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

Raheleh Ahmadpour[1], Jamil Vaezi[2*], Ahmad Reza Bahrami[1], Farshid Memariani[3]

[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; Tell: +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

1. INTRODUCTION

The genus Juncus L. (Juncaceae) is a widely distributed rushes 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

No	Taxon	Life cycle	Kirschner (2002a, 2002b, 2002c)	Locality	Collection Date
1	*J. articulatus* L.	Perennial	Subgen. *Juncus* Sect. *Ozophyllm*	Iran, South Khorassan province, Beyhood village	2011
2	*J. fontanesii* subsp. *kotschyi* (Boiss.) Snogerup	Perennial	Subgen. *Juncus* Sect. *Ozophyllm*	Iran, Razavi Khorassan province, Kalat Naderi	2011
3	*J. turkestanicus* V.Krecz & Gontsch	Annual	Subgen. *Agathryon* Sect. *Tenageia*	Iran, Razavi Khorassan province, Daregaz	2007
4	*J. rechingeri* Snogerup	Annual	Subgen. *Agathryon* Sect. *Tenageia*	Iran, North khorassan province, Bojnourd	2006
5	*J. bufonius* L.	Annual	Subgen. *Agathryon* Sect. *Tenageia*	Iran, Razavi Khorassan province, Chenaran	2006
6	*J. inflexus* L.	Perennial	Subgen. *Agathryon* Sect. *Juncotypus*	Iran, North khorassan province, Bojnourd	2008

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).

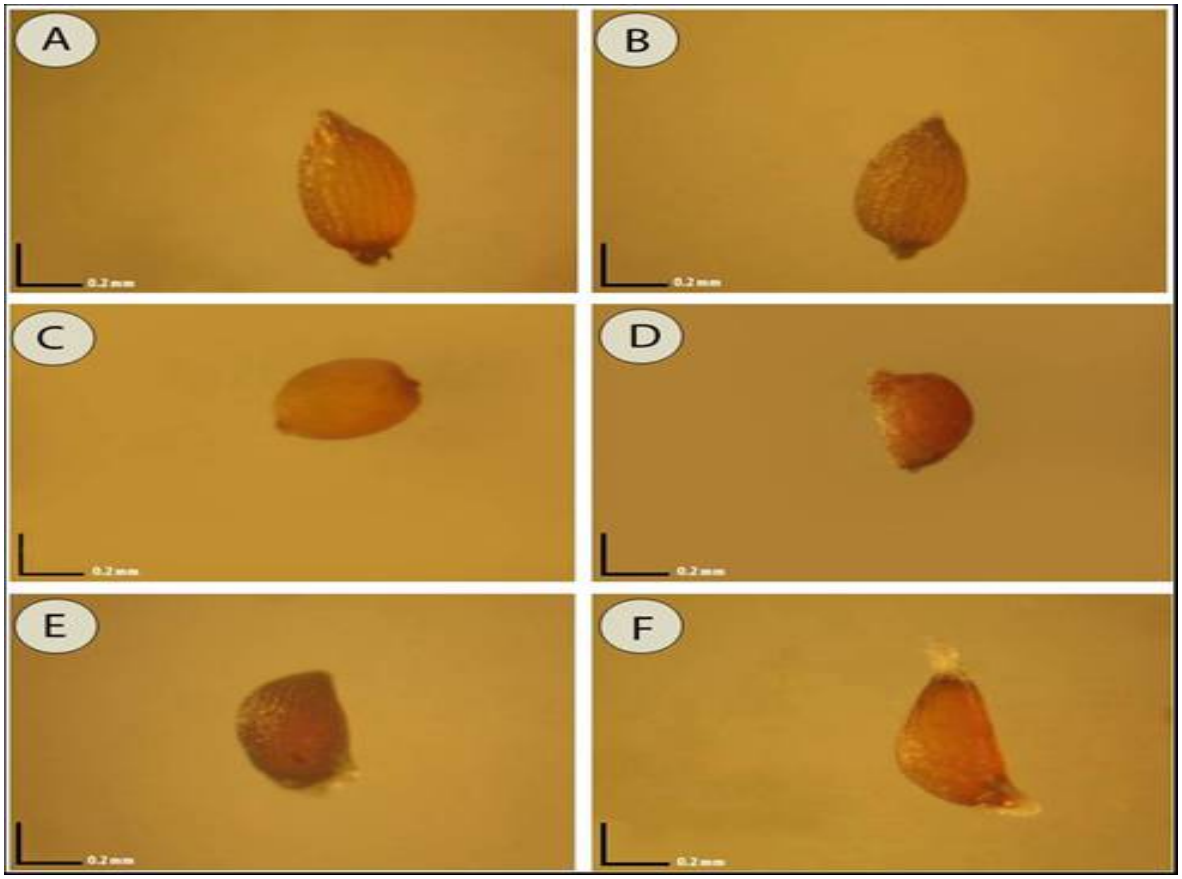

Figure 1. LM photographs representing morphology of seeds in the genus *Juncus*. A - *J. articulatus*, B - *J. fontanesii*subsp. *Kotschyi* C - *J. turkestanicus*, D - *J. rechingeri*, E - *J. bufonius*, F - *J. inflexus*

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 2. Morphological characters of seeds of the Juncus species used in the current study

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

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).

