

**Diagnostic leaf anatomical features of four *Silene* (Caryophyllaceae, *Auriculatae*) species in Iran**Maryam KESHAVARZI ^{*1}, Neda ATAZADEH ¹, Masoud SHEIDAI ²¹ Biology dept., Faculty of Science, Alzahra University, Vanak, Tehran, Iran² Faculty of Biologica sciences, Shahid Beheshti University, Evin, Tehran, Iran**Abstract**

The *Silene* (Caryophyllaceae) species are distributed throughout the northern hemisphere, Europe, Asia and northern parts of Africa. The section *Auriculatae* containing about 35 species, from which 21 are endemic to Iran. Leaf anatomical structure of 18 accessions of four *Silene* species as: *S. commelinifolia*, *S. lucida*, *S. nurensis* and *S. eremicana* was studied. Cross sections were made by hand and studied after double coloration. 14 qualitative and quantitative features were measured and evaluated. Statistical analysis revealed that features as collenchymas presence, hair density at dorsal and ventral leaf surface, shape of vascular bundle, abaxial shape of midrib, cuticle thickness at adaxial surface, leaf diameter at midrib and cortex diameter are significant characters. Present study shows that leaf anatomical features are of diagnostic importance. There is a close relationship between *S. eremicana* and *S. lucida* due to the similarity in collenchymas present and midrib shape. *S. commelinifolia* and *S. nurensis* show similarity due to absence of collenchymas. *S. commelinifolia* var. *comelinifolia* and *S. commelinifolia* var. *ovatifolia* inter-population variation was observed. These two varieties have different midrib and vascular bundle shape.

Key words: leaf anatomy, *Silene*, population**1. Introduction**

The genus *Silene* L. (Caryophyllaceae) is a very large genus of world-wide distribution, containing about 700 species which are mostly hermaphrodite, although a few species are dioecious or gynodioecious (Bari, 1973; Greuter, 1995). *Silene* species are mostly distributed throughout the northern hemisphere, Europe, Asia and northern parts of Africa. annual, biennial, or perennial herbs with the basic chromosome number $x = (10) 12$. The section *Auriculatae* (Boiss.) Schischkin is the largest section of the genus containing about 35 species, from which 21 species are endemic to Iran (Melzheimer, 1980). The members of this section are caespitose mountainous plants with large flowers placed at the end of short stems. Metcalfe and Chalk (1950) presented some anatomical features of Caryophyllaceae family containing some minute details of *Silene*. Mentioned details were about stomata main type which recorded diacytic (few records of anisocytic), glandular hairs and oxalate crystal presence. Diacytic type is a diagnostic feature in Pink family (Sahreen et al., 2010). Jafari et al. (2008) used epidermis features in their taxonomic studies of *Silene* species in Iran. Yildiz and Minareci (2008) recorded glandular hairs and stomata in both leaf sides in *Silene urvillei*. Sahreen et al. (2010) studied leaf anatomical features of some *Silene* species in Pakistan. They found a great variation in leaf epidermis features as shape and size of epidermal cells, hairs (glandular, eglandular, simple or multicellular) and crystals (Shape and composition) which were of diagnostic value. Sahreen et al. (2010) recognized differences between adaxial and abaxial leaf surface. Kiliç (2009) studied leaf and stems anatomical structure of *Silene* species in Turkey and found that cuticle thickness, hair types of epidermis, sclerenchyma layers, cortex and vascular bundle diameter showed differences in studied taxa. In present study leaf anatomical structure of 18 accessions of four *Silene* species as: *S. commelinifolia*, *S. lucida*, *S. nurensis* and *S. eremicana* are considered for the first time in Iran.

2. Materials and methods

18 populations of *S. commelinifolia*, *S. lucida*, *S. nurensis* and *S. eremicana* have been gathered from different habitats in Iran and studied accordingly (Table 1). From each population three specimens were taken and three

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replications were used for each specimen. Vouchers are deposited in the Herbarium of Alzahra University (AUH). Samples were taken from the middle part of the leaf blade. Leaf materials were prepared by hand-made cross sections and double coloration by methyl green and Congo red. Photos were taken by a light microscope with an Olympus DP12 digital camera. The quantitative characteristics were measured by UTHSCSA Image tool, version 3.0 (2002). The terminology of Cullen (1978) and Stearn (1983) was followed for the general outline of leaf cross section. Analysis of variance (ANOVA) was applied to detect the significant differences in the studied characters of various species. To reveal the species relationships, we used cluster analysis, principal component analysis (PCA) (Ingrouille, 1986) and principal coordinate analysis (PCO). For multivariate analysis, the mean values of the quantitative characters were used, while the qualitative characters were coded as binary/multi-state characters. Standardized variables were used for a multivariate statistical analysis. The average taxonomic distances and squared Euclidean distances were used as dissimilarity coefficient in the cluster analysis of anatomical data. In order to determine the most variable anatomical characters among the studied species, a factor analysis based on the principal components analysis was performed. We have used SPSS ver. 9 (1998) and PAST: PAleontological STatistics, ver. 2.17b (4.1MB) (2012) soft wares for statistical analysis.

