Scape anatomy of Allium sect. Allium (Alliaceae) in Iran

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Abstract

The genus *Allium* encompasses over 750 species which have been classified into 15 subgenera and 72 sections. Section *Allium* is one of the most economically important sections of the genus, containing several medicinal and edible species. The section includes those species of *Allium* with a well developed bulb, stem (never basal) leaves and filaments in two distinct whorls, the outer nearly always simple and the inner tricuspidate. The objectives of the current study were to compare the scape anatomical features of some species of the section *Allium*. Some characteristics such as diameter of the cross section, palisade parenchyma, spongy parenchyma, and vascular bundles as well as number of vascular bundles, and sclerenchymatous layers were studied. On the basis of the results obtained, although the species of *Allium* section *Allium* form a relatively homogeneous group, some distinctions among cross-sections of scapes are evident. In fact, our findings offer a comprehensive survey using anatomical traits for delimitation and diagnosing species of the section *Allium* and provide a platform for further taxonomic investigations.

Keywords: *Allium*; Alliaceae, Scape anatomy; Taxonomy, Iran

Introduction

Allium L. (Alliaceae) is the largest genus of the petaloid monocots, encompassing over 750 species (Friesen *et al.* 2006). This divers and taxonomically difficult genus is characterized by having bulbs enclosed in membranous tonics, free or almost free tepals, and often a subgynobasic style. The largest numbers of species are found primarily throughout the temperate, semi-arid and arid regions of northern hemisphere (Fritsch & Friesen 2002). The main centre of diversity of the genus is considered in the mountainous areas of southwest and central Asia including the territory of Iran (Fritsch & Friesen 2002). Here the genus seems to be typical for the Irano-Turanian phyto-geographical region and displays a high level of specific endemism (Matin 1992). One of the most recent classifications proposes 15 subgenera and 72 sections for the genus *Allium* (Friesen *et al*. 2006). Accordingly, the recognized *Allium* species in Iran fit in 7 subgenera and 29 sections.

Section *Allium*, the subject of the present study, includes species of *Allium* with a well developed bulb, stem (never basal) leaves, campanulate to cup-shaped (never stellate) flowers, and filaments in two distinct whorls, the outer three nearly always simple and the inner three markedly tricuspidate (rarely 5-7 cuspidate) with the anther attached to the median cuspid (Mathew 1996). The section comprises about 115 species worldwide; at least 30 of which including 6 endemics grow in Iran (Wendelbo 1971). Based on the morphology, anatomy, karyology and molecular systematics of the species, section *Allium* is a homogeneous, well-defined and probably monophyletic group and therefore is of high taxonomic interest (Mathew 1996; Fritsch & Astanova 1998; Fritsch). Interestingly, this section includes a number of economically important species such as *A. porrum* (leek), *A. sativum* (garlic), *A. ampeloprasum* var. *ampeloprasum* (Russian garlic or elephant garlic) and *A. ampeloprasum* var. *kurrat* (kurrat, Egyptian leek).

Most important anatomical studies on *Allium* were focused on the structure of the bulb scales (Fritsch 1988) and the leaf structure (De Mason 1990; Fahn 1967; Fritsch, 1988; 1993; Mathew, 1996; Miceli et al., 1984; Saghir & Mann, 1969; Tanker & Kurucu, 1981; Traub, 1968). Intriguingly, anatomy of scape and its potential use in the systematics and delimitation of the species of *Allium* received little attention (Friedlander 1988; Jacobsen 1979; Fritsch 1992; Uysal 1999). The main scope of the present study is to provide a comprehensive scape anatomical study in *Allium* sect. *Allium* in Iran, to find useful characters in taxonomy of the species, and to contribute towards the taxonomic information available on the section. Another long term goal is to examine the scape anatomy of some

species of the section in detail, as a basis for further anatomical studies of other members of the genus.

Materials and methods

Scape anatomical characters of 12 *Allium* species of the section *Allium* were studied by means of light microscope. Plant materials of some species were collected from their natural habitats and other samples (3 species) were taken from the herbarium material housed at Tehran University Herbarium (TUH). A list of voucher specimens of the species is presented in Table 1.

The plant materials were fixed in 70% ethanol for 3 days, and the cross-sections were prepared from the upper 1/3 of scape using commercial razor blades. The sections were stained with safranin and fast green (Gerlach 1977) prior to be dehydrated through a graded ethanol series. Thereafter, they were cleared with xylene and mounted in Canada balsam. The sections were then studied using a Nikon E-1000 light microscope. A number of characters such as shape and diameter of cross-sections, number of rows of the vascular bundles, number of vascular bundles in each row, number of rows of parenchyma and the diameter of vascular bundles were scored by examining of at least 5 cross-sections.

