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Scape anatomy of *Allium* (Alliaceae) in Iran and its systematic application

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Abstract: Cross section of scapes in 20 *Allium* species representing 6 subgenera and 12 sections were investigated. The following characters were determined to be informative: the cross section shape and diameter, number of vascular bundles and vascular bundles diameter. The cross section shape in the members of subgenera *Allium*, *Cepa*, *Reticulatobulbosa* and *Polyprason* is circular whereas in the members of subgen. *Melanocrommyum* and *A. paradoxum* (subgen. *Amerallium*) the cross sections are ellipse and triangular, respectively. Our results show that scape anatomical studies on *Allium* are useful in circumscribing the species and in the subgeneric classification of the genus.

Keywords: *Allium,* scape anatomy, subgeneric classification, Iran.

Introduction

The genus *Allium* L. (Alliaceae) comprises approximately 750 species (STEARN 1992) which are naturally distributed throughout the temperate, semi-arid and arid regions of northern hemisphere (FRITSCH & FRIESEN 2002). The main diversity centre of the genus is considered in the mountainous areas of Southwest and Central Asia (FRITSCH & FRIESEN 2002). Based on molecular data, 15 subgenera and 56 sections have been proposed for the genus (FRIESEN et al. 2006). According to "Flora Iranica" (WENDELBO 1971) and subsequent

additions (FRITSCH et al. 2006, KHASSANOV et al. 2006, KHASSANOV & MEMARIANI 2006) approximately 90 species of *Allium* are distributed in Iran, among those, 28 species are endemic to the country. Based on FRIESEN et al. (2006), Iranian members of the genus can be classified in seven subgenera and 29 sections. Hitherto, most of previous anatomical studies were focused on leaf structure (FAHN 1967, TRAUB 1968, SAGHIR & MANN 1969, KRAHULEC 1980, TANKER & KURUCU 1981, MICELI et al. 1984, FRITSCH 1988 & 1993, DE MASON 1990, MATHEW 1996) whereas only few anatomical studies were conducted on scape anatomy of *Allium* (JACOBSEN 1979, FRIEDLANDER 1988, UYSAL 1999). These studies followed a descriptive style, instead of providing a comprehensive tool for separating species or group of species. The present study deals with scape anatomical characters of some *Allium* species in order to find how these characters are useful for the taxonomy of the genus.

Material and methods

Scape anatomical characters of 20 *Allium* species representing six subgenera and 12 sections were studied by optical microscope. Plant materials of some species were collected from the natural habitats. The remainder was prepared from herbarium material housed at herbaria of Research Institute of Forests and Rangelands, Tehran (TARI), and Tehran University Herbarium (TUH). A list of voucher specimens including systematic positions of the species is presented in Tab. 1. Following the plant samples were fixed in ethanol alcohol 70% for 3 days, cross sections from the lower third in the upper part of scape tissue were made using commercial razor blades. The sections were stained with safranin and fast green (GERLACH 1977) with some modifications, and then mounted on microscopic slides using Canada balsam. The samples were studied using Olympus VANOX AHBS3 optical microscope with 125X to 412.5X magnifications. Some characters such as presence of trichomes, the cross section shape and diameter, number of rows of vascular bundles, number of vascular bundles in each row, number of parenchyma rows and maximum diameter of vascular bundles were selected and investigated. Although most of these characters have been used in former anatomical studies of *Allium* (JACOBSEN 1979, FRIEDLANDER 1988, UYSAL 1999), their potential application in the systematics of the genus have been ignored.