Table 1. Voucher details of studied *Silene* specie

Taxon	Locality
<i>S. commelinifolia</i> var. <i>ovatifolia</i>	Pyranshahr to Nagadeh, Gerdekashaneh, Likbin village, Kouhe Lande sheikhan, 36 41 7.5 N 45 26 27.1 E, 2400 m.
<i>S. commelinifolia</i> var. <i>ovatifolia</i>	Tehran, Darakeh mountain, 35 49 37.3 N 51 22 47.3 E, 1925 m.
<i>S. commelinifolia</i> var. <i>ovatifolia</i>	West Azarbayejan, Urumiieh, Anhar, Marmisho, Solok, 37 29 0.33 N 44 45 0.22 E, 2327 m.
<i>S. commelinifolia</i> var. <i>ovatifolia</i>	West Azarbayejan, Takab.
<i>S. commelinifolia</i> var. <i>commelinifolia</i>	Mazandaran, Baladeh, Kamarbon, Gosfandsarai-e chai khaksar, 36 14 16.1 N 51 22 17.1E, 2852m.
<i>S. commelinifolia</i> var. <i>commelinifolia</i>	Tehran, Haraz Road, Polur, 35 48 899 N 52 01 643 E, 2405 m.
<i>S. commelinifolia</i> var. <i>commelinifolia</i>	Hamadan, Alisadr cave.
<i>S. cf commelinifoli</i>	Ardebil, Km 30 Ardebil to Kivi, before Neor lake, 38 00 549 N 48 55 225 E, 2590 m.
<i>S. cf commelinifolia</i>	East Azarbayejan, Sarab, Shaleghon village , Bozghoosh mt., 37 45 54 N 47 35 31 E, 2650-3000 m.
<i>S. eremicana</i>	Hamadan, Alvand mountain, Ganjnameh, 34 43 475 N 48 25 039 E, 2800 m.
<i>Silene lucida</i>	East Azarbayejan, Sarab, Shaleghon village , Bozghoosh mt., 37 45 54 N 47 35 31 E, 2650-3000 m.
<i>S. lucida</i>	Guilan, Kelachai, Rahim Abad, Eshkevarat, Chakol, Boza kouh, 2800-3100 m.
<i>S. lucida</i>	East Azarbayejan, Bostan Abad to Mianeh, km 75.
<i>S. lucia</i>	Urumiyeh, Silvana, Khalilkouh, 37 22 44.5 N 44 48 3.8 E, 2594 m.
<i>S. lucia</i>	Urumiyeh, Silvana, Khalilkouh.
<i>S. cf commelinifolia</i>	Chaharmahal-o Bakhtiari, Farsan, Kuhrang, Zardkouh, 32 18 704 N 50 08 574 E, 3300-3400 m.
<i>S. nurensis</i>	Chaharmahal-o Bakhtiari, Farsan, Kuhrang, Zardkouh, 32 18 704 N 50 08 574 E, 3300-3400 m.
<i>S. nurensis</i>	Lurestan, Azna, Drreh tacht, Oshtorankouh, 33 20 473 N 49 20 347 E, 2680 m.

3. Results

In leaf cross section of *S. commelinifolia* var. *commelinifolia* accessions shape of abaxial midrib was dome-shaped and shape of midrib vascular bundle was ellipsoid (Fig. 1 & 2). In Alisadr population there were higher hairs densities in leaf cross sections. In *S. commelinifolia* var. *ovatifolia* populations abaxial leaf surface at midrib showed a rounded shape with ellipsoid to round vascular bundle (Figs 3- 5). Kivi, Neor and Bozghoosh populations of *S. cf commelinifolia* abaxial leaf surface at midrib was crenate with round vascular bundle (Fig 6). In *S. commelinifolia* there was no collenchymas under epidermis. In Zardkouh population of *S. nurensis* abaxial shape of midrib is swollen (Fig. 11) but in Oshtorankouh population of same species the abaxial surface is smooth with long hairs (Fig. 10). Shape of vascular bundle is round or ellipsoid in this species. In this species collenchyma was absent too. In *S. eremicana* abaxial midrib surface and vascular bundle was round and collenchyma was present under epidermis (Fig. 7). In leaf cross sections of *S. lucida* populations abaxial midrib shape is round or acute and midrib vascular bundle shape was ellipsoid or round. Khalilkouh accession showed long hairs. All accessions of *S. lucida* had collenchyma under abaxial leaf epidermis (Figs 8 & 9).