Results

Scape anatomical features of species studied are summarized in Table 2. In cross-sections of scapes, which were mostly circular and rarely elliptic (i.e. in *A. qaradaghense*) (Fig. 1 & 2), fundamental tissues such as epidermis, parenchyma, sclerenchyma, and vascular bundles were observed.

Table 1. Collection data and voucher number of *Allium* specimens examined here from scape anatomical point of view. The voucher specimens are located in TUH.

Species	*Collection data*	*Herbarium*
A. affine Ledeb.	E Azarbaijan, 50-60 km to Zanjan from Mianeh	28027-TUH
A. atroviolaceum Boiss.	E Azarbaijan, SW Tabriz, E Azarbaijan, Basmenj road, Liqvan village	37047-TUH
A. borszczowii Regel	????	9908-TUH
A. dictyoscordum Vved.	E Azarbaijan, Tabriz university	37036-TUH
A. erubescens C. Koch	W. Azarbaijan, between Naghadeh and Piranshahr	4197-TUH
A. iranicum (Wendelbo) Wendelbo	E Azarbaijan, SW Tabriz, E Azarbaijan, Basmenj road, Liqvan village	37035-TUH
A. laeve Wendelbo & Von Bothmer	Lorestan, Khorramabad, Keshvar road, 5 km to Nuzhan pass	21829-TUH
A. longicuspis Regel	E Azarbaijan, SW Tabriz, ????? E Azarbaijan, Basmenj road, Liqvan village	37037-TUH
A. phanerantherum Boiss. &	E Azarbaijan, SW Tabriz,????	37046-TUH
A. qaradaghense Feinbr.	E. Azarbaijan, 5 km N Tabriz, Dand mountains	37051-TUH
A. rotundum L.	E Azarbaijan, Tabriz to Marand, ca. 15 km to Marand, SW Marand, Mishu-dagh mountains	37040-TUH
A. subvineale Wendelbo	E Azarbaijan, Maraghe, Chenar (NW of Maraghe)	37042-TUH

Table 2. Details of scape anatomy of *Allium* species examined here. All sizes in columns 1, 2, 3, 4, and 12 represent the mean and are in µm. Column 1: Diameter of the section, Column 2: Diameter of outer parenchymatous layer (palisade), Column 3: Diameter of inner parenchymatous layers (spongy), Column 4: Diameter of outer layers of pith parenchyma, Column 5: Epidermis state (sinuate, smooth), Column 6:Number of vascular bundles, Column 7: Number of circles of vascular bundles, Column 8: Number of vascular bundles in outer circle, Column 9: Number of vascular bundles in median layer, Column 10: Number of vascular bundles in the innermost circle, Column 11:Number of sclerenchymatous layers, Column 12: Diameter of Vascular bundles, Column 13: Mean of number of metaxylem in each bundles.

Spieces	*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*	*12*	*13*
A. affine Ledeb.	1714.2	64.86	54.05	81.08	sinuate	14	2	6	-	8	5	100	9
A. atroviolaceum Boiss	5603.5	81.08	54.05	54.05	sinuate	27	3	11	10	6	4	114.28	6
A. borszczowii Regel	2428.57	54.05	27.02	135.13	sinuate	18	2	10	-	8	6	178.57	
A. dictyoscordum Vved	1500	27.02	27.02	54.05	smooth	13	2	5	-	8	5	128.57	5
A. erubescens C. Koch	1142.85	81.08	54.05	121.62	smooth	19	2	11	-	8	3	85.71	4
A. iranicum Wendelbo	3000	285.71	108.10	135.13	smooth	26	3	10	9	7	5	180	8
A. laeve Wendelbo & Von Bothmer	1500	81.08	54.05	40.54	sinuate	10	1	-	-	10	3	92.85	6
A. longicuspis Regel	1500	77.77	66.66	111.11	sinuate	22	2	14	-	8	4	121.62	8
A. phanerantherum Boiss. &	4857.14	135.13	67.56	135.13	sinuate	48	3	16	20	12	3	200	10
A. qaradaghense Feinber	3175.67	81.08	162.16	108.10	sinuate	48	3	26	13	9	7	108.10	6
A. rotundum L	1625	55.55	66.66	133.33	sinuate	7	1	-	-	7	5	100	6
A. subvineale Wendelbo	1875.14	64.86	54.05	54.05	sinuate	18	2	11	-	7	4	114.28	10

