ANATOMICAL AND POLLEN CHARACTERS IN ACANTHOPHYLLUM C. A. MEY. (CARYOPHYLLACEAE) FROM NORTHEAST OF IRAN

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Received 28.07.2012. Accepted for publication 08.11.2012.

Mahmoudi Shamsabad, M., Ejtehadi, H., Vaezi, J., Memariani, F. 2013 06 30: Anatomical and Pollen Characters in *Acanthophyllum* C. A. Mey. (Caryophyllaceae) from northeast of Iran. *–Iran. J. Bot. 19 (1): 107-118.* Tehran.

Palynological and anatomical features of 11 species from NE Iran belonging to *Acanthophyllum* sect. *Oligosperma* were carried out and evaluated by numerical methods to determine the taxonomic value of endomorphic characters. The cross section of stems, peduncles and leaves were prepared and stained with Safranin and Fast-green and pollen morphology has been examined by light and scanning electron microscope. The principal component analysis and Cluster Analysis results showed that the type of stomata, shape and size of epidermal cells, trichomes, the number of epidermal cells and stomata, the shape of stem cross section, arrangment of xylem elements in peduncle, arrangment of mesophyll in leaf and the number of sclerenchymatous layers are significant to separating species. The pollen grains were spheroid, pantoporate, ornamentation was scabrate-punctate and exine structure was spinulose-punctate.

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Key words. Anatomy, Pollen Morphology, *Acanthophyllum*, Caryophyllaceae, Numerical Analysis, Iran.

بررسی صفات تشریحی و گرده شناسی جنس چوبک (Acanthophyllum C. A. Mey) در شمال شرق ایران

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صفات تشریحی و گرده‌شناسی ۱۱ گونه *Acanthophyllum* از بخش *Oligosperma* Sect. در شمال شرقی ایران با استفاده از تاکسونومی عددی مورد بررسی قرار گرفت. برش‌هایی از سطح مقطع ساقه، دمگل و برگ تهیه و سپس با سافرانین و فست‌گرین رنگ آمیزی شد و مورفولوژی دانه‌های گرده با استفاده از میکروسکپ نوری و الکترونی بررسی شدند. نتایج نشان داد صفات نوع روزنه، شکل و اندازه سلول‌های اپیدرمی، نوع کرک، تعداد سلول‌های اپیدرمی و روزنه در واحد سطح، شکل سطح مقطع ساقه، آرایش آوندهای چوب در دمگل‌آذین، آرایش مزوفیل در برگ و تعداد لایه‌های اسکلرانشیمی در تفکیک گونه‌های این بخش مفید بودند. دانه‌های گرده در این جنس کروی، چند منفذی، زبرنقطه‌ای و ساختار اگزین خاردار نقطه‌ای است.

INTRODUCTION

Acanthophyllum C. A. Mey. belongs to the family Caryophyllaceae, subfamily Caryophylloideae. *Acanthophyllum* with about 60 species is distributed in the Irano-Turanian region (Takhtajan 1986, Schiman-Czeika 1988) and adapted to deserts, mountains and temperate areas (Ghaffari 2002). The northeast of Iran and adjacent regions in Afghanistan and Turkmenistan are the main centers of diversification for the genus (Ghaffari 2004). *Acanthophyllum* is represented in Iran with 33 species, 23 out of which are endemics. However, in recent investigation on the Iranian *Acanthophyllum* species, Basiri & al. (2011) have introduced seven synonyms and five reductions to the

Table 1. Voucher specimens of *Acanthophyllum* species used in the study.

