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MACRO AND MICRO MORPHOLOGICAL STUDY OF FRUIT AND SEEDS IN THE GENUS *ALYSSUM* (BRASSICACEAE)

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Abstract

In this study, the fruit and seed characteristics of twelve species belonging to 5 sections of the genus *Alyssum* (Brassicaceae) were investigated in 12 populations from different localities in Eskişehir/Turkey. Morphological and micromorphological studies were carried out using stereomicroscope and scanning electron microscopy (SEM) to evaluate the nutlet and fruit characteristics to determine the systematic relationships of 12 species belonging to the genus Alyssum from the family Brassicaceae (Cruciferae). The methods used in the numerical analysis included clustering [unweighted pair group method with arithmetic mean (UPGMA)] and principal component analysis (PCA). PCA allowed for most of the among the seed micromorphology to be highlighted and provided possible explanations for them. According to the UPGMA analysis, the most related taxa were *A. linifolium* with *A. obtusifolium* and *A. niveum* with *A. simplex* As a result of the PCA, it was determined that the extension width as well as the extension length minimum and maximum values are important characters to be used in the classification of *Alyssum*.

Key words: *Alyssum*, Seed, Fruit, SEM, UPGMA, PCA.

Introduction

Brassicaceae is among the largest angiosperm families, and it is represented worldwide by 340 genera and 3350 species. (Rechinger, 1968; Al-Shehbaz, 1986; Khalik & Maesen, 2002; Al-Shehbaz, 2007; Al-Shehbaz, 2012; Abbasian & Keshavarzi, 2016). The genus *Alyssum* L. consists of 195 species native to Europe, Asia, and North Africa. (Al-Shehbaz, 1987; Warwick *et al*., 2006; Al-Shehbaz *et al*., 2007; Bolourian & Pakravan, 2011; Li *et al*., 2014). Most of the species in the genus *Alyssum* L. grow in regions that are rocky and arid (Rechinger, 1968). The genus Alyssum is divided with in the followig 5 sections.

In this study, some of the taxa of the genus *Alyssum*, were in section Meniocus; *A. linifolium* and *A. dasycarpum* in section Psilonema; *A. desertorum*, *A. minitum*, *A. simplex*, and *A. strigosum* in section *Alyssum* p.p.; *A. niveum* in section Gamosepalum; *A. pateri*, *A. murale*, *A. flotusifolium*, and *A. sibiricum* in section Odontarrhena. *Alyssum* siliculae are dehiscent or indehiscent, loculi 1-8-ovulate with nearly apical or distinctly lateral placentation (Davis *et al*., 1988). The micromorphological features of seeds and fruit are important in taxonomic problems (Brochmann, 1992; Khalik & Maesen, 2002). Variations in the seed coat are important factors to be used in infrageneric classification (Barthlott, 1981). There are few taxonomic studies on the seed structure of *Alyssum* (Mirzadeh *et al*., 2015; Cecchi *et al*., 2013; Bulbul *et al*., 2019).

The clustering [unweighted pair group method with arithmetic mean (UPGMA)] results allow for the creation of a hierarchical. The results of principal component analysis (PCA) allow for the reduction of a large number of characters into fewer components and provide an explanation of the relationship that exists between the characters (Karamura, 1999; Lawrey, 2001; Dega & Ercal, 2015; Sarıçam & Müştak, 2015). The aim of the study was testing the morphological as well as the micromorphological characters of the fruit and seeds of some *Alyssum* taxa (Rohlf, 1998).

Methods

Plant material: The seeds and fruit of 12 *Alyssum* taxa were used in macro and micro morphological studies. Samples we collected from natural populations.A total of 20 mature seeds were collected for each taxon. A list all examined taxa is given in (Table 1 and Fig. 1).

Table 1. Locality information of examined taxa.