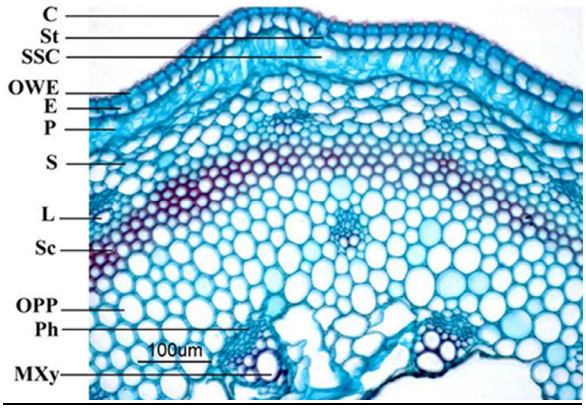

Fig. 1: A typical cross-section of scape including different fundamental tissues. C: cuticle, St: stomate, SSC: sub stomata cavity, OWE: outer wall of epidermis, E: epidermis, P: parenchyma, s: spongy cell, L: laticifere, Sc: sclerenchyma, OPP: outer layer of pith parenchyma, ph: phloem, MXy: metaxylem.

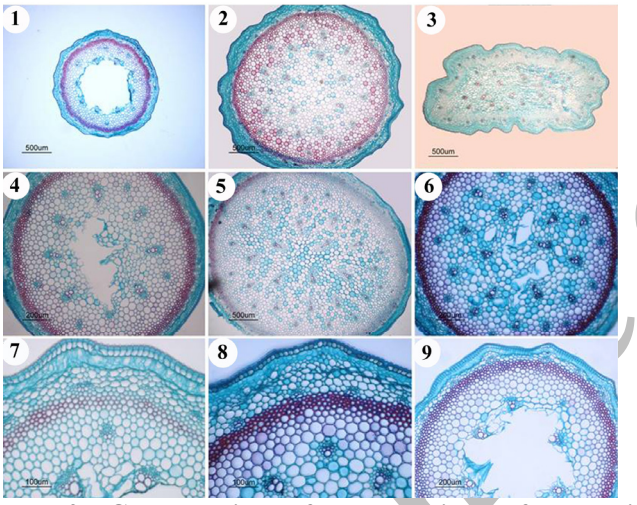

Figure 2- General view of cross-sections of scape in different species of *Allium* sect. *Allium* including: a) A. subvineale, b) *A. iranicum*, c) *A. qaradaghense*, , d) *A. dictyoscordum*, e) *A. phanerantherum* f) *A. atroviolaceum*, g) *A. longicuspis*, h) *A. dictyoscordum*, i) *A. phanerantherum,* j) *A. rotundum* k) *A. atroviolaceum*, l) *A. borszczowii.*

Epidermis: In all species investigated the scape epidermis was consisted of a single cell layer. The cells were compact, radially elongated, and covered by a thick cuticle layer at outer surface (Fig. 1). Noticeably, the outer epidermal wall was much thicker than the inner wall (Fig. 1, 3g-i). The type of stomata with distinctive guard cells was recognized as xerophytic in all species studied (Fig. 2g, 2j, 3i).

Parenchyma: Underlying the single-layered epidermis were few layers of chlorenchyma containing one to two row(s) of extremely curved palisade cells and 2-3 layers of isodiametric spongy cells (Fig. 1). In most specimens some secretory ducts were distributed throughout spongy parenchyma in distinctive arrangement and distribution patterns (Fig. 1). Obviously, one circle of distinct vascular bundles was located throughout the inner parts of parenchyma just before sclerenchyma (Fig. 1, 2).

Sclerenchyma: Sclerenchyma contained three (*A. erubescens*, *A. laeve* and *A. phanerantherum*: Fig. 2-e), four (*A. atroviolaceum*: Fig. 2f, *A. longicuspis* and *A. subvineale*: Fig. 2a), five (*A. affine*, *A. dictyoscordum*: Fig. 2d and *A. rotundum*: Fig. 2j) or rarely six (*A. borszczowii*: Fig. 2l) and seven (*A. qaradaghense*: Fig. 2c) layers of cells. In all species, the sclerenchyma formed a closed circle under spongy cells (Fig. 1, 2). Centripetally, wall thickness and lignification-grade of sclerenchymatous cells were decreased but their diameter was notably increased.