species	localities
A. adenophorum Freyn	Khorassan, NE Bojnourd, between Ali Muhammad and Robat, 1500 m, 4/7/1993, 23578, Faghihnia and Zangooei
A. borsczowii Bunge ex Boiss.	Khorassan, S Sabzevar, Hares Abad desert park, 800 m, 1/6/1991, 20553, Joharchi and Zangooei
A. korshinskyi Schischk.	Khorassan, NE Qayen, Tikab, 1200 m, 28/5/1995, 25602, Rafei and Zangooei
A. speciosum Rech. f. & Schiman- Czeika	Khorasan, NE Kalat Naderi, 1200 m, 24/5/1994, 24041, Faghihnia and Zangooei
A. laxiusculum Schiman-Czeika	Khorassan, E Bajestan, between Hojat Abad and Helali, 1250 m, 9/5/1998, 30525, Rafei and Zangooei
A. heratense Schiman- Czeika	Khorassan, N Bojnurd, 8 km north of Jow-Darreh, 1600 m, 11/6/1996, 27190, Rafei and Zangooei
A. lilacinum Schischk	Khorassan, NW Gonabad, 1300 m, 14/5/1997, 26895, Rafei and Zangooei
A. squarrosum Boiss.	Khorassan, NE Bojnurd, between Naveh & Qatlish, ca. 3 km on Izman bifurcation road, 1250 m, 17/6/2009, 43145, Memariani and Zangooei
A. diezianum Hand-Mzt.	Khorassan, NW Qayen, 6 km east of Karghand village, 1700 m, 19/5/1998, 30764, Joharchi and Rafei
A. brevibracteatum Lipskyi	Khorassan, N Kashmar, 10 km south of Ataieh, 1700 m, 25/5/1999, 32615, Hojjat and Zangooei
A. pachystegium Rech. f.	Khorassan, NW Bojnurd, Jargalan area, 3 km from Baqleq towards Guy-Nik, 1430 m, 11/6/2008, 40772, Memariani and Zangooei

rank of variety for the genus; accepting 21 species in Iran. Based on the Flora Iranica (Schiman-Czeika 1988), the genus has been divided into seven sections. The sect. *Oligosperma,* with 23 species worldwide, stand as the largest section of the genus of which 16 occur in Iran (Schiman-Czeika 1988). The members of this section are identified by dense flowers, spherical terminal heads, calyx (4) 6-12 mm long, calyx-teeth 1-2 mm long and 4-ovuled ovary (Schiman-Czeika 1988, Shishkin, 1936).

Anatomical characters are less influenced by environmental condition than morphological characters and are more uniform from one population to another (Bokhari 1987). Generally, variation of morphological characters within family Caryophyllaceae makes taxa complicated to be delineated and identified (Fior & al. 2006). In *Acanthophyllum* species like other Caryophyllaceae genera there are extreme variety in population that causes difficalties to distinguish. In order to resolve problems, with respect to effect of environmental codition on morphology characters, the need of survey on endomorphic characters is nessesary and helps to identify species (Sahreen & al. 2008, Kilic 2009).

There have been no anatomical and palynological studies on this genus so far. The previous studies that carried out on anatomy of Caryophyllaceae had been done by Metcalfe and Chalk (1983). Schwingruber (2007) described and analysed the xylem and phloem of 88 species from Caryophyllaceae. Kilic (2009) investigated anatomical and pollen characters in the genus *Silene* from Turkey. The earlier palynology studies on Caryophyllaceae had been done by Perveen and Qaiser (2006); they studied 74 species of the family Caryophyllaceae from Pakistan.

The aim of this paper is to do anatomical and palynological studies for 11 species of the *Acanthophyllum* sect. *Oligosperma* in northeastern Iran including *A. borsczowii* Litw., *A. speciosum* Rech. f. & Schiman-Czeika, *A. korshinskyi* Schischk., *A. pachystegium* Rech. f., *A. adenophorum* Freyn., *A. lilacinum* Schischk., *A. brevibracteatum* Lipsky., *A. diezianum* Hand.-Mzt., *A. laxiusculum* Schiman-Czeika, *A. squarrosum* Boiss., and *A. heratense* Schiman-Czeika and to evaluate their taxonomical application

MATERIALS AND METHODS

The anatomical and palynological investigations are based on the herbarium specimens obtained from FUMH (Ferdowsi University of Mashhad Herbarium). The list of these species is given in Table 1. The pollen grains extracted from the anther and dehydrated by acetic acid, then acetolyzed according to Erdtman (1960). Pollen grains were mounted in glycerine-jelly to make permanent slides and observed with Olympus BH-2 microscope under oil immersion. For scanning electron microscope studies, the pollen grains coated

with gold and examination was carried out on LEO1450VP microscope. Pollen terminology followed here is based on Punt & al. (1994). Measurements were based on 20 pollen grains per specimen. For anatomical study, leaves and stem were taken from the middle parts of specimens and epidermal surface were taken from the lower parts of leaves. The number of stomata and epidermal cell was considerd in 1mm².