Section	Taxa	Location
Odontarrhena	*Alyssum murale* Waldst. & Kit.	Mihalıcık; Mihalıcık Road, 39°51'02"N-31°28'00"E, 1194 m, 25.06.2020.
	A. sibiricum Willd.	Sivrihisar; Ballıhisar Village, 39°19'29"N 31°34'43"E, 928 m, 06.05.2020.
	A. pateri Nyar	Alpu; Bozan surroundings, 39°47'25"N 31°06'50"E, 866 m, 12.06.2020.
	A. floribundum Boiss.	Sarıcakaya; İğdir Village, 40°03'00"N 30°38'37"E, 287 m, 20.08.2020.
	A. obtusifolium Steven ex DC.	Sivrihisar; Eskişehir-İzmir Road Slopes, Yeşilköy Village Surroundings, 39°18'26"N 31°27'30"E, 884 m, 06.05.2020.
Alyssum p.p.	*A. desertorum* Stapf	Alpu; Bozan surroundings, 39°47'42"N 31°07'15"E, 910 m, 12.06.2020.
	A. strigosum Banks & Sol.	Günyüzü; Kavuncu Village, 039°25'57"N 31°56'28"E, 727 m, 06.05.2020.
	A. simplex Rudolph	Sivrihisar; Yeşilköy Village, 39°18'19"N 31°29'20"E, 863 m, 06.05.2020.
	A. minutum Schltdl. ex DC.	Günyüzü; Fatih Village, 39°25'33"N 31°52'29"E, 806 m, 06.05.2020.
Psilonema	*A. dasycarpum* Stephan ex Willd.	Günyüzü; Yazır Village, 39°26'16"N 31°48'55"E, 815 m, 06.05.2020.
Meniocus	*A. linifolium* Stephan ex Willd.	Sivrihisar; Yukarıkepen Village 39°25'12"N 31°28'57"E, 983 m, 06.05.2020.
Gamosepalum	*A. niveum* Dudley	Alpu; Bozan surroundings, Southern Western Slope, 39°48'12"N 31°07'38"E, 950 m, 12.06.2020.

Fig. 1. Geographical distribution of the 12 examined species and their locations (1. *A. murale*, 2. *A. sibiricum*, 3. *A. dasycarpum*, 4. *A. desertorum*, 5. *A. floribundum*, 6. *A. linifolium*, 7. *A. minutum*, 8. *A. niveum*, 9. *A. obtusifolium*, 10. *A. pateri*, 11. *A. simplex*, 12. *A. strigosum*).

Optical observation: Optical observation 10 samples of each species, examination of the color and appearance characteristics of the fruit and seeds were conducted under a BX51 stereomicroscope and a camera to study the morphology of the seeds and fruit.

Scanning electron microscopy (SEM): The seeds and fruit were directly mounted and coated with gold using a spray coater for scanning electron microscopy (SEM). SEM examination was performed at Eskişehir Osmangazi University using a JEOL 5600 LV-SEM microscope (JEOL Ltd., Akishima, Tokyo, Japan). Photographs of the live material were taken using a Nikon D5200 digital camera (Nikon Corp., Minato City, Tokyo, Japan).

Quantitative analysis: The numerical analysis results of the *Alyssum* samples are shown in Table 4, the characters used in the numerical analysis (seed length and width) are shown in Fig. 14, and the dendrogram resulting from the UPGMA analysis is shown in Fig. 15. The average of 20 seed measurements from each population was used. Quantitative characters were summarized as the minimum–maximum (mean). Determination of the length-to-width ratio was conducted using the formula LW, in which L is the length and W is the width of the seeds. The length and width of the seeds were measured using ImageJ software (National Institutes of Health, Bethesda, Maryland, USA).