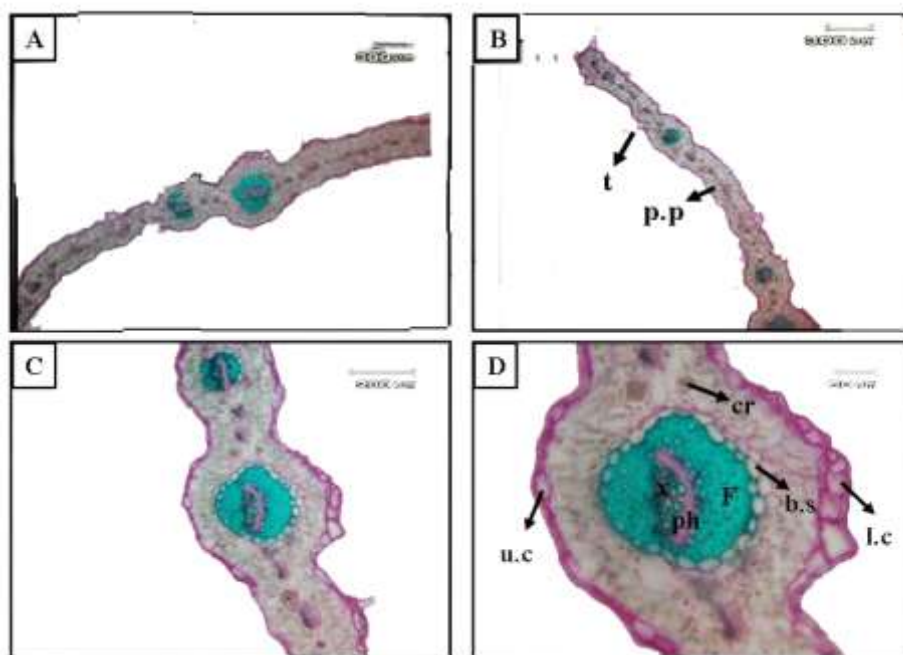


Figure 1. leaf cross section in Alisadr population of *S. commelinifolia* var. *comelinifolia*. Lc: lower epidermis, U.C: upper epidermis, P.P.: palisade parenchyma, Ph: Phloem, X: Xylem, F: Fiber, b.s.: Bundle sheath, cr: crystal, t: trichome

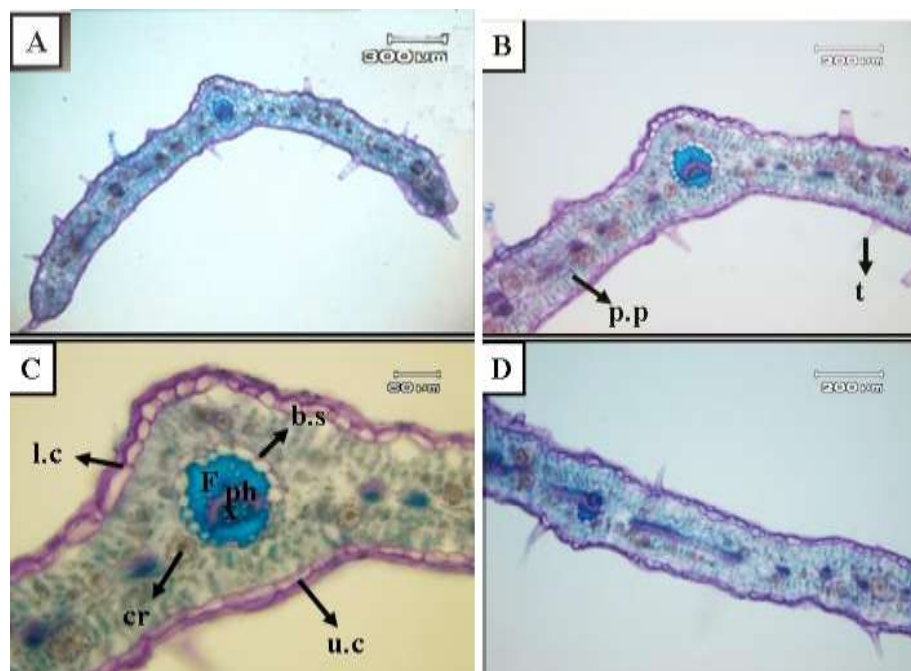


Figure 2. leaf cross section in Mazandaran population of *S. commelinifolia* var. *comelinifolia*. Lc: lower epidermis, U.C: upper epidermis, P.P.: palisade parenchyma, Ph: Phloem, X: Xylem, F: Fiber, b.s.: Bundle sheath, cr: crystal, t: trichome.

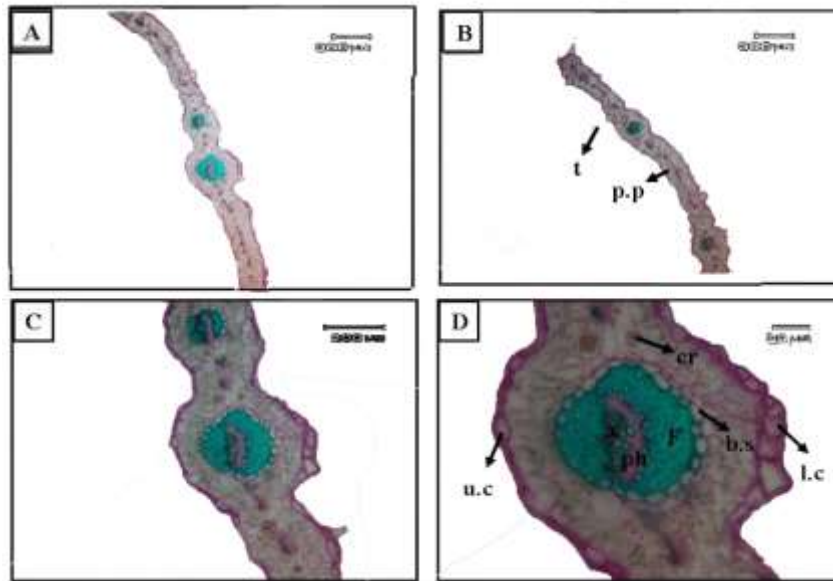


Figure 3. leaf cross section in Marmisho population of *S. commelinifolia* var. *ovatifolia*. Lc: lower epidermis, U.C: upper epidermis, P.P.: palisade parenchyma, Ph: Phloem, X: Xylem, F: Fiber, b.s.: Bundle sheath, cr: crystal, t: trichome.