For soften, all materials were embeded in glycerine: ethanol: distilled water for three months, then fixed in FAA for 72 h. All sections cut by hand and staind with Safranin and Fast-green and mounted in entellan to make permanent slides. Pictures were taken by Olympus BH-2 microscope that connected to a Dino lite cammera. Qualitative and quantitative characters were based on 10 observations

Anatomical and palynological characters. A total of 63 including 53 quantitative and 10 qualitative characters were examined on each specimen (Table 2). Qualitative characters were scored as binary or multistate characters. In this investigation missing data replacement were made with the means of variables (Legendre and Legendre 1998).

Numerical methods. For collection, the characters that found to be functional in separating an apriori group several runs of PCA were carried out. Only those quantative characters allow to be contributed that variability of the first three axes of the PCA ($r>0.5$) and had the least correlation ($r<0.5$) were used to differentiate specimens from each other. The results of these investigations are based on Principal Component Analysis (PCA) and Cluster Analysis (CA) that carried out based on UPGMA method and Euclidian distance as a disimilarity cofficience and was performed using NTSYS software Version 2 (Rohlf 1998). For selecting diagnostic characters, PCA was performed using CONACO software Version 4.5 (Ter Braak 1988).

RESULTS

Leaf epidermal anatomy

Stomata. A. speciosum, *A. korshinskyi*, *A. pachystegium*, *A. adenophorum*, *A. lilacinum*, *A.brevibracteatum*, *A. diezianum*, *A. laxiusculum* and *A. heratense* have diacytic, anomocytic and anisocytic types of stomata while *A. borsczwii* has only anomocytic and *A. squarrosum* has diacytic type of stomata (Fig. 1).

Trichomes. Acanthophyllum pachystegium, A. adenophorum and *A. lilacinum* have glandular and eglandular hairs, *A. brevibracteatum, A. diezianum, A. laxiusculum, A. heratense* and *A. squarrosum* have multicellular and unicellular eglandular hairs while *A. korshinskyi* has short glandular hairs. *A. speciosum* has 2-4 celled glandular hairs and in *A. borsczowii* unicellular eglandular hairs are observed.

Shape of epidermal cells. The shape of epidermal cells is irregular, rectangular and polygonal with crenate or entire subsidary cells. *Acanthophyllum pachystegium*, *A. adenophorum*, *A. lilacinum*, *A. brevibracteatum*, *A. speciosum* and *A. korshinskyi* have irregular epidermal cells with crenate walls and *A. borsczowii* has polygonal cells with entire walls while *A. heratense*, *A. laxiusculum*, *A. squarrosum* and *A. diezianum* have rectangular cells with entire walls.

Size of epidermal cells. The lenght of epidermal cells in *Acanthophyllum* species are diffrent from .053 µm (smallest) in *A. heratense* to 0.12 µm (largest) in *A. laxiusculum* (Table 3)

Palynological analysis

Pollen shape, type, size, ornamentation and structure. Pollen grains are radially symmetrical and spheroidal; the type of pollen grain is pantoporate. The grains have median size (23-31 µm), this character is diffrent from 24.5µm polar axes and 23.8 µm equatorial axis in *A. borsczowii* (smallest) to 30.7 µm polar axes and 30.3µm equatorial axis in *A. adenophorum* (largest) (Table 4). Pollen ornamentation is scabrate-punctate and exin structure is spinulose-punctate (Figs. 2 and 3)

Anatomical characters

Cuticular layer 2-4 µm thick. Epidermis consist of a single layer of orbicular or rectangular cells located close to several parenchymatous layers with druse crystals. Several sclerenchymatous layers including thick and thin walled cells that surrounded vascular bundle. The pith is hollow or filled with large thin walled cells in some species contain druse crystals. The important indicative anatomical characters that we found for separation of taxa are as follows:

Stem cross section shape and size. In *A. borsczowii* the stem in cross section is rectangular-elliptical and have the greatest size (1.8 mm length and 1.135 mm width), in other species stem cross section is more or less elliptical-circular and *A. pachystegium* has the smallest stem cross section (0.979 mm length and 0.793 mm width) (Fig. 4).