Results

The macromorphological and micromorphological data for the fruit and seed structures of the *Alyssum* indicated considerable variation (Tables 2 and 3). The color, size, shape, indumentum, and surface ornamentation were examined macromorphologically. The seeds were ovoid (*A. murale*, *A. sibiricum*, *A. simplex*, *A. pateri*, *A. linifolium*, *A. strigosum*, *A. niveum*, and *A. obtusifolium*) and ovoid-oblong (*A. minutum*, *A. floribundum*, *A. dasycarpum* and *A. desertorum*) and light brown–dull yellowish (*A. murale*, *A. desertorum*, *A. dasycarpum*, *A. pateri* and *A. floribundum*), light brown–yellowish (*A. sibiricum* and *A. minutum*), dark brown (*A. simplex*), dull yellowish (*A. linifolium*), dull brown–dull yellowish (*A. strigosum* and *A. niveum*) in color. Five different seed surface were observed: undulate in *A. murale*, *A. sibiricum*, *A. strigosum*, *A. niveum*, *A. obtusifolium* and *A. minium*; ruminate in *A. desertorum*; tuberculate in *A. simplex*; reticulate in *A. dasycarpum*, *A. pateri*, and *A. linifolium*; and smooth in *A. floribundum*. The most common ornamentation type was reticulate, while the least common were tuberculate and ruminate (Table 2). The seed sizes varied in length from 0.8 to 2.4 mm and in width from 0.4 to 2.1 mm. These characters were found to be important in delimiting taxa within the genus. The siliculae of the studied taxa were orbicular, ovate, obovate, or obcordate. The most common shape was orbicular-ovate, which was seen in 5 taxa. The length of the fruit ranged from 2.00 to 8.00 mm and the width ranged from 0.8 mm to 4.5 mm. The smallest fruit (*A. pateri*) had a length of 2 mm and a width of 3.5 mm. The largest fruit (*A. floribundum*) had a length of 8 mm and a width of 4.5 mm (Table 2), (Oran, 1996; Abdel, 2005; Bulbul *et al*., 2019).

Table 2. Comparison of the morphological characteristics of the seeds examined of the 12 *Alyssum* taxa.

Taxa	Seed shape	Size (length × width) in mm	Ornamentation on the surface	Color
Alyssum murale Waldst. & Kit.	Ovoid	1−1.2 × 0.7−0.9	Undulate	Light brown−dull yellowish
A. desertorum Stapf	Ovoid-oblong	1−1.4 × 0.5−0.7	Ruminate	Light brown−dull yellowish
A. sibiricum Willd.	Ovoid	1.4−1.7 × 1−1.3	Undulate	Light brown−yellowish
A. simplex Rudolph	Ovoid	2−2.4 × 1.7−2.1	Tuberculate	Dark brown
A.dasycarpum Stephan ex Willd.	Ovoid-oblong	1.1−1.5 × 0.6−0.9	Reticulate	Light brown−dull yellowish
A. pateri Nyar	Ovoid	1−1.3 × 0.8−1	Reticulate	Light brown−dull yellowish
A. linifolium Stephan ex Willd.	Ovoid	0.8−1.1 × 0.4−0.7	Reticulate	Dull yellowish
A. strigosum Banks & Sol.	Ovoid	1.3−1.6 × 1.1−1.2	Undulate	Dull Brown−dull yellowish
A. floribundum Boiss.	Ovoid-oblong	1.4−1.8 × 0.9−1.3	Smooth	Light brown−dull yellowish
A. niveum Dudley	Ovoid	1.8−2 × 1.5−1.9	Undulate	Dull Brown−dull yellowish
A. obtusifolium Steven ex DC.	Ovoid	1.3−1.4 × 1−1.2	Undulate	Light brown
A. minutum Schltdl. ex DC.	Ovoid-oblong	1−1.2 × 0.7−0.9	Undulate	Light brown−yellowish

Table 3. Comparison of the morphological characteristics of the examined fruit of the 12 *Alyssum* taxa.