Results

Scape anatomical features of studied samples are summarized in Tab. 2. On the outer side of scape, there is a single layer of cutinized epidermis, followed by sclerenchyma. Under these layers, there is a parenchyma zone with small and thick walled cells. Toward the center the size of these cells become larger and the thickness of the walls decreases (see Fig. 1a). Interestingly, the sclerenchyma and thick walled parenchyma are absent in *A. paradoxum* G. Don (Fig. 1e). Vascular bundles are arranged in one to three circles and the bundles of the outer circles are smaller than the inner ones (Fig. 1b). Phloem lies on the

outer side of bundles whereas xylem lies on the inner side (Fig. 1a). Pith in the studied species is filled with thin walled parenchyma. The cross section shape in the studies samples are mostly circular (e.g. members of subgen. *Allium* represented in Figs 1a and 1b), ellipse (in members of subgen. *Melanocrommyum*, Figs. 1c and 1d) or triangular (*A. paradoxum*, Fig. 1e). Cross section diameter varies between 0.93 mm in *A. montelburzense* R. M. FRITSCH, Y. SALMAKI & SH. ZARRE to 7.15 mm in *A. cristophii* TRAUTV. The margin of the scape in all members except for *A. iranicum* (WENDELBO) WENDELBO is serrate. In the latter species, that is quite smooth (Fig. 1a). Trichomes are observed only in two species on the epidermis: *A. derderianum* REGEL and *A. scabriscapum* BOISS & KOTSCHY. The parenchyma zone with small and thick walled cells is ranged from 3 rows (e.g. in *A. rubellum* M. BIEB.) to 6 rows (*A. subvineale* WENDELBO) and the parenchyma zone with thin walled cells is also ranged from 3 rows (e.g. *A. schoenoprasum* L.) to 6 rows (e.g. *A. hymenorrhizum* LEDEB.). The number of vascular bundles in the peripheral layer varies between 5 (*A. montelburzense* and *A. stamineum* BOISS.) to 21 (*A. elburzense* WENDELBO, Fig. 1c). The minimum diameter of vascular bundles was measured in *A. montelburzense* and *A. tuchalense* F. O. KHASS. & NOROOZI (0.07 mm, Fig. 1b) whereas the maximum diameter of vascular bundles was measured in *A. xiphopetalum* AITCH. & BAKER (Fig. 1f).

Discussion

Members of subgen. *Melanocrommyum* (WEBB et BERTH.) ROUY are characterized by broad leaves which sheathing the scape only at basal parts. Despite of the diversity, members of the subgenus have been considered to form a monophyletic group (DUBOUZET & SHINODA 1998, FRIESEN et al. 2006). Considering the type of leaves, WENDELBO (1971) attributed *A. cristophii*, *A. dederianum* and *A. elburzense* to this subgenus. This approach is also confirmed by recent classification based on nrDNA ITS sequences (FRIESEN et al. 2006). Because of ellipse shape of scape in cross section (Figs. 1c-d, Tab. 2), the representatives of the subgenus (*A. cristophii*, *A. dederianum* and *A. elburzense*) are similar to each other but different from all the studied species. Therefore, this character can represent a synapomorphy sharing by the species of this subgenus. On the other hand, among the studied species of this subgenus, some characters such as cross section diameter, number of vascular bundles and vascular bundles diameter reveal a close relation between *A. cristophii* and *A. elburzense* (Tab. 2). With respect to morphological aspects, there are also some characters such as scape height, undulant margin of leaves and presence of trichomes on the scape of *A. derderianum* make this species distinct from *A. cristophii* and *A. elburzense*. The recent molecular systematic studies using nrDNA ITS sequences (FREISEN et al. 2006) confirms the classical position of *A. derderianum* and *A. elburzense* in sect. *Acanthoprason* WENDELBO, and separated from sect. *Kaloprason* C. KOCH to that *A. cristophii* belongs. From biogeographical point of view, both former species are very common in Alborz

mountain range in northern Iran, while *A. cristophii* is distributed in North East of Iran and adjacent countries. The number of vascular bundles and arrangement of these elements were considered as characteristic features for the taxonomy of *Allium* in previous scape anatomical researches (TRAUB 1968, JACOBSEN 1979). However, the high number of vascular bundles is the most important characteristic feature of *A. elburzense* which is not shared by its relative species, i.e. *A. derderianum*. Therefore, the importance of this feature in the systematics of the genus finds no support from the present study.