The number of sclerenchymatous and parenchymatous layers in stem. Sclerenchymatous thin walled cells layers are well developed in *A. borsczowii* (8-9 layers) and occupied about 70% of stem radious whereas sclerenchymatous thick walled cell layers are expanded in *A. korshinskyi* and *A. adenophorum*, this character defined in the fewest amount in *A. lilacinum*, *A. pachystegium*, *A. borsczowii* and *A. brevibracteatum* (0-2 layers). Parenchymatous cell layers defind in the greatest amount in *A. brevibracteatum* and *A. speciosum* and occupied 30-40% of stem radious (about 6 layers).

Table 2. Anatomical and palynological characters used in this study, followed by their abbreviations. The qualitative characters denoted by asterisks, character states and considered scores are given in square brackets.

Pollen	Equatorial axis	EQAP
	Polar axis	POAP
	Length of pores	LEPP
	P/E rate	PERP
	Exine thickness	EXTP
	Average distance between pores	ADBP
	Number of micropores (mm²)	NMIP
Leaf epidermis	*type of epidermal cells [entire:1, crenate:2]	TEPE
	Length of epidermal cells	LECE
	Width of epidermal cells	WECE
	Number of epidermal cells	NUCE
	Length/width of epidermal cells	LWIE
	Length of stomata	LSTE
	Width of stomata	WSTE
	Length/Width of stomata	LWSE
	Guard cell length	GCLE
	Guard cell width	GCWE
	Length/Width of guard cell	LWGE
	Number of stomata	NSTE
Indumentum	*Unicellular simple hair [absent:0, present:1]	UNSH
	*Multicellular simple hair [absent:0, present:1]	MUSH
	*Short glandular hair [absent:0, present:1]	SGLH
	*Long glandular hair [absent:0, present:1]	LGLH
Stem	*Crystal in parenchymatous layer [absent:0, present:1]	CRPS
	*Shape of stem cross section [Rounded = 0, elliptic = 1]	SHAS
	Length of stem cross section	LCRS
	Width of stem cross section	WCRS
	Number of sclerenchymatus layers cell	NSCS
	Number of parenchymatous layer	NPAS
	Size of phloem	SPHS
	Size of xylem	SXYS
	Width of vascular bundle	WVAS
	Size of xylem/phloem ratio	SXPS
	Size of pith	SPIS
	Length of vascular bundle	LVAS
	Size of greatest xylem element	SGXS
	Size of cuticle	SICS
Peduncle	Length of peduncle cross section	LCRI
	Width of peduncle cross section	WCRI
	Length/width of peduncle cross section	LWCI
	Size of cuticle	SCUI
	Size of epidermal layer	SEPI
	Size of parenchymatous layer	SPAI
	Number of sclerenchymatus layer	NSCI
	Number of parenchymatous layer	NPAI
	Size of phloem	SPHI
	Size of xylem	SXYI
	Size of pith	SPII
	Width of vascular bundle	WVAI
	Length of vascular bundle	LVAI
	Length/width of vascular bundle	LWVI
	Size of greatest xylem element	SXYI
	*Arrangment of xylem elements [solitary:1, radial chain pore:2, cluster:3]	AXYI
	Size of greatest xylem element	SGXI
Leaf	Number of sclerenchymatus layers cell	NSCL
	Size of lower epidermal cell	SLEL
	Size lower parenchymatous layer	SLPL
	Lenght of leaf cross section	LCRL
	Width of leaf cross section	WCRL
	Length/width of leaf cross section	LWCL
	*Crystal in leaf [scarcely:0, densely:1]	CRYL
	*Arrangment of mesophyll [isolateral 1, dorsiventral 2]	ARML
	Length of lamina	LELL

Table 3. Leaf epidermal features of *Acanthophyllum* species in adaxial surface. The measurements are in mm.