Taxa	Section	Fruit shape	Fruit (length × width) in mm	Indumentum
Alyssum murale Waldst. & Kit.	Odontarrhena	Orbicular−ovate	2.5−5 × 0.8−4	Stellate
A. desertorum Stapf	*Alyssum* p.p.	Orbicular−ovate	(2−)2.5 × 3−4	Glabrous
A. sibiricum Willd.	Odontarrhena	Obovate−obcordate	3−4 × 2−3.5	Stellate
A. simplex Rudolph	*Alyssum* p.p.	Orbicular	4−6 × 4.5−5.5	Stellate
A.dasycarpum Stephan ex Willd.	Psilonema	Orbicular−ovate	2.5−4 × 2−3.5	Stellate
A. pateri Nyar	Odontarrhena	Obovate	2−3.5(−4) × 2−3.5	Stellate
A. linifolium Stephan ex Willd.	Meniocus	Obovate	3.5−7 × 2−4.5	Glabrous
A. strigosum Banks & Sol.	*Alyssum* p.p.		2−3(−4) × 2−3	Stellate
A. floribundum Boiss.	Odontarrhena	Obovate	5−8 × 4−4.5	Glabrous
A. niveum Dudley	Gamosepalum	Orbicular−ovate	3.5−5 × 3−4	Stellate
A. obtusifolium Steven ex DC.	Odontarrhena	Obovate−orbicular	2−3.5 × 2.5−4.5	Stellate
A. minutum Schltdl. ex DC.	*Alyssum* p.p.	Orbicular−ovate	3−4(−6.5) × 3−4	Glabrous

Table 4. Seed morphology measurements of *Alyssum* (values in mm).

Taxon	$L._{Max}$	$L._{Min}$	$L._{M}$	$W._{Max}$	$W._{Min}$	$W._{M}$
A. murale	1.79	0.92	**1.28**	1.2	0.61	**0.85**
A. sibiricum	1.69	1.38	**1.57**	1.23	0.99	**1.1**
A. dasycarpum	1.55	1.2	**1.43**	1.16	0.68	**0.98**
A. desertorum	1.63	1.42	**1.54**	1.41	1.16	**1.28**
A. floribundum	1.64	0.98	**1.3**	1.33	0.67	**0.96**
A. linifolium	1.68	1.01	**1.34**	1.44	0.7	**0.97**
A. minutum	1.91	1.19	**1.57**	1.67	0.77	**1.08**
A. niveum	2.47	1.77	**2.11**	1.96	1.23	**1.6**
A. obtusifolium	1.54	1.14	**1.34**	1.09	0.85	**1**
A. pateri	1.22	1.03	**1.14**	0.97	0.77	**0.87**
A. simplex	2.44	1.86	**2.14**	1.89	1.48	**1.71**
A. strigosum	1.93	1.28	**1.58**	1.52	0.87	**1.2**

*M: Mean, Min: Minimum, Max: Maximum, L: Length, W: Width

Discussion

The seed and fruit morphological and indumentum characteristics of the genus *Alyssum* in Eskişehir were investigated. The taxonomic importance of the trichomes, which were used to examine the morphology of *A. borzaeanum* Nyar, and showed the formation of stellate hairs, was emphasized by Heywood (1971) and Toma (1977). Inamdar & Rao (1983) examined the trichomes of *A. maritimum* Lam. along with of the Brassicaceae taxa. In the study of Oran (1996), the studied trichomes of 12 taxa belonging to the genus *Alyssum* using a stereomicroscope and SEM (Figs. 2-13).

Result of PCA revealed that morphological characters seed and fruit characteristics were related to the taxa of the genus. *A. desertorum*, *A. simplex* was closely related on two basic seeds and fruit characters where on *A. niveum* showed isolated locations due to gypsum and marly habitat and ecology (Fig. 14). These 3 isolated species exhibited very distinctive morphological characters due to the difficulties brought about by their ecological conditions (Ullah *et al*., 2019).

The UPGMA method produced three clusters. The first UPGMA cluster (Fig. 15) comprised all species populations classified in section *Alyssum*. According to the UPGMA analysis, the most related taxa were *A. linifolium* with *A. obtusifolium*, *A. murale* with *A. pateri*, *A. desertorum* with *A. strigosum*, *A. sibiricum* with *A. minutum*, and *A. niveum* with *A. simplex*. Based on the PCA results, it was determined that the extension width value, as well as the minimum extension length value, were considered to be important characters to be used in the classification of *Alyssum*.

