Seed coat morphology of the genus *Juncus* L. (Juncaceae) and its systematic significance in Northeast of Iran

Raheleh Ahmadpour1, Jamil Vaezi2, Ahmad Reza Bahrami1, Farshid Memariani3

1 Department of Biology, Faculty of Sciences, Ferdowsi University of Mashhad, Mashhad, Iran
2 Department of Biology, Faculty of Sciences, Shahid Chamran University of Ahvaz, Ahvaz, Iran
3 Research Centre for Plant Sciences, Ferdowsi University of Mashhad, Mashhad, Iran

*correspondence should be addressed to Jamil Vaezi, Department of Biology, Faculty of Sciences, Shahid Chamran University of Ahvaz, Ahvaz, Iran; Tel: +986113331045; Fax: +986113331045; Email: J.vaezi@Scu.ac.ir.

**ABSTRACT**

The seed morphology of six species of the genus *Juncus* L. (Juncaceae), involving wild annuals and perennials was studied in Northeast of Iran using Light and Scanning Electron Microscope (SEM). Seed morphology (light microscope) of the investigated species exhibits certain variations in shape, size and surface ornamentation. Results of SEM analysis reveals that there are different patterns of seed surface. Seed shape and surface structure exhibit some diversity among taxa indicating systematic significance in species differentiation. An identification key to the investigated taxa is provided based on seed characters in this research.

**Key words:** Seed coat morphology, SEM, light microscopy, Juncus, systematic significance, Iran

Copyright © 2012 Raheleh Ahmadpour et al. This is an open access article distributed under the Creative Commons Attribution License.

1. **INTRODUCTION**

The genus *Juncus* L. (Juncaceae) is a widely distributed rush with nearly 220 species identified worldwide and 20 species in Iran (1). Rushes are found in diverse habitats and occupy areas of every continent except Antarctica (2). Most diversity of the genus is in mesophytic and boreal regions of the world. For the first time, this genus was described by Linnaeus in 1753 (2). He reported 15 species of *Juncus* and divided them into two groups, according to stem type. After Linnaeus, several authors (e.g., Boissier (1881); Buchenau (1875, 1890, 1906)) have studied this genus based on the morphological characters and tried to provide a system to divide the genus into subgenera, sections and subsections (3, 4). Revisionary studies revealed that systematic significance of seed coat morphology, rarely investigated in *Juncus*. Buchenau (1867) and Engelmann (1866) investigated the seed morphology of *Juncus* using light microscopy (5-7). These studies formed the basis of *Juncus* seed investigations (6, 7). Brooks and Khun (1986) studied seed morphology of 15 species of four subgenera of *Juncus* from Kansas by using light and Scanning Electron Microscopy (SEM) (8). Knapp and Naczi (2008) investigated taxonomy, morphology, and geographic distribution of the *Juncus longii* complex and studied the seeds of three species of *Juncus* by SEM. Results of this study revealed that the seeds of these species differ in size and shape (9). Abdel Khalik (2010) studied seed coat morphology and systematic significance for 10 species of *Juncus* from Egypt and presented a key based on the seed coat morphology for identification of the investigated taxa (10). In the present study we used Light and Scanning Electron Microscopy in order to compare seed morphology and seed-coat traits among species of the genus *Juncus* distributed in the Northeast of Iran (North, South, and Razavi Khorassan provinces). The aim is to determine if seed-coat morphology provides useful characters in interspecies differentiation.