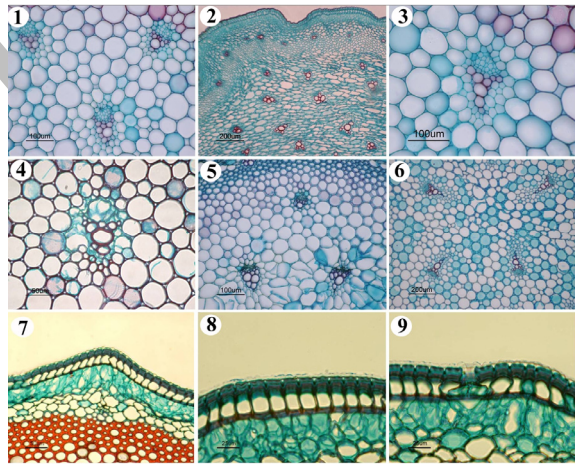

Fig. 3: Enlarged view of cross-sections of scape in different species of *Allium* sect. *Allium* highlighting vascular systems (a-f) and epidermis plus thick cuticle (g-i). a) *Allium iranicum*, b) *A. qaradaghense*, c) *A. iranicum* d) *A. erubescens* , e) *A. atroviolaceum*, f) *A. borszczowii*, g) *A. rotundum*, h) *A. subvineale.*

Vascular bundles: The scapes of all species possessed collateral vascular bundles with adaxial xylem (Fig. 1, 3a-f). One circle of somewhat large vascular bundle at distance of three to four cellular layers from sclerenchyma was located in central parenchyma (Fig. 2). The bundles were collateral in all species and the phloem and the xylem were in contact with each other directly. The central pith parenchyma with relatively large cells and large intercellular spaces were observed only in early

stages of development (Fig. 2b, e, 3f). About 30 percent (in the mature state until 80 percent) of the transverse sections was devastated by maturation of scapes (Fig 2). There were also internal vascular bundles in the pith region (Fig. 2b, e, f). Number, size and location of the bundles might be different in species of the genus whereas thick scapes (Fig. 2e, f) usually have more vascular bundles than thin ones (Fig. 2a). Generally, the inner vascular bundles were larger than the outer ones and they owned more cells of phloem and xylem (Fig. 2e, f). In cross-sections, vascular bundles usually were surrounded by vascular sheaths consisting of some layers of small parenchyma cells that were gradually converted to collenchyma or scleranchyma (Fig. 3a-f). Although inner vascular bundles were arranged in zigzag or circular patterns, internal vascular bundles were almost in the all parts of central parenchyma.

Discussion

The species of *Allium* section *Allium* form a relatively homogeneous and well characterized group regarding taxonomical and molecular systematical studies (Mathew, 1996; Friesen *et al.* 2006). Previous investigations revealed that anatomical investigations offer valuable features for characterizing natural groups or distinguishing species from each other within sections (Fritsch 1988, Uysal 1999). Accordingly, the current study provides a comprehensive survey for probable application of anatomical findings in delimitation and diagnosing species of the section *Allium*.

The shape of the cross-sections was circular in most species studied. The only exception was observed for *A. qaradaghense* with elliptic-oblong sections. However, this unusual shape might be caused by preparing mistreatments and represents probably an artifact. The margin of the scape in cross-sections provides another diagnostic character. The form of the scape epidermis of nine species including *A. affine, A. atroviolaceum*, *A. borszczowii*, *A. leave*, *A. longicuspis*, *A. phanerantherum*, *A. qaradaghense*, *A. rotundum* and *A. subvineale*'s was sinuate, whereas it was smooth in *A. dictyoscordum* , *A. erubescens,* and *A. iranicum*. Study of several sections confirmed that this form is invariable within a given species and, therefore, is useful criteria for grouping the species.

In all species palisade and spongy parenchyma in the scape were totally differentiated. In fact, the inner tissues of scapes were completely surrounded by two or three palisade parenchyma layers. The xerophytic stomata coincide with the distributional characteristics of *Allium* species. Notably, all species owned laticifer tubes in scape mesophyle that have been previously found in leaves, bulb scales as well as in scape of *Allium* taxa (Fahn 1967; Huang & Sterling 1970; Yentür 1984). Three different patterns of laticifer distribution were distinguished: a) at inner boundary of palisade tissue (between palisade and spongy cells) (in *A. qaradaghense*, *A. iranicum* and *A. phanerantherum*), b) at inner boundary of isodiametric spongy cells (between parenchyma and sclerenchyma) (in *A. laeve*, *A. subvineale*, *A. borszczowii* and *A. rotundum*), and c) throughout the spongy cells such as in *A. atroviolaceum*, *A. erubescens* and *A. longicuspis*. Because the places of laticifers are inconsistent among different species, it seems to be an important anatomical character for taxonomic studies.

So far, the anatomy of vegetative organs and scapes of *Allium* have been used for taxonomical purposes in different hierarchical levels. However, our work provides the first detailed anatomical findings on the species of the section *Allium* and represents some distinctions among cross-sections of scapes. Hence, these results can be used in addition to the data obtained from other anatomical studies of *Allium* species (Saghir & Mann 1969; Fritsch 1988; Mathew 1996). Besides, the anatomical survey of section *Allium* offer new tools for delimitation and diagnosing of the species and provide a prospective for further taxonomic analysis of the genus.

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