The cross sections in subgenera *Reticulatobulbosa* (KAMELIN) N. FRIESEN, *Polyprason* RADIC and *Cepa* (MILL.) RADIC. are circular in shape. Based on the presence of rhizome, WENDELBO (1971) had classified all species of these subgenera in subgen. *Rhizirideum* (KOCH) WENDELBO, while molecular data, divided them into three different subgenera (FRIESEN et al. 2006). With respect to diameter of vascular bundles, *A. xiphopetalum* and *A. scabriscapum* (both of subgen. *Reticulatobulbosa*) are distinguished from *A. hymenorrhizum* (subgen. *Polyprason*) and *A. schoenoprasum* (subgen. *Cepa*). Presence of trichomes on the scape of *A. scabriscapeum* is another important feature that distinct these species from *A. xiphopetalum*. Morphologically, *A. xiphopetalum* possesses some characteristic features such as purple and acuminate tepals with a basal gibbosity that separates it from other relatives. Beside these morphological differences, *A. xiphopetalum* is characterized by the maximum diameter of vascular bundles, but the importance of this character should be re-assessed after including more species of *Allium* in a more inclusive study in future.

The cross section shape in all representatives of subgen. *Allium* is circular and there is no trichome on the scape of the studied species. In the members of sect. *Allium* (*A. affine* LEDEB., *A. atroviolaceum* BOISS., *A. erubescens* C. KOCH, *A. iranicum* and *A. subvineale*), except for the number of vascular bundles rows, other anatomical features are quite uniform. The variation in the number of vascular bundles does not show any significant relationship between the studied species of this section. The most characteristic feature of the sect. *Allium* is the filament type (presence of two filiform apical appendages at both sides of stamens) (WENDELBO 1971). Following that the classification presented by FRIESEN et al., (2006) has also confirmed this classification. From anatomical point of view, *A. montelburzense* (subgen. *Allium*) possesses the minimum scape diameter and vascular bundles diameter. The distribution of *A. montelburzense* has been reported only in Alborz protected area (FRITSCH et al. 2006). This species is very similar to *A. capitellatum* BOISS. especially in term of morphological features. However, *A. montelburzense* possesses some characteristic features such as pink tepals and long filaments which separate it from *A. capitellatum.*

According to WENDELBO (1971), *A. capitellatum*, *A. rubellum* M. BIEB. and *A. umbilicatum* BOISS. were classified in sect. *Scorodon* C. Koch based on morphological features. However, based on molecular data, *A. capitellatum* and *A. montelburzense* are classified in sect. *Caerulea* (FRIESEN et al. 2006). *A. rubellum* and *A. umbilicatum* are very similar particularly in morphological

features such as the height of scape, length of tepals and ratio of filaments to tepal length. The anatomical characters also confirm the similarity of two latter species. Although, the number of vascular bundles is more frequent in *A. umbilicatum* and also the size of vascular bundles is larger than *A. rubellum* but according to modern classification of the genus, both are classified in the sect. *Avulsea*. The anatomical investigation conducted by UYSAL (1999) on two *Allium* species (subgen. *Allium*), could also not reflect any significant differences on the scape anatomical features. Hence, it seems that anatomical characters are quite uniform for the species of the subgen. *Allium*.

Allium paradoxum is the only representative of sect. *Briseis* of subgen. *Amerallium* TRAUB. in Iran which shows triangular cross sections. This feature has also reported in some other species of subgen. *Amerallium* (JACOBSEN 1979). The number of vascular bundles is another characteristic feature which has been considered as useful feature for taxonomy of the genus at the species level. Due to the character, JACOBSEN (1979) has distinguished *A. cernuum* ROTH and *A. validum* WATS which have classified in subgen. *Amerallium* (WENDELBO 1971). Unfortunately, *A. paradoxum* is the only member of subgen. *Amerallium* which has studied here and we are not able to compare it with other species of the subgenus. The most important anatomical features of *A. paradoxum* that characterize this species from all studied samples are lacking of sclerenchyma beneath epidermis and thick walled parenchyma cells. Moreover it is morphologically distinguished from other species of *Allium* based on white flowers accompanied by bulbils in the inflorescences and triangular cross section of the scape.