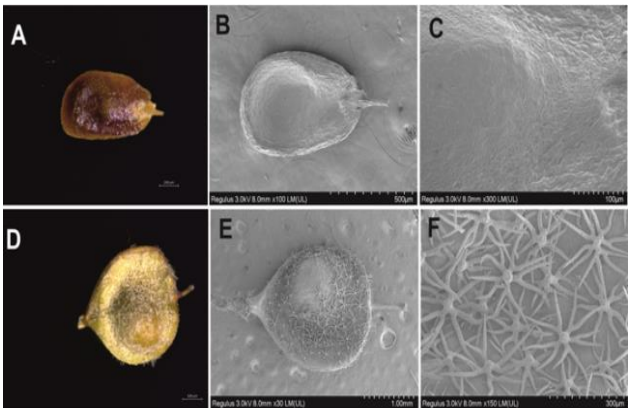

Fig. 2. Section: *Alyssum* p.p., A. desertorum.

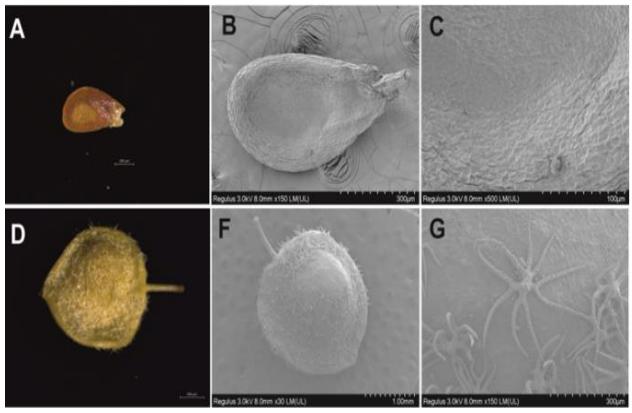

Fig. 3. Section: *Alyssum* p.p., A. *minutum.*

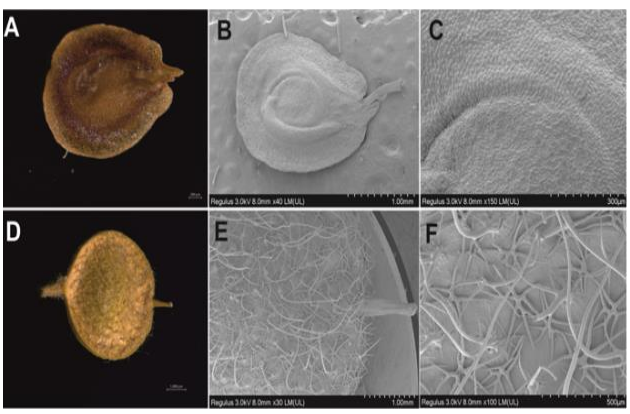

Fig. 4. Section: *Alyssum* p.p., *A. simplex.*

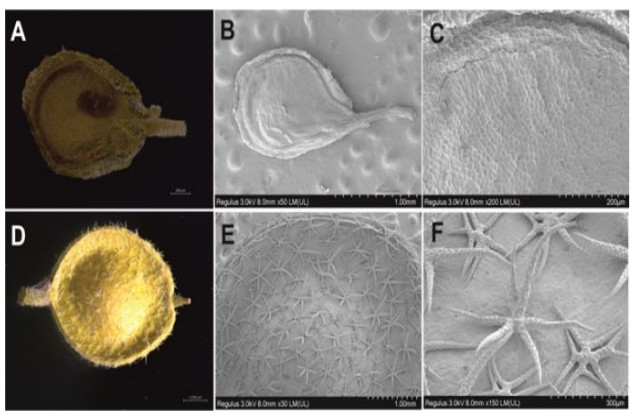

Fig. 5. Section: *Alyssum* p.p., *A. strigosum.*

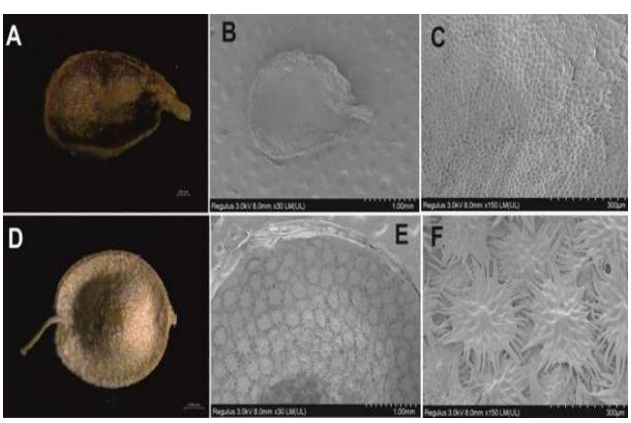

Fig. 6. Section: Gamosepalum *A. niveum.*