Taxon	Epidermal cells lenght	Epidermal cells width	Number of epidermal cell in 1mm²	Stomata width	Stomata lenght	Number of stomata in 1mm²	Guard cell lenght	Guard cell width	Stomata type
A. adenophorum	0.1	0.051	14	0.02	0.03	4	0.025	0.014	diacytic, anomocytic, anisocytic
A. brevibracteatum	0.106	0.051	7	0.029	0.043	2	0.041	0.012	diacytic, anomocytic, anisocytic
A. diezianum	0.082	0.066	11	0.03	0.033	3	0.028	0.013	diacytic, anomocytic, anisocytic
A. borsczowii	0.054	0.071	11	0.026	0.027	3	0.027	0.013	anomocytic
A. heratense	0.053	0.037	16	0.027	0.031	5	0.029	0.012	diacytic, anomocytic, anisocytic
A. korshinskyi	0.11	0.058	12	0.032	0.034	3	0.035	0.01	diacytic, anomocytic, anisocytic
A. laxiusculum	0.12	0.051	11	0.028	0.035	2	0.031	0.029	diacytic, anomocytic, anisocytic
A. lilacinum	0.093	0.039	13	0.029	0.036	2	0.032	0.01	diacytic, anomocytic, anisocytic
A. pachystegium	0.07	0.062	12	0.028	0.035	3	0.035	0.013	diacytic, anomocytic, anisocytic
A. speciosum	0.095	0.032	16	0.026	0.035	5	0.028	0.012	diacytic, anomocytic, anisocytic
A. squarrosum	0.082	0.055	15	0.024	0.026	5	0.029	0.011	diacytic

Table 4. General pollen characters of the examined *Acanthophyllum* species.

Taxon	Polar axis	Equatorial axis	P/E	Pores diameters	Exine thickness	Pores distance
A. adenophorum	30.7	30.3	1.013201	4.5	1.4	10.2
A.brevibracteatum	28.9	28.2	1.024823	4.5	1.5	8.5
A.diezianum	24.7	24.6	1.004065	3.2	1.4	8
A. borsczowii	24.5	23.875	1.026178	3.7	1.6	7
A.heratense	25.4	24.9	1.02008	3.7	1.9	8.3
A.korshinsky	27.6	26.8	1.029851	3.6	1.5	6.4
A.laxiusculum	26.1	25.2	1.035714	4.5	1.6	8.7
A.lilacinum	29.2	28.9	0.989726	5.2	1.9	10.8
A.pachystegium	29.4	29.4	1	6	2	8.6
A.speciosum	30.6	30.25	1.001637	5	2	11.5
A.squarrosum	26.75	26.4	1.013258	4.1	1.47	7.92

Pith size and distribution of crystals. Size of pith in *A. adenophorum* and *A. borsczowii* is the greates amount (about .28 mm) and druse crystals are seen densly in the pith of *A. laxiusculum*.

The arrangment of xylem elements in peduncle and size of them. This character is different among the *Acanthophyllum* species and divided this section into three groups as i) *A. borsczowii* with solitary elements ii) *A. diezianum*, *A. lilacinum*, *A. heratense*, and *A. squarrosum* have radially chain pore arrangement iii) cluster or radially chain pore arrangement with cluster elements are observed in *A. laxiusculum*, *A. pachystegium*, *A. brevibracteatum*, *A. korshinskyi,* and *A. adenophorum* (Fig. 5). The size of xylem elements is maximum in *A. borsczowii*, *A. adenophorum*, *A. lilacinum,* and *A. korshinskyi* (> 0.02 mm). This