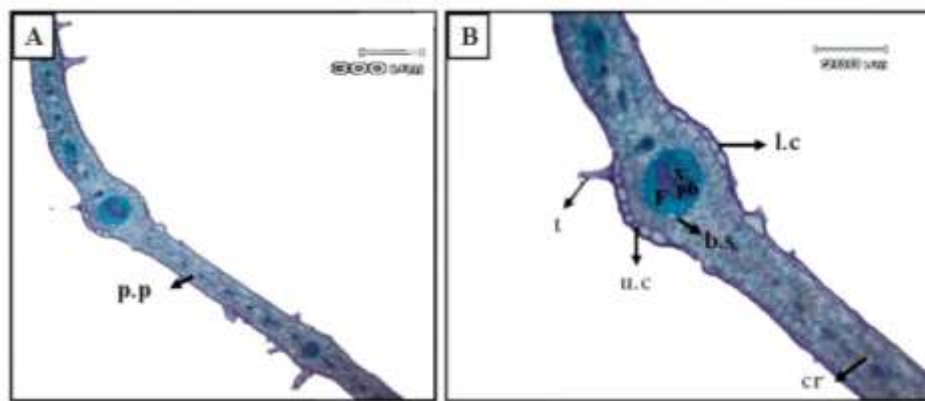


Figure 4. leaf cross section in Darakeh population of *S. commelinifolia* var. *ovatifolia*. Lc: lower epidermis, U.C: upper epidermis, P.P.: palisade parenchyma, Ph: Phloem, X: Xylem, F: Fiber, b.s.: Bundle sheath, cr: crystal, t: trichome.

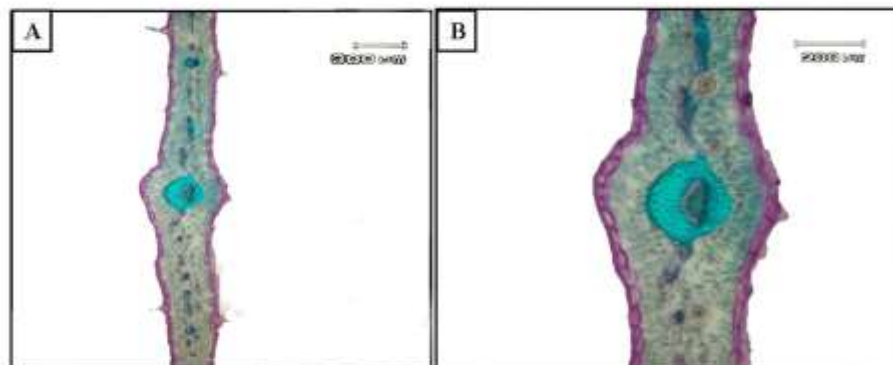


Figure 5. leaf cross section in Pyranshahr- Lande sheikhan population of *S. commelinifolia* var. *ovatifolia*. Lc: lower epidermis, U.C: upper epidermis, P.P.: palisade parenchyma, Ph: Phloem, X: Xylem, F: Fiber, b.s.: Bundle sheath, cr: crystal, t: trichome.

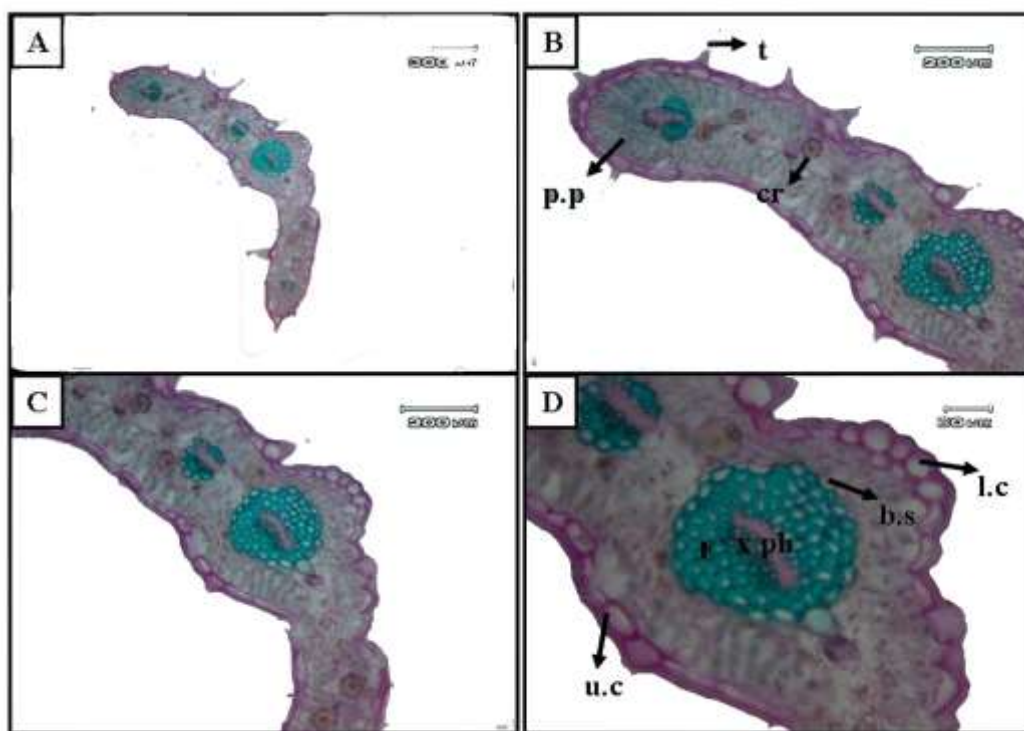


Figure 6. leaf cross section in Sarab- Bozghoosh population of *S. cf commelinifolia* Lc: lower epidermis, U.C: upper epidermis, P.P.: palisade parenchyma, Ph: Phloem, X: Xylem, F: Fiber, b.s.: Bundle sheath, cr: crystal, t: trichome.