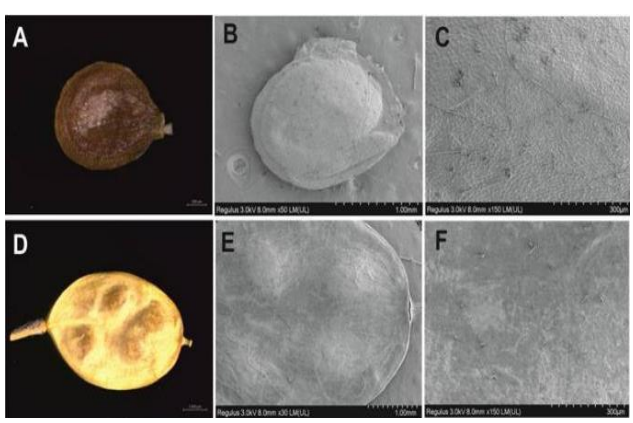

Fig. 7. Section: Odontarrhena, *A. linifolium.*

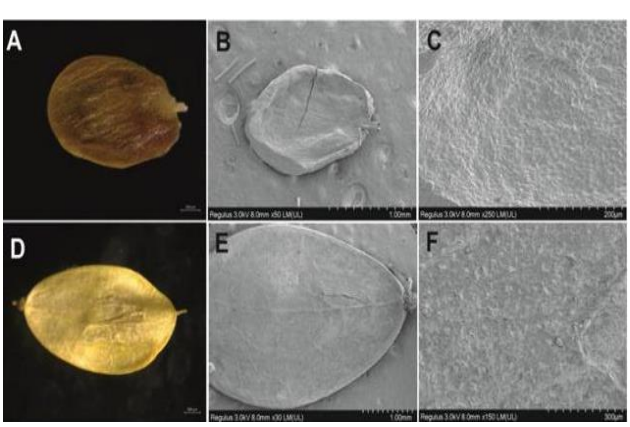

Fig. 8. Section: Odontarrhena, *A. floribundum.*

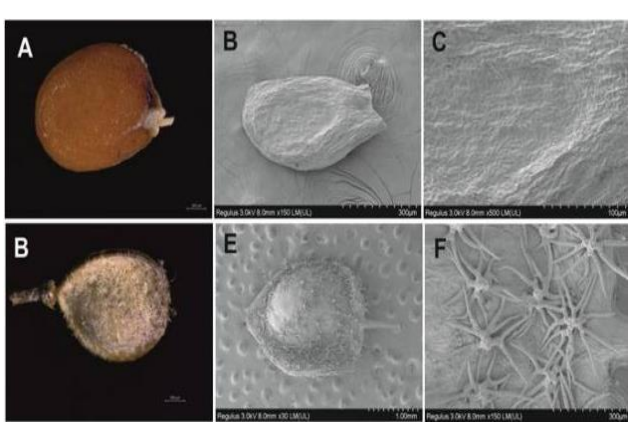

Fig. 9. Section: Odontarrhena., *A. obtusifolium.*

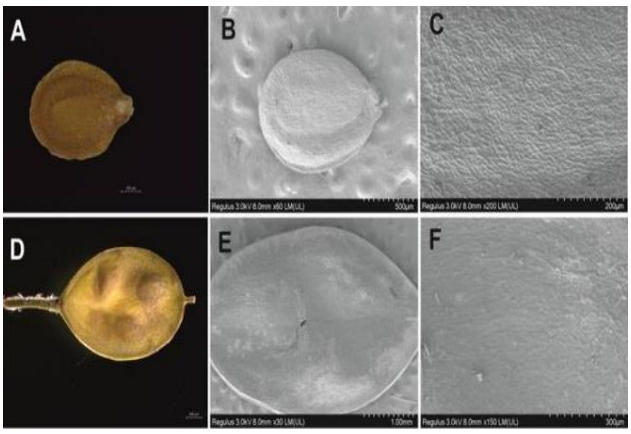

Fig. 10. Section: Odontarrhena, *A. pateri.*

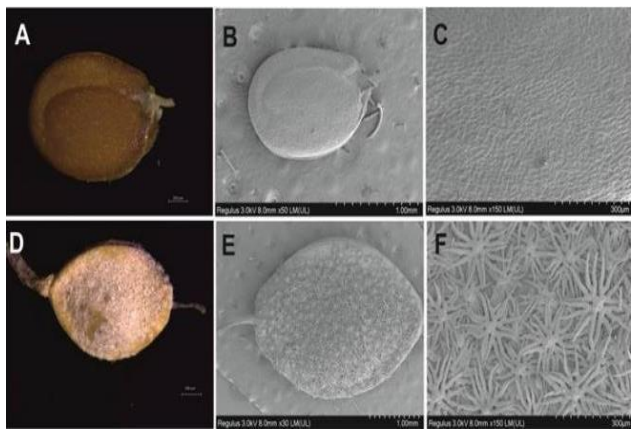