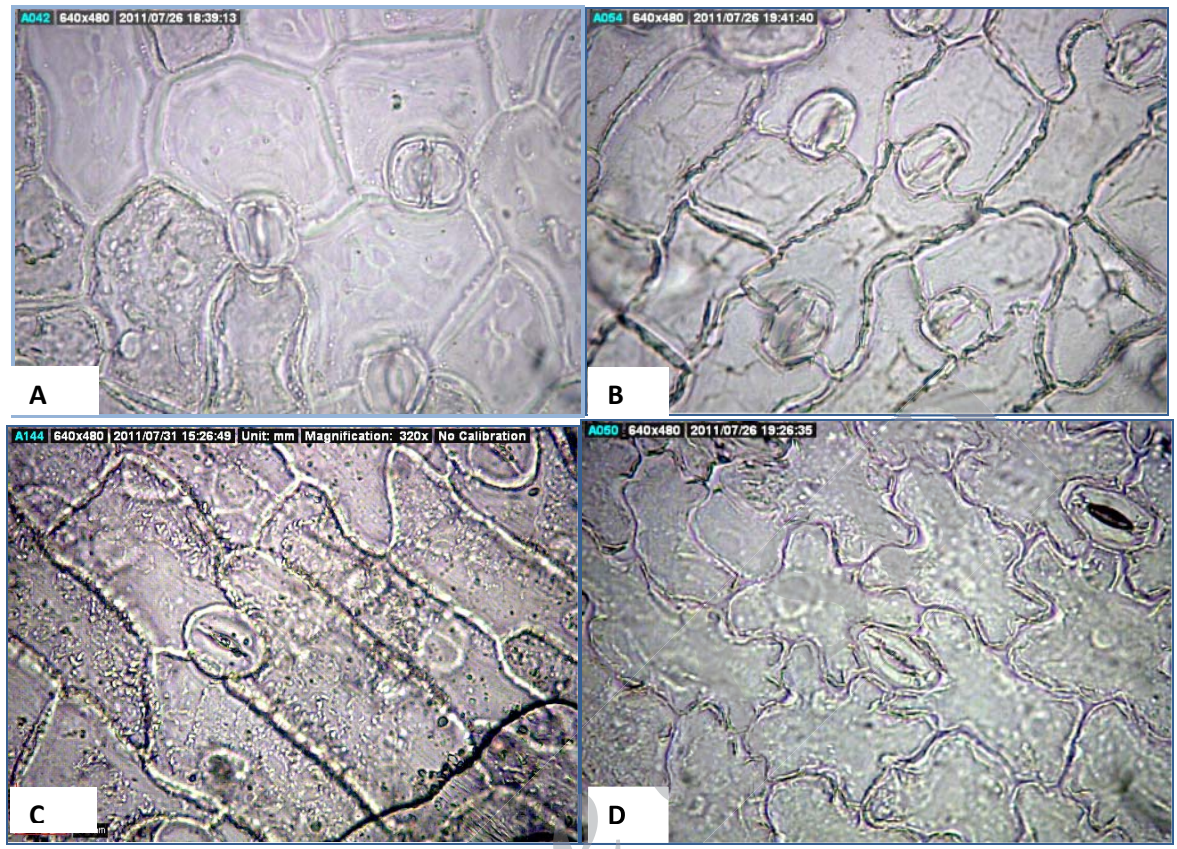

Fig. 1. Epidermal surface of some *Acanthophyllum* species. A: *A. borsczowii*, B: *A. squarrosum*, C: *A. laxiusculum*, D: *A. lilacinum*.

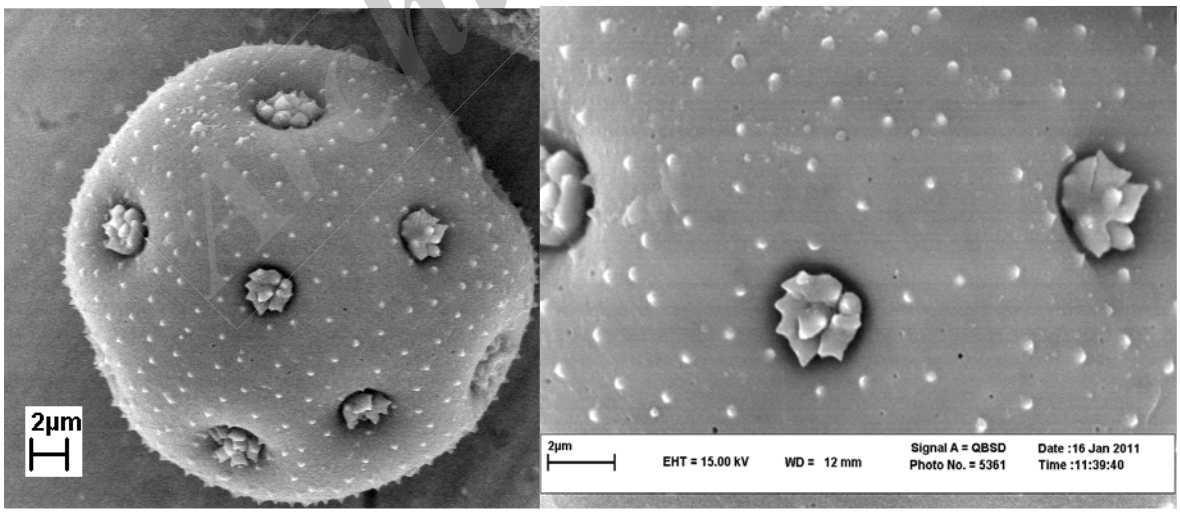

Fig. 2. The SEM micrographs of pollen grains in *Acanthophyllum adenophorum*.