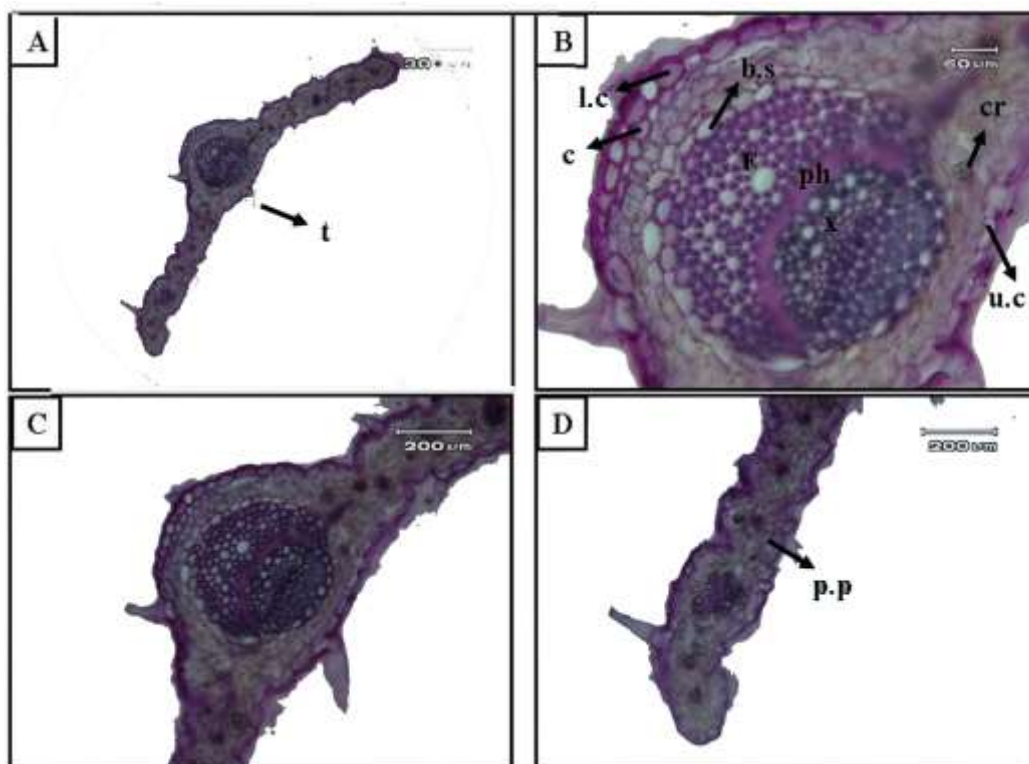


Figure 7. leaf cross section in Alvand population of *S. eremicana* Lc: lower epidermis, U.C: upper epidermis, P.P.: palisade parenchyma, Ph: Phloem, X: Xylem, F: Fiber, b.s.: Bundle sheath, cr: crystal, t: trichome, c: collenchyma.

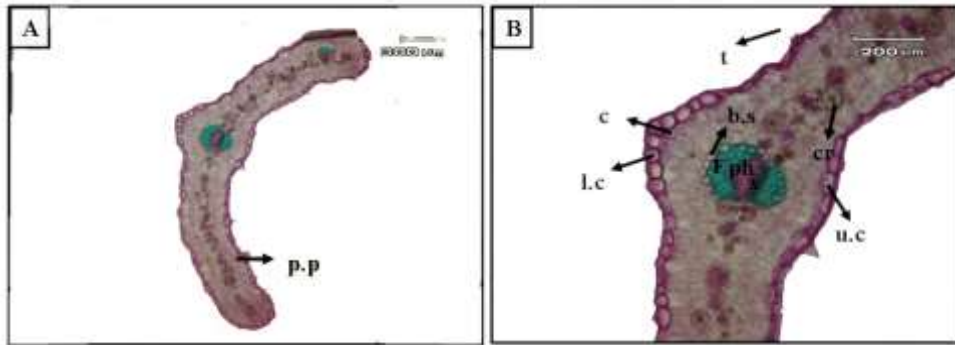


Figure 8. leaf cross section in Sarab- Bozghoosh population of *S. Lucida* Lc: lower epidermis, U.C: upper epidermis, P.P.: palisade parenchyma, Ph: Phloem, X: Xylem, F: Fiber, b.s.: Bundle sheath, cr: crystal, t: trichome, c: collenchyma.