Fig. 11. Section: Odontarrhena, *A. sibiricum.*

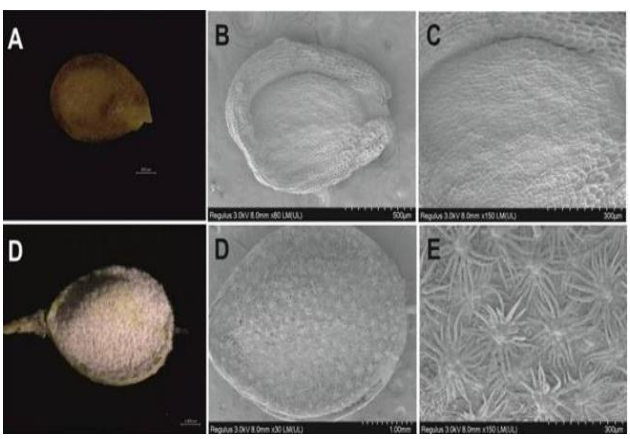

Fig. 12. Section: Odontarrhena, *A. murale.*

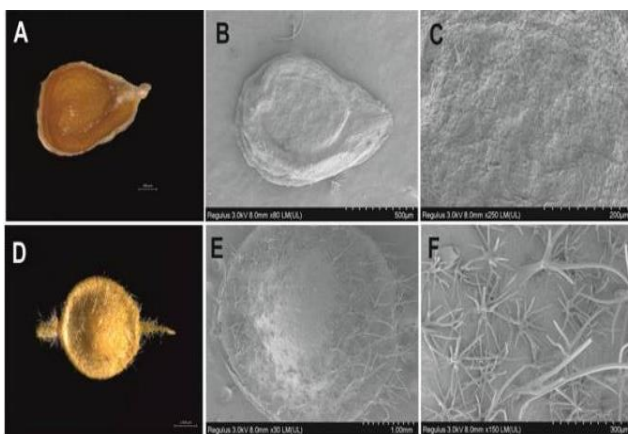

Fig. 13. Section: Psilonema, *A. dasycarpum.*

(A- Stereomicroscope micrographs of the seeds, D- Stereomicroscope micrographs of the fruit, B-C Scanning electron micrographs (SEMs) of the seeds, E- SEMs of the fruit, F- SEM photographs of the indumentum).

