4-5</td>
<td>5-6</td>
<td></td>
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<td>Nutlet orientation</td>
<td>Vertical</td>
<td>curve</td>
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<tr>
<td>Nutlet beak</td>
<td>1-1.5</td>
<td>1.6-2</td>
<td>2.1-3</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Nutlet shape</td>
<td>Trapeziform</td>
<td>Ovate</td>
<td>Deltoid</td>
<td>Wide ovate</td>
<td>Linear</td>
<td></td>
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<tr>
<td>Nutlet color</td>
<td>Black-yellow</td>
<td>Brown-white</td>
<td>Brown</td>
<td>Green-white</td>
<td>White</td>
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<td></td>
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<tr>
<td>Nutlet surface</td>
<td>Smooth</td>
<td>Wrinkle</td>
<td>Verrucate</td>
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Onosma procerum, Onosma dichoroanthum, Onosma straussii, Onosma sericeum and Onosma pachypodum comprised the fourth cluster. Two species of Onosma longilobum and Onosma microcarpum showed morphological affinity and form a separate cluster. The same is true for two species of Onosma dasytrichum and Onosma macrophyllum showing close affinity and form a distinct cluster. The last two species showing similarity to each other were Onosma albo-roseum and Onosma rostellatum which stand far from the other species (Figure 1).

**ISSR analysis**

Out of the 17 ISSR primers used (alone and in combination), 6 primers produced 41 polymorphic and reproducible bands. Bands No. 4 and (450 and 750 bp respectively) of the primer 807, band No. 6 (450 bp) of the primer 834 and band No. 8 (750 bp) of the primer 849 were specific for O. rostellatum, band No. 8 (480 bp) of the primer 834, occurred only in O. procerum. Few bands were present only in two species, for example, band No. 4 of the primer 834 (400 bp) occurred in O. bistonenosis and O. bulbotrachum, while the bands No. 5 and 7 (420 and 460 bp respectively) occurred in, O. rostellatum and O. sericeum. UPGMA and NJ trees of combined morphological and ISSR data produced similar results and therefore NJ tree is only discussed thus (Figure 2). O. araraticum and O. straussii from the subsect. Haplotricha showed affinity and stand far from the other species forming the first major cluster. The second major cluster contains three subclusters. O. armenum, rasychaenem (subsect. Asterotricha) and O. orientale (sect. Protonosma), O. demavendicum and O. cornatum (subsect. Haplotricha) and O. macrophyllum (subsect. Heterotricha) are placed close to each other comprising the first subcluster, while O. orientale (subsect. Asterotricha), O. dichoroanthum, O. kilouyense and O. elwedicum (subsect. Heterotricha) comprise the second subcluster. The third subcluster is formed by O. dasytrichum (subsect. Asterotricha) and O. sericeum
Figure 2. UPGMA tree of ISSR data.

(subsect. Haplotricha).

Combined data analysis

We made similar analysis on the combined data set of morphological and ISSR data (Figure 3). The first major cluster is comprised two subclusters. O. araraticum, O. straussii and O. bulbotrichum form the first subcluster, while O. kilouyense, O. kotschy, O. platyphyllum, O. elwedicum, O. pachypodum, O. sabalanicum, O. albo-roseum, O. longilobum and O. procerum form the second subcluster. These species all are from the section Onosma, subsect. Haplotricha.

The second major cluster contains 3 subclusters. The first subcluster is formed by O. armenum, O. rasycchaenem (both from the sect. Onosma, subsect. Asterotricha), O. demavendicum (subsect. Heterotricha), and O. bodeanum (subsect. Haplotricha).

O. bistonensis (subsect. Asterotricha), O. stenosiphon and O. microcarpum (both from subsect. Haplotricha) comprise the second subcluster, while O. dichoroanthum, O. sericeum and O. cornotum (all from the subsect. Haplotricha) as well as O. olivieri (subsect. Heterotricha) form the third subcluster.

O. orientale (sect. Podnosma) stands alone in a separate cluster joining the members of second major cluster with some distance. Two species of O. dasytrichum (subsect. Asterotricha) and O. macrophyllum (subsect. Haplotricha) show affinity and form a cluster while, O. rostellatum (sect. Protonosma) stands far from the other species in a single cluster.

DISCUSSION

The variable qualitative morphological characters among Onosma species studied, like caule leaf shape, bract shape, corolla shape, venation may serve as useful taxonomic characters for the species delimitation which can also be supported by some quantitative characters including the caule leaf size, nutlet length, corolla teeth size and anther length.

Morphometric study was also used to study the species relationship in Onosma echioides complex (Peruzzi and Passalacqua, 2008), concluding to treat this complex as a single species subdivided in four subspecies. The names O. angustifolia Leh., O. canescens J. and C. Presl, O. dalmatica Scheele, O. echioides var. columnae Lacaita, O. echioides var. veronensis Lacaita and O. javorkae Simonski were typified and a new combinations of O. echioides subsp. angustifolia (Lehm.) Peruzzi and N. G. Passal., O. echioides subsp. canescens (J. and C. Presl) Peruzzi and N. G. Passal. and O. echioides subsp. dalmatica (Scheele) Peruzzi & N. G. Passal., were proposed.
The specific ISSR bands obtained in *O. rostellatum*, *O. procerum*, showed the occurrence of genetic material change during species diversification and that such molecular markers may be used in the species delimitation. Possibly, by using more number of ISSR markers we may find more specific bands for the other species too. Moreover, presence of common bands in two or more species, for example ISSR bands obtained in *O. bistonensis* and *O. bulbotrichum*, as well as in *O. rostellatum* and *O. sericeum* indicate the presence of synapomorphic characters to be used in sister group identification.

The NJ and UPGMA trees obtained from morphological and molecular data partly agrees with each other. In both trees, *O. dasytrichum* and *O. microcarpum* are placed close to each other, *O. procerum* and *O. pachypodum* showed affinity to each other and *O. araraticum*, *O. bodeanum*, *O. bistonensis*, *O. stenosiphon* and *O. bulbotrichum* are placed close to each other while *O. rostellatum* stands far from the other species.

The tree of combined morphological and ISSR data (Figure 3), clearly separates the members of three sections of *Onosma*, *Podnosma* and *Protonosma* from each other. Moreover, almost a good separation of different subsections in the sect. *Onosma* occurs in this combined tree. For example all members of the first major cluster are from the subsect. *Haplotricha*. Therefore, molecular data along with morphological characters may be of use in taxonomic treatment of the genus *Onosma*.

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