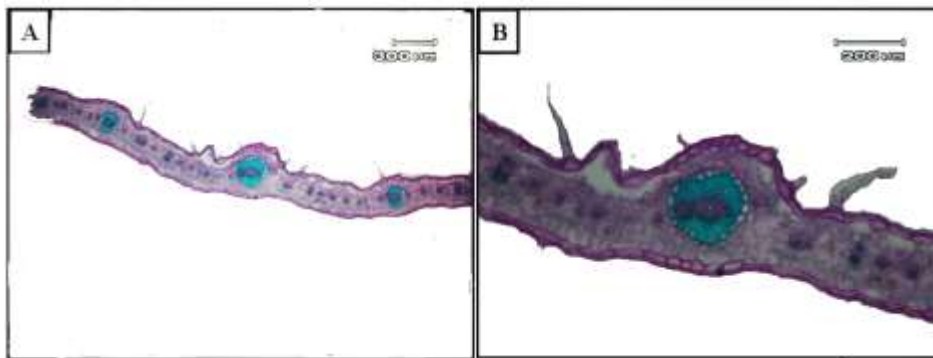


Figure 9. leaf cross section in Khalilkouh population of *S. lucida* Lc: lower epidermis, U.C: upper epidermis, P.P.: palisade parenchyma, Ph: Phloem, X: Xylem, F: Fiber, b.s.: Bundle sheath, cr: crystal, t: trichome, c: collenchyma.

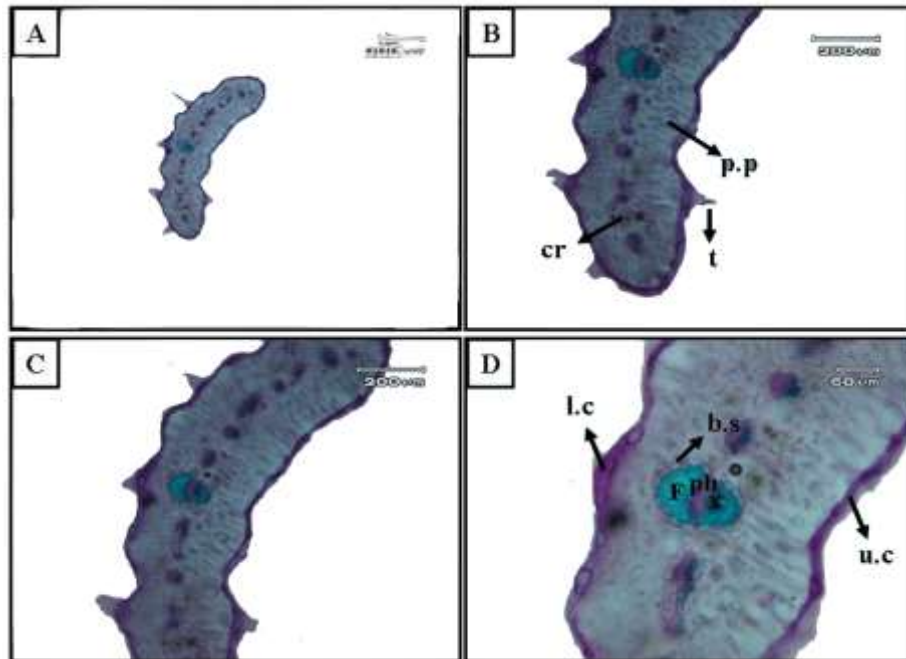


Figure 10. leaf cross section in Oshtorankouh population of *S. cf commelinifolia* Lc: lower epidermis, U.C: upper epidermis, P.P.: palisade parenchyma, Ph: Phloem, X: Xylem, F: Fiber, b.s.: Bundle sheath, cr: crystal, t: trichome.