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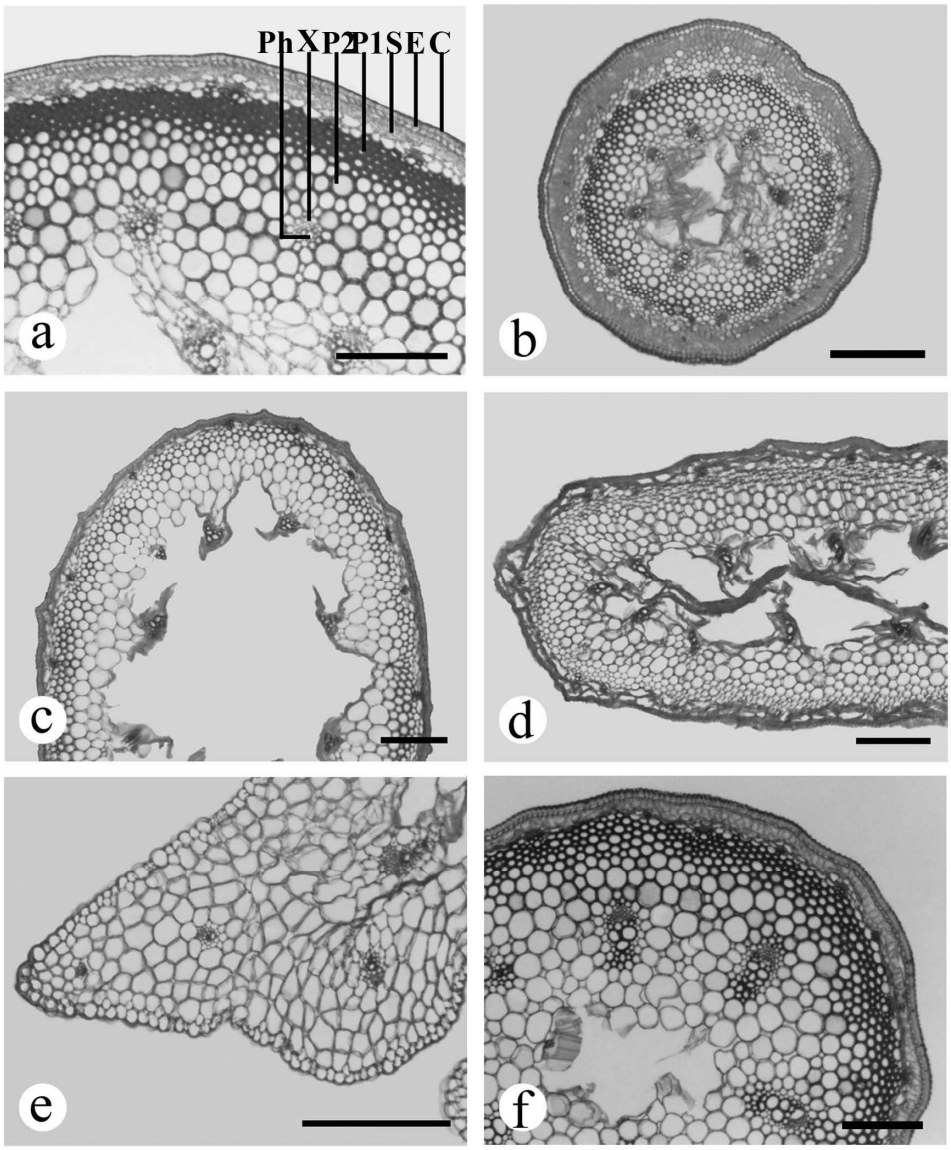

Fig. 1a-f. Cross sections of scape in selected species of *Allium*. a. *A. iranicum*, b. *A. tuchalense*, c. *A. derderianum*, d. *A. elburzense*, e. *A. paradoxum*, f. *A. xiphopetalum*. Scale bar = 0.3 mm. Abbreviations: C = Cuticle, E = Epidermis, S = Sclerenchyma, P1 = Parenchymatous thick walled cells, P2 = Parenchymatous thin walled cells, X = Xylem, Ph = Phloem.