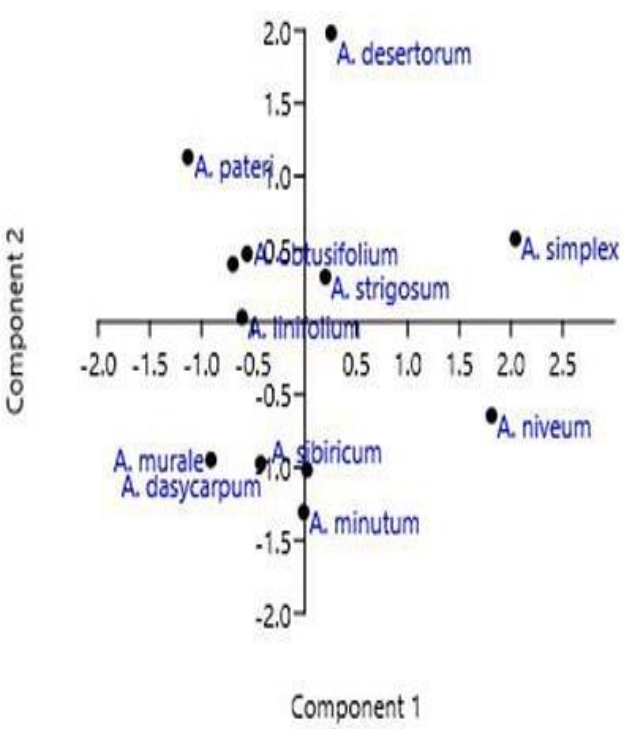

Fig. 14. Principal component axes showing the ordination of the *Alyssum* specimens from the 12 populations.

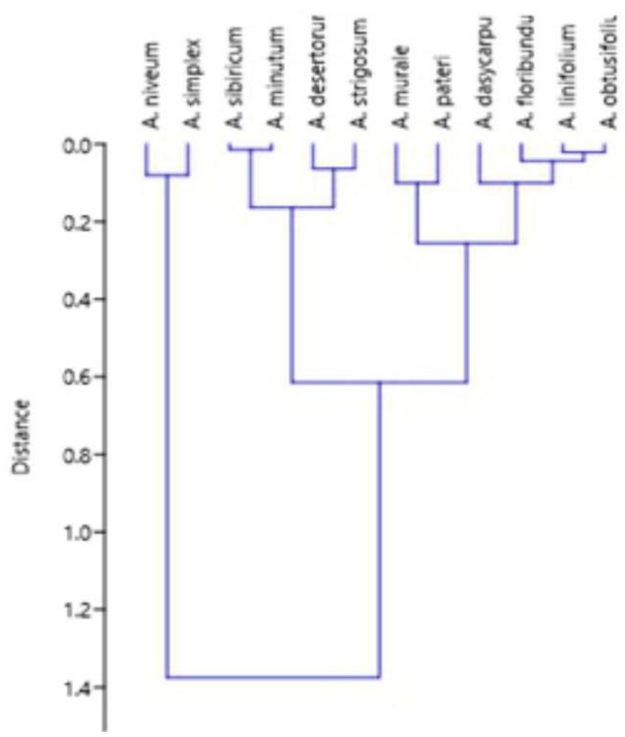

Fig. 15. Phenogram (Ward's method) based on seed micromorphological characters of the twelve *Alyssum* species.

Conclusions

In this study, the seed and fruit morphological (micro and macro) characters assisted in the identification and classification of the taxa in *Alyssum*. The SEM results showed that significant taxonomic variations were present among the different species. The seed and fruit surface morphology provided effective data on their hair structure. The results herein showed which of the characters, i.e., the seed and fruit shape, color, length, and width, provided important characteristics to be used in distinguishing seeds of various species of *Alyssum*. The systematics of the seed and fruit micro and macro morphological characters was detailed with the differences in the selected species. Herein, stereomicroscope macromorphological and SEM micromorphological research of the seeds and fruit, and additional comparative studies conducted on the seed ultrastructural characters of the taxa within the genus *Alyssum* species are needed. The results suggested that it would be useful to the current systematic classification of this complex genus.

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