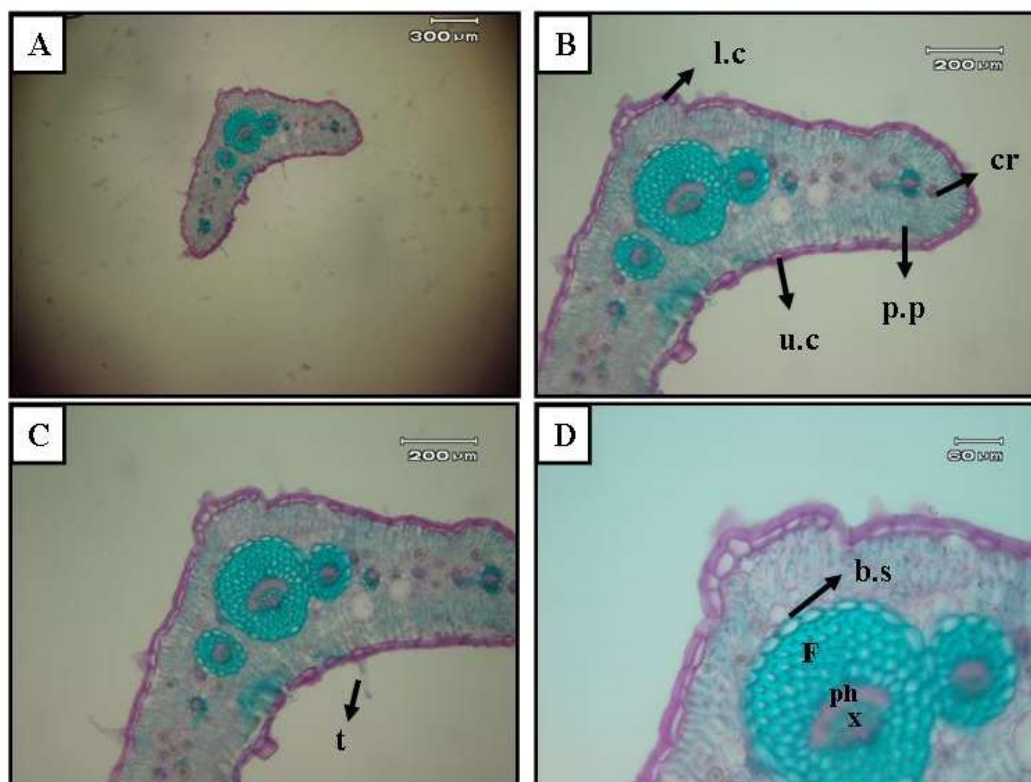


Figure 11. leaf cross section in Zardkouh population of *S. nurensis* Lc: lower epidermis, U.C: upper epidermis, P.P.: palisade parenchyma, Ph: Phloem, X: Xylem, F: Fiber, b.s.: Bundle sheath, cr: crystal, t: trichome.

In order to clarify the species and accessions relationships, cluster analysis by WARD method (Minimum Variance Spherical Cluster) and UPGMA (Unweighted Paired Group With Average Method) were used. To define the most variable character between species and populations Factor analysis was used. Results were plotted by Principle component analysis (PCA) and Principal coordinates analysis (PCO). In UPGMA dendrogram (Figure 12) there are two clusters; main cluster is divided in two subsets. Alvand population of *S. eremicana* and Bozghoosh (luc2), Khalilkouh (luc4) and Guilan (luc1) populations of *S. lucida* are grouped in first subset. Second cluster composed of Mazandaran (Com5), Haraz (Com 6) of *S. Commelinifolia* var. *commelinifolia* and Oshtorankouh (nur4) Population of *S. cf. commelinifolia*, Zardkouh1 (nur 1) and Zardkouh2 (nur 2) of *S. nurensis*, Marmisho population (Com10), Darakeh (Com11), Takab (Com 13) from *S. commelinifolia* var *ovatifolia*, Kivi (Com1), Bozghooh(Com3) from *S. cf. commelinifolia* and two populations of Landesheikhan (com14) of *S. commelinifolia* var *ovatifolia* and Alisadr cave (Com 8) from *S. commelinifolia* var. *commelinifolia* which is somehow far from other populations. Cluster analysis by WARD method is in concordant with UPGMA results (Fig 12). In order to study and define the most variable characters in studied populations' factor analysis was conducted. Four first factors revealed 90.56% of total variation. PCA diagram revealed population relationships based on two coordinates. In first factor with 34.98% of total variation shape of vascular bundle and collenchymas presence show highest correlation(>0.7). In second factor with 23.4% of total variation leaf width in midrib and cortex diameter show the highest correlation (>0.7). in third factor with 16.72% of observed variation, midrib shape has the highest correlation. In fourth factor with 15.46% of variation hair density is most important feature. Dendrogram of cluster analysis is agreed with PCA and PCO graphs (Fig 13).

4. Conclusions

Leaf anatomical study in *S. commelinifolia*, *S. eremicana*, *S. lucida*, *S. nurensis* is presented here for the first time. Results of present study showed that selected features are of taxonomic importance in species and varieties delimitations. There is a close relationship between *S. eremicana* and *S. lucida* due to the similarity in collenchymas present and midrib shape. Between *S. commelinifolia* and *S. nurensis* is a similarity due to absence of collenchymas. Midrib shape, shape of vascular bundle and some other qualitative features show a little variation. *S. commelinifolia* var. *commelinifolia* and *S. commelinifolia* var. *ovatifolia* inter-population variation was observed. These two varieties have different midrib and vascular bundle shape. These features with collenchymas condition, width of leaf at midrib,

cortex diameter and hair density are of diagnostic importance in *Silene* species. These results are in concordant with Kiliç (2009) and Jafari et al. (2008) findings for other species of this genus.