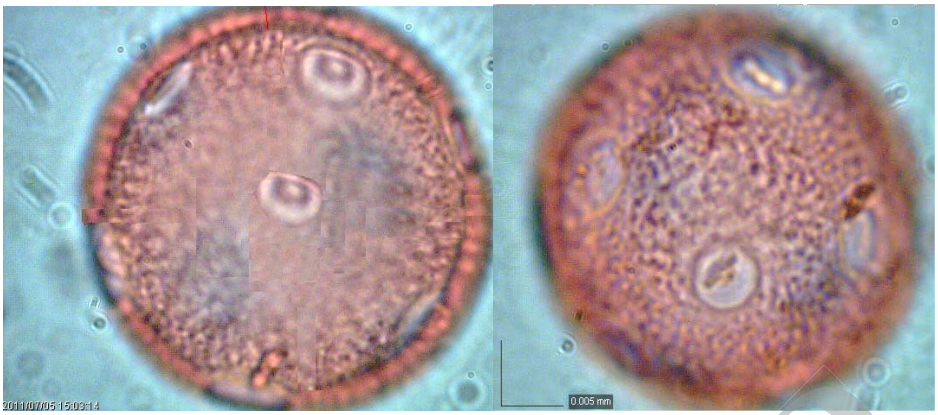

Fig. 3. The LM micrographs of pollen grains in *Acanthophyllum adenophorum.*

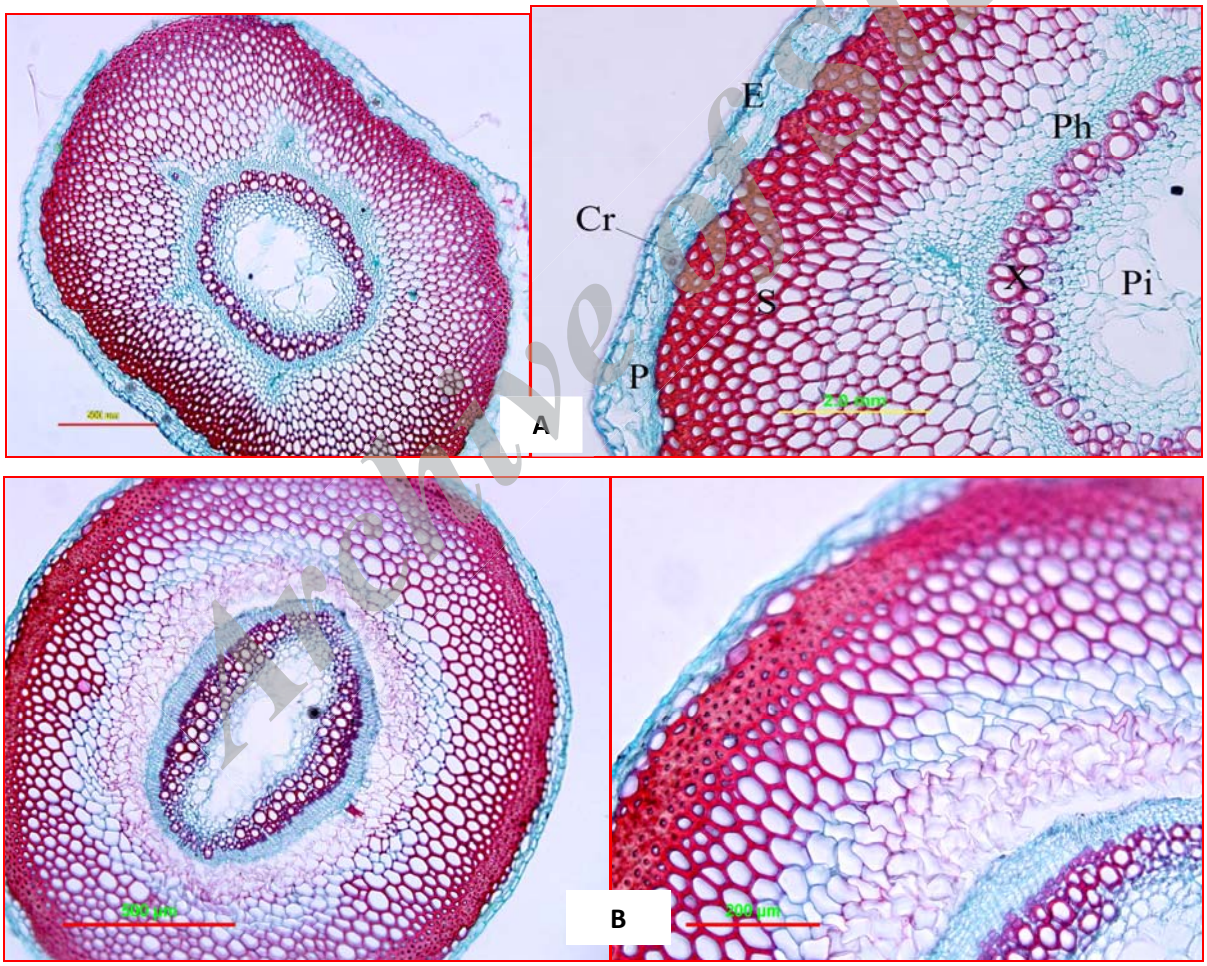

Fig. 4. The cross section of stem in *Acanthophyllum borsczowii* (A) and *A. korshinskyi* (B). E= Epidermis, P= Parenchymatous