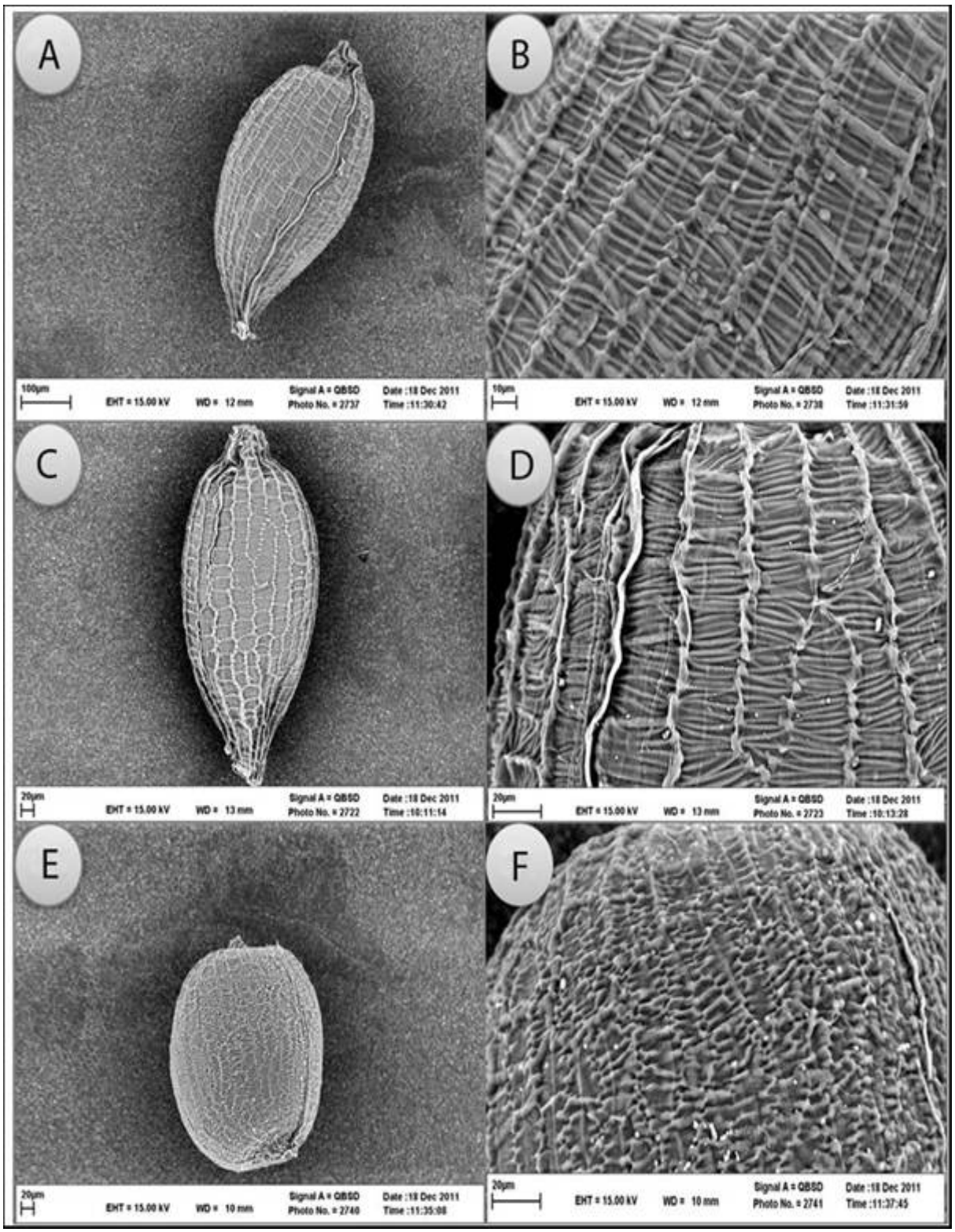

Figure 2. SEM photographs representing seed surface patterns in the genus Juncus. A and B - J. articulatus C and D - J. fontanesii subsp. kotschyi E and F - . J. turkestanicus (left ×500 magnification, right ×2000 mag.)