Tab. 1. Collection data of *Allium* species examined here from anatomical point of view. Species endemic to Iran are indicated by an asterisk (*). The subgeneric classification follows FRIESEN et al. (2006).

Species	Subgenus / Section	Collection data
Allium affine LEDEB.	*Allium / Alllium*	Iran: Lorestan, 24 km from Shoul abad to Aligodarz, Alt. 2100–2350 m, 19.08.1982, Mozaffarian & Sardabi, 42487-TARI.
A. atroviolaceum BOISS.		Iran: Yasouj, 25 km to Meymand from Yasouj, Alt. 1400 m, 28.05.1995, Ghahraman & Attar, 19078-TUH.
A. erubescens C. KOCH*		Iran: Semnan, Shahroud, Abr to Soultan Meydan, Alt. 2200 m, 13.07.1998, Mozaffarian, 78110-TARI.
A. iranicum (WENDELBO) WENDELBO		Iran: Mazandaran, Alborz Protected Area, 2 km to Arange, Alt. 1600 m, 15.05.2006, Hosseinzadeh & Moazzeni, 35934-TUH.
A. subvinale WENDELBO*		Iran: Tehran, Lar valley, Alt 2420 m, 02.07.1974, Wendelbo & Assadi, 13361-TARI.
A. rubellum M. BIEB.	*Allium* / *Avulsea*	Iran: Mazandaran, after Kandovan tunnel, Siyah Bishe, Alt. 1800 m, 10.04.2006, Zarre & Moazzeni, 35940-TUH.
A. umbilicatum BOISS.		Iran: Golestan, Golestan to Bojnord, 25.08.1969, Turgobuf, 4256-TUH.
A. capitellatum BOISS.*	*Allium* / *Caerulea*	Iran: Tehran, south slope of Tuchal mountains, Alt. 2900 to 3650 m, 22.07.1998, Mozaffarian, 78292-TARI.
A.montelburzense R.M. FRITSCH, Y. SALMAKI & SH. ZARRE		Iran: Mazandaran, Alborz Protected Area, 2 km after Kandovan tunnel to Siyah Bishe, Alt. 2780 m, 17.07.2005, Zarre & Moazzeni 36583-TUH.
A. lenkoranicum MISCZENKO	*Allium* / *Codonoprasum*	Iran: Mazandaran, Alasht Lin, Alt. 1000 m, 22.08.1972, Rowshan, 5844 (TARI)
A. stamineum BOISS.		Iran: Hormozgan, Bandar Abbas, Abe Garm-e Genu, 150 to 250 m, 11.03.1986, Ghahreman & Mozaffarian, 5430-TUH.
A. tuchalense F.O. KHASS. & NOROOZI	*Allium* / *Pallasia*	Iran: Tehran, Tuchal mountain, Alt. 3705 m, 14.07.2006, Noroozi, 35917-TUH.
A. paradoxum (M. BIEB.) G. DON	*Amerallium* / *Briseis*	Iran: Mazandaran, Alborz Protected Area, Pol- e Zanguleh, Alt. 2300 m, 01.05.2007, Zarre & Moazzeni, 35937-TUH.
A. schoenoprasum L.	*Cepa* / *Schoenoprasum*	Iran: Azarbayejan, 40 km to north of Sarab & Sabalan, Alt. 3000 m, 11.08.1973, Moeinodin, 9327-TARI.
A. derderianum REGEL*	*Melanocrommyum* / *Acanthoprason*	Iran: Tehran, Tuchal mountain, Alt. 2700 m, 14.06.1959, 4182-TUH.
A. elburzense WENDELBO*		Iran: Ghazvin, 50 km to Alamout from Ghazvin, Alt. 1800-2000 m, 18.05.2007, Hosseinzadeh, Moazzeni & Salmaki, 35930-TUH.
A. cristophii TRAUTV.	*Melanocrommyum* / *Kaloprason*	Iran: Khorassan, Kotal Yek Chenar, 10.04.2006, Moazzeni, 35938-TUH.
A. hymenorrhizum LEDEB.	*Polyprason* / *Falcatifolia*	Iran: Semnan, 50 km north of Semnan between Sheli & Hikuh village, Alt. 2400 m, 28.07.1982, Assadi & Mozaffarian, 40569-TARI.
A. xiphopetalum AITCH. & BAKER	*Reticulatobulbosa* / *Campanulata*	Iran: Mazandaran, 10 km after Kandovan tunnel, Alt. 1800–1900 m, 15.05.2006, Hosseinzadeh & Moazzeni, 35935-TUH.
A. scabriscapum BOISS. & KOTSCHY	*Reticulatobulbosa* / *Scabriscapa*	Iran: Esfahan, Tiran, Ghameshlo to Chale Siyah, Alt. 2100 m, Feyzi, 5461-TUH.