2. **MATERIALS AND METHODS**

2.1. **Authentication of plant material**

Some seed samples were obtained from collected plants (*Juncus articulatus* L., and *J. fontanesii* subsp. kotschyi (Boiss.) Snogerup) during 2011 from different localities of the Northeast of Iran (Table 1). The others (*J. turkestanicus...
V. Krecz and Gontsch., J. rechingeri Snogerup, J. bufonius L., and J. inflexus L.) were taken from herbarium specimens of FUMH (Ferdowsi University of Mashhad Herbarium) (Table 1). The seeds were selected from the specimens of the species from different populations of the Northeast of Iran. The taxa were identified using Flora Iranica, Flora of Pakistan, Flora of Turkey, Flora of Iraq, Flora of USSR, Flora of Palestine, and Flora of Egypt. First the mature seeds were soaked in ETOH 50% for 48 hours. Then air dried seeds were examined for shape, size and color using Light microscope and stereomicroscope (Dino-Lite) with the help of DinoCapture eye and DinoCapture 2.0 Software (Electronics Corporation). All photographs of seeds were saved in TIF format. For SEM studies, the seeds enveloping were removed and were acetolyzed in a 1:9 sulfuric acid–acetic anhydride solution. The seeds were vigorously shaken for 5 min. Then, they were left for 24–48 h in the solution. After this time, seeds were again shaken for 5 min and then washed in distilled water by shaking for a further 5 min. The seeds were dried overnight and then were mounted on stubs and covered with Au-Pd by sputter coater model SC 7620. After coating, coated seeds were photographed with a LEO 1450 VP Scanning Electron Microscope. All photographs were taken at the central laboratory (Faculty of Sciences, Ferdowsi University of Mashhad, Iran). The terminology of Abdel Khalik and Maesen (2002), Abdel Khalik and Osman (2007) and Abdel Khalik (2010) was adopted to describe the seed surface patterns (3, 10, 11).

Table 1. List of seed specimens used in LM and SEM studies

<table>
<thead>
<tr>
<th>No</th>
<th>Taxon</th>
<th>Life cycle</th>
<th>Kirschner (2002a, 2002b, 2002c)</th>
<th>Locality</th>
<th>Collection Date</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>J. articulatus L.</td>
<td>Perennial</td>
<td>Subgen. Juncus Sect. Ozophyllm</td>
<td>Iran, South Khorassan province, Beyhood village</td>
<td>2011</td>
</tr>
<tr>
<td>2</td>
<td>J. fontanesii subsp. kotschyi (Boiss.) Snogerup</td>
<td>Perennial</td>
<td>Subgen. Juncus Sect. Ozophyllm</td>
<td>Iran, Razavi Khorassan province, Kalat Naderi</td>
<td>2011</td>
</tr>
<tr>
<td>3</td>
<td>J. turkestanicus V. Krecz &amp; Gontsch</td>
<td>Annual</td>
<td>Subgen. Agathryon Sect. Tenageia</td>
<td>Iran, Razavi Khorassan province, Daregaz</td>
<td>2007</td>
</tr>
<tr>
<td>4</td>
<td>J. rechingeri Snogerup</td>
<td>Annual</td>
<td>Subgen. Agathryon Sect. Tenageia</td>
<td>Iran, North khorassan province, Bojnourd</td>
<td>2006</td>
</tr>
<tr>
<td>5</td>
<td>J. bufonius L.</td>
<td>Annual</td>
<td>Subgen. Agathryon Sect. Tenageia</td>
<td>Iran, Razavi Khorassan province, Chenaran</td>
<td>2006</td>
</tr>
</tbody>
</table>

3. RESULTS AND DISCUSSION

3.1. Light Microscopy investigation

A wide range of variations were found in shape of seeds among the investigated taxa (Figure 1). Seeds are ovoid in J. articulatus and J. fontanesii subsp. kotschyi, kidney-shaped in J. rechingeri, ovoid to elliptic in J. bufonius, orbicular to elliptic in J. turkestanicus, and fusiform in J. inflexus (Table 2 and Figure 1). Seed dimensions vary more or less among the examined taxa as the largest fusiform seed belongs to J. inflexus, with 0.5-0.6 mm in length (Figure 1), and ovoid to elliptic seed of J. bufonius, with 0.3-0.4 in diameter. In overall, the smallest seed belongs to J. fontanesii subsp. kotschyi, with 0.1-0.2 × 0.3-0.4 mm, while the other species have slightly larger seeds (Table 2 and Figure 1). Seed color seems to be of less diagnostic and of systematic interest among species. The color of seed varies from yellowish-brown in J. turkestanicus, reddish-brown in J. inflexus, pale brown in J. fontanesii subsp. kotschyi to brown in J. articulatus and J. rechingeri, while it is dark-brown in J. bufonius (Table 2).
3.2. Scanning Electron Microscopy investigation
The surface patterns and epidermal cell shapes of seeds are of highly diagnostic and of systematic value among the species. The surface pattern is reticulate in *J. articulatus* and *J. inflexus*, faveolate-reticulate in *J. fontanesii* subsp. *kotschyi*, alveolate-reticulate in *J. bufonius*, rugose in *J. rechingeri* and smooth in *J. turkestanicus* (Figure 2, Figure 3 and Table 2).