layers, Cr= Crystal, S= Sclerenchymatous layers, Ph= phloem, X= xylem, Pi= pith

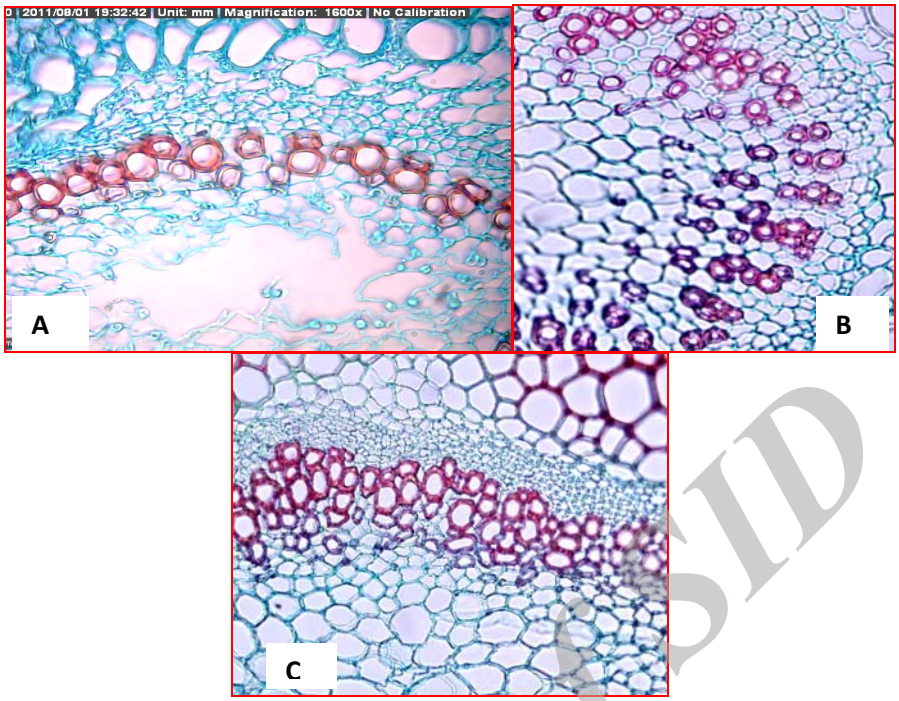

Fig. 5. The peduncle cross section of some *Acanthophyllum* species. A: ***A. borsczowii***, B: *A. squarrosum*, C: *A. adenophorum*.