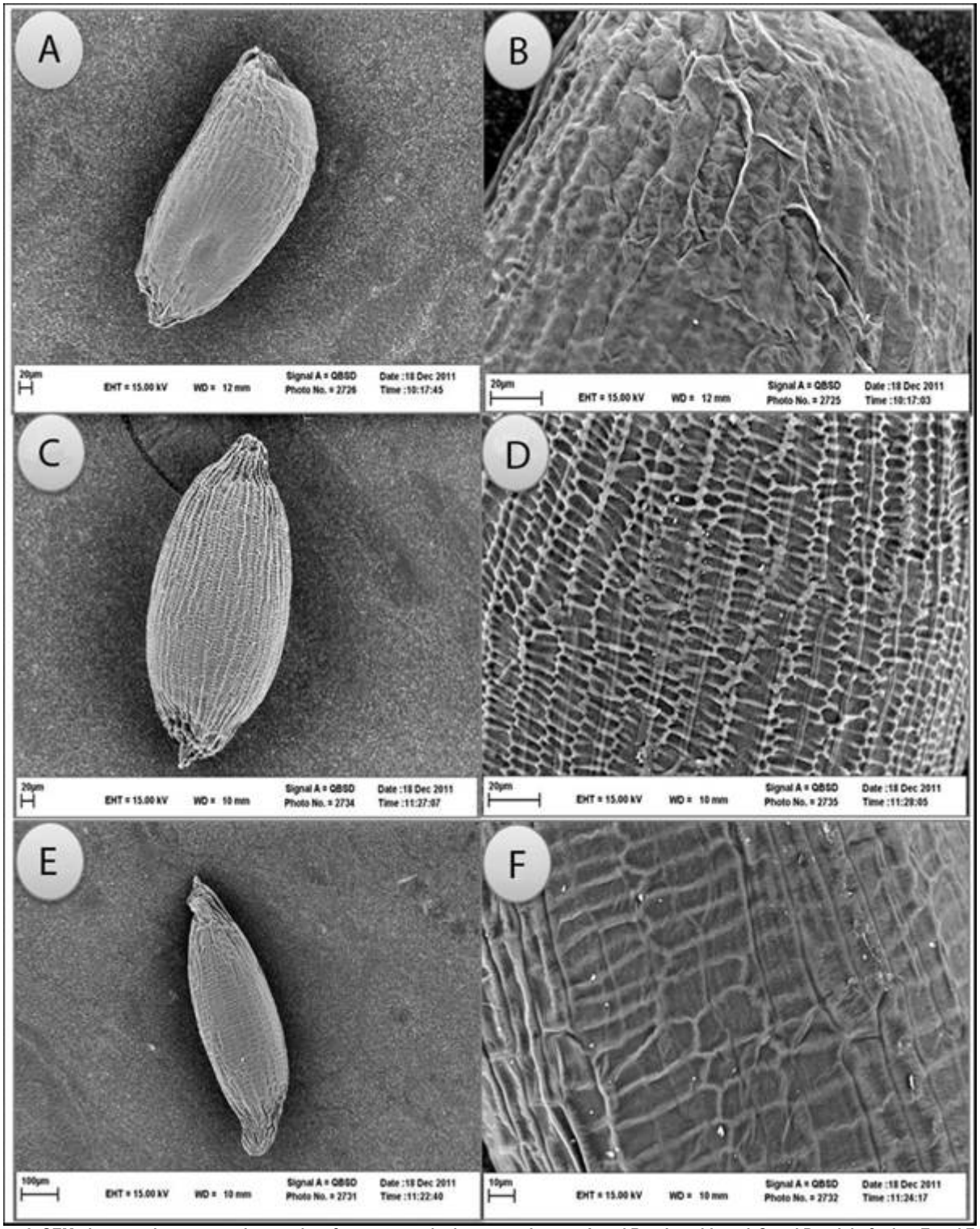

Figure 3. SEM photographs representing seed surface patterns in the genus *Juncus*. A and B - *J. rechingeri*, C and D - *J. bufonius*, E and F - *J. inflexus* (left ×500 magnification, right ×2000 mag.)

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 sub generic 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 *Juncotypus* 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 *Jucus*. 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.

***Attachment**

***Identification key to the Juncus species in Northeast of Iran based on seed characters*

1a. Seeds with smooth surface or with no clear longitudinal streaks.....2

b. Seeds reticulate or with clear longitudinal streaks.....5

2a. Seeds orbicular to elliptic shape; yellowish brown; epidermal cell shape polygonal to elongate in one direction; 0.2-0.3 × 0.3-0.4 mm.....*J. turkestanicus*

b. Seeds fusiform and elongated or elliptic.....3

3a. Seeds fusiform and elongated; reddish-brown; with two appendages at both ends; anticlinal cell wall raised, straight, and smooth.....*J. inflexus*

b. Seeds ovoid-elliptic or kidney shape.....4

4a. Seeds kidney shape; brown; partly reticulate; anticlinal cell wall slightly raised channeled, straight, smooth to fine folded; 0.2-0.3 × 0.4-0.5 mm.....*J. rechingeri*

b. Seeds ovoid to elliptic; dark brown; raised, anticlinal cell wall straight to sinuous, smooth to fine folded; 0.3-0.4 × 0.4-0.6 mm .....*J. bufonius*

5a. Seeds pale brown; 0.1-0.2 × 0.3-0.4 mm; periclinal cell wall flat, obscurely striate.....*J. fontanesii* subsp. *kotschyii*

b. Seeds 0.2-0.3 × 0.4-0.5 mm; periclinal cell wall flat to concave, smooth to microreticulate.....*J. articulatus*

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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.

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