<table>
<thead>
<tr>
<th>No</th>
<th>Species</th>
<th>Shape</th>
<th>Size (mm) 3D measurement required</th>
<th>Color</th>
<th>Surface pattern</th>
<th>Epidermal cell shape</th>
<th>Anticlinal cell wall</th>
<th>Periclinal cell wall</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><em>J. articulatus</em> L.</td>
<td>Ovoid</td>
<td>0.2-0.3×0.4-0.5</td>
<td>Brown</td>
<td>Reticulate</td>
<td>Irregular to 4-5 gonals</td>
<td>Raised, sinuous, folded, with clear longitudinal streaks</td>
<td>Flat to concave, smooth to microreticulate</td>
</tr>
<tr>
<td>2</td>
<td><em>J. fontanesii</em> subsp. <em>kotschyi</em> (Boiss.) Snogerup</td>
<td>Ovoid</td>
<td>0.1-0.2×0.3-0.4</td>
<td>Pale brown</td>
<td>Faveolate-reticulate</td>
<td>Irregular to 4-5 gonals</td>
<td>Raised, sinuous, folded, with clear longitudinal streaks</td>
<td>Flat, obscurely striate</td>
</tr>
<tr>
<td>3</td>
<td><em>J. turkestanicus</em> V.Krecz &amp; Gontsch</td>
<td>Orbicular to elliptic</td>
<td>0.2-0.3×0.3-0.4</td>
<td>Yellowish brown</td>
<td>Smooth</td>
<td>Polygonal to elongate in one direction</td>
<td>Raised, sinuous, smooth to fine folded</td>
<td>Flat to concave, smooth to microreticulate</td>
</tr>
<tr>
<td>4</td>
<td><em>J. rechingeri</em> Snogerup.</td>
<td>Kidney shaped</td>
<td>0.2-0.3×0.4-0.5</td>
<td>Brown</td>
<td>Rugose</td>
<td>Ambigious</td>
<td>Slightly channeled, raised, straight, smooth to fine folded</td>
<td>Flat to slightly reticulate</td>
</tr>
<tr>
<td>5</td>
<td><em>J. bufonius</em> L.</td>
<td>Ovoid to elliptic</td>
<td>0.3-0.4×0.4-0.6</td>
<td>Dark brown</td>
<td>Alveolate-reticulate</td>
<td>Polygonal to elongate in one direction</td>
<td>Raised, straight, smooth to fine folded</td>
<td>Flat to concave, smooth to microreticulate</td>
</tr>
<tr>
<td>6</td>
<td><em>J. inflexus</em> L.</td>
<td>Fusiform</td>
<td>0.2-0.3×0.5-0.6</td>
<td>Reddish brown</td>
<td>Reticulate</td>
<td>Elongate in one direction</td>
<td>Raised, smooth</td>
<td>Flat to concave, microreticulate</td>
</tr>
</tbody>
</table>

The shapes of epidermal cells show significant variations among the species of the genus. They are irregular with 4-5 sides in *J. articulatus* and *J. fontanesii* subsp. *kotschyi* (Figure 2). The epidermal cells are polygonal to elongate in one direction in *J. turkestanicus* and *J. bufonius* (Figure 2, and Figure 3), elongate in one direction in *J. inflexus* and ambiguous in *J. rechingeri* (Figure 3). Anticlinal and periclinal cell walls can also serve as good diagnostic characters for
Juncus species. Anticlinal cell walls are generally well-developed in the species under study. In this investigation, four types of anticlinal cell boundaries were observed: 1) Raised, sinuous, folded with longitudinal streaks in J. articulatus and J. fontanesii subsp. kotschyi (Figure 2); 2) Raised, straight to sinuous, smooth to fine folded in J. turkestanicus and J. bufonius (Figure 2, Figure 3); 3) Slightly raised channeled, straight and smooth to fine folded in J. rechingeri (Figure 3); and 4) Raised, straight and smooth in J. inflexus (Figure 3).

There are four different shapes for the periclinal cell wall including 1) Flat to concave, smooth to micro-reticulate in J. articulatus, J. turkestanicus, and J. bufonius (Figures 2 and 3); 2) Flat, obscurely striate in J. fontanesii subsp. kotschyi (Figure 2); 3) Flat to slightly reticulate in J. rechingeri (Figure 3); and 4) Flat to concave, micro-reticulate in J. inflexus (Figure 3).

Figure 2. SEM photographs representing seed surface patterns in the genus Juncus. A and B - J. articulatus C and D - J. fontanesii subsp. kotschyi E and F - J. turkestanicus (left ×500 magnification, right ×2000 mag.)
Morphological characters of seeds provide valuable information on the evolutionary classification of flowering plants. Brooks and Khun (1986) and Abdel Khalik (2010) presented and discussed some results from macro- and micro-morphological studies of *Juncus* (8, 11). In our observation seed characters of *Juncus* vary among different species, particularly with regard to seed shape, size, coat pattern, epidermal cell shape and anticlinal and periclinal cell wall (Table 2). The seed morphology, especially surface ornamentation, is useful in suggesting relationships at both the sub generic and specific levels (9, 12, 13). Different patterns of seed morphology are helpful in distinguishing various species and partially confirm the subgeneric and sectional classification of the genus *Juncus*.

The micro-morphological studies of seeds presented here reveal that their surface features are reticulate, faveolate-reticulate, alveolate-reticulate, and rugose patterns. The reticulate type of seed surface sculpturing is more common among the species studied. The seeds that were used in this study were from different subgenera and sections of *Juncus* (Table 1). *Juncus articulatus* and *J. fontanesii* subsp. *kotschyi* are two species that belong to the subgen. *Juncus* sect. *Ozophyllum* (Table 1). These species are obviously similar in morphology and outline seed shape but differ from each other in ornamentation of seed coat (Table 2; Figure 2). *Juncus inflexus* (subgen. *Agathryon* sect. *Juncotypus*, Table 1), has fusiform seeds (Figure 3). This character can easily differentiate this species from the other...
taxa. Brook and Khun (1986) indicated that the ornamentation of seeds in sections Juncocytus and Tenageia (Figures 2 and 3) is similar, while in this research the species of these sections have no similarity in ornamentation and were easily differentiated, especially with regard to characters such as the seed shape, epidermal cell shape, and anticlinal-periclinal cell walls (14, 15). Although J. turkestanicus, J. rechingeri and J. bufonius (subgen. Agathryon sect. Tenageia) are greatly similar in other aspects of their morphology they differ from each other on the basis of seed shape and seed size. Therefore, these characters can be useful for separation of the taxa in the vast region of Northeast of Iran. Abdel Khalik (2010) investigated seed coat morphology of 10 species of Juncus from Egypt (10, 11). Their results, concerning the species J. inflexus and J. bufonius, are in disagreement with those of our study. As indicated in the materials and methods section we identified and verified the examined taxa using various floras. All these taxa were also included in the morphometric investigation to better delimit the species boundaries. Therefore we reached the conclusion that the previous identification (Abdel Khalik 2010) of these species was not valid. The results of this study are consistent primarily with the morphological classification in Flora Iranica (Snogerup, 1971) (8, 15, 16).

4. CONCLUSION

In conclusion, the present study supports the application of seed morphological characters as a parameter for species identification of genus Juncus. As a result, seeds in Juncus have partly unique features that facilitate species recognition and description. Therefore seed morphological characters are very important in separating species and these features can be used in preparing an identification key.

ACKNOWLEDGMENT

Authors are very much thankful to the Department of Biology, Ferdowsi University of Mashhad and also Department of Biology, Lurestan University, Khorram Abad for providing us the facilities to carry out the research work.

Funding/ Support

Not mentioned any Funding/ Support by authors

AUTHORS CONTRIBUTION

This work was carried out in collaboration among all authors.

CONFLICT OF INTEREST

The authors declared no potential conflicts of interests with respect to the authorship and/or publication of this article.

REFERENCES

4. CENTURIA X. EXSICCATA DE FLORA IBERO-MACARONESICA SELECTA.