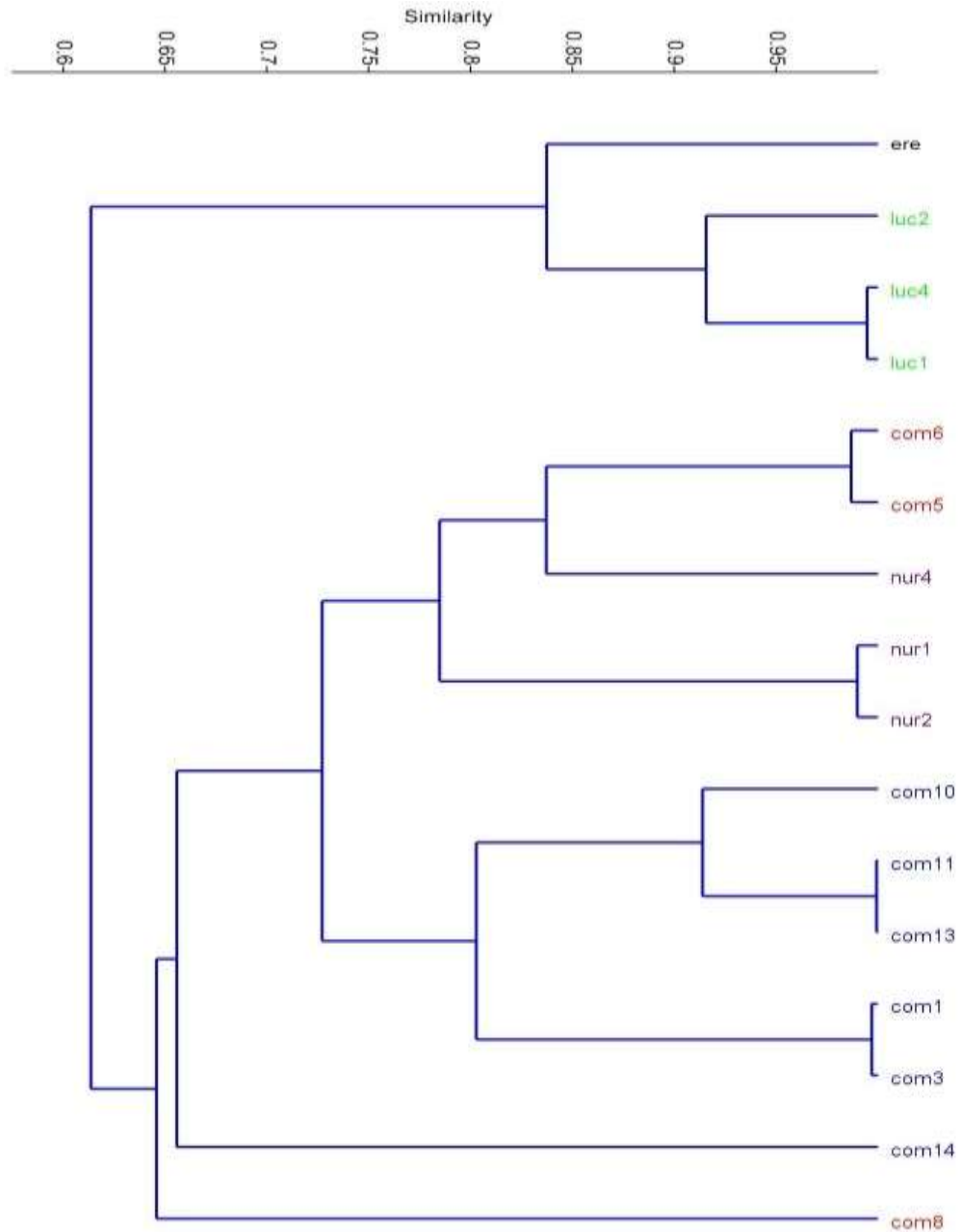


Figure 12. Dendrogram of cluster analysis by UPGMA method, based on leaf cross section. *S. nurensis*(nur1: Zardkouh1, nur2: Zardkouh2), *S. commelinifolia* (Com1: Kivi, Com5: Mazandaran, Com6: Haraz, Com8: Alisadr cave, Com10: Marmisho, Com11:Darakeh91, Com13: Takab, Com14: Pyranshahr- Landesheikhan), *S. cf commelinifolia* (nur4: Oshtorankouh, com3: Sarab-Bozghoosh) *S. lucida* (luc1: Guilan, luc2: Bozghoosh, luc4: Khalilkouh1), *S. eremicana* (ere: Alvand population)

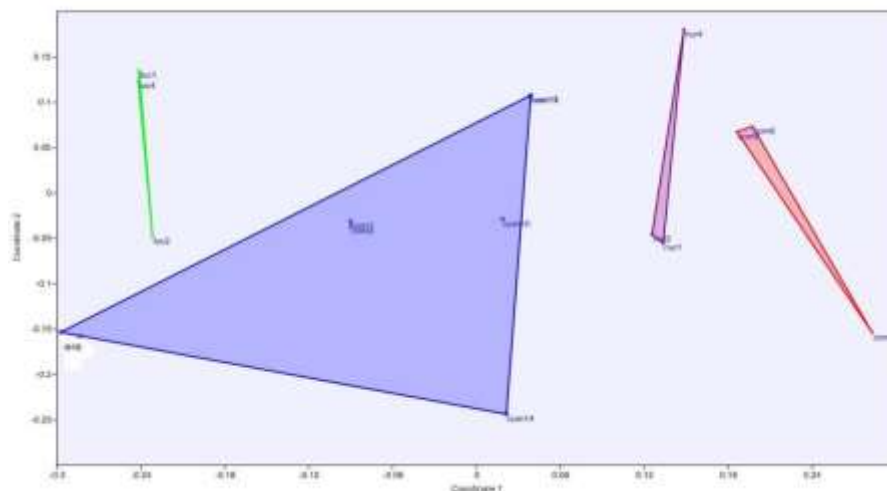


Figure 13. PCO diagram based on leaf cross section. *S. nurensis*(nur1: Zardkouh1, nur2: ZardKouh2), *S. commelinifolia* (Com1: Kivi, Com5: Mazandaran, Com6: Haraz, Com8: Alisadr cave, Com10: Marmisho, Com11:Darakeh91, Com13: Takab, Com14: Pyranshahr- Landesheikhan), *S. cf commelinifolia* (nur4: Oshtorankouh, com3: Sarab- Bozghoosh *S. lucida* (luc1: Guilan, luc2: Bozghoosh, luc4: Khalilkouh1), *S. eremicana* (ere: Alvand population).

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