Tab. 2. Scape anatomical characters inferred from the cross sections in some *Allium* species. Abbreviations: Diam = Diameter of the cross section in mm, VBR = number of rows of vascular bundles, VBN = number of vascular bundles in the peripheral layer, TKP = number of layers of thick walled parenchyma, TNP = number of layers of thick walled parenchyma, VDM = Diameter of the largest vascular bundles. The numbers represent the means.

Species	Subgenus / section	Trichome	Shape	Margin	Diam	VBR	VBN	TKP	TNP	VDM
Allium affine	*Allium / Alllium*	-	Circular	Serrate	3.5	3	9	5	4	0.12
A. atroviolaceum		-	Circular	Serrate	2.57	3	9	5	5	0.14
A. erubescens		-	Circular	Serrate	1.42	1	6	4	5	0.12
A. iranicum		-	Circular	Smooth	2.55	2	9	5	3	0.14
A. subvinale *		-	Circular	Serrate	1.87	2	7	6	4	0.11
A. rubellum	*Allium / Avulsea*	-	Circular	Serrate	1.34	1	7	3	4	0.09
A. umbilicatum		-	Circular	Serrate	2.05	1	10	4	4	0.12
*A. capitellatum**	*Allium / Caerulea*	-	Circular	Serrate	1.42	2	7	3	4	0.12
A.montelburzense		-	Circular	Serrate	0.93	1	5	3	4	0.07
A. lenkoranicum	*Allium / Codonoprasum*	-	Circular	Serrate	1.56	1	6	3	6	0.10
A. stamineum		-	Circular	Serrate	1.37	1	5	4	4	0.12
A. tuchalense	*Allium / Pallasia*	-	Circular	Serrate	1.23	1	7	4	4	0.07
A. paradoxum	*Amerallium / Briseis*	-	Triang.	Serrate	2.55	2	6	0	5	0.15
A. schoenoprasum	*Cepa / Shoenoprasum*	-	Circular	Serrate	1.68	1	8	4	3	0.08
*A. derderianum**	*Melanocrommyum / Acanthoprason*	+	Ellipse	Serrate	1.90	1	9	3	4	0.09
*A. elburzense**		-	Ellipse	Serrate	3.75	1	21	3	4	0.12
A. cristophii	*Melanocrommyum / Kaloprason*	-	Ellipse	Serrate	7.15	2	15	4	4	0.13
A. hymenorrhizum	*Polyprason / Falcatifolia*	-	Circular	Serrate	1.98	1	6	4	6	0.10
A. xiphopetalum	*Reticulatobulbosa / Campanulata*	-	Circular	Serrate	1.52	1	6	5	3	0.20
A. scabriscapum	*Reticulatobulbosa / Scabriscapa*	+	Circular	Serrate	2.03	1	8	5	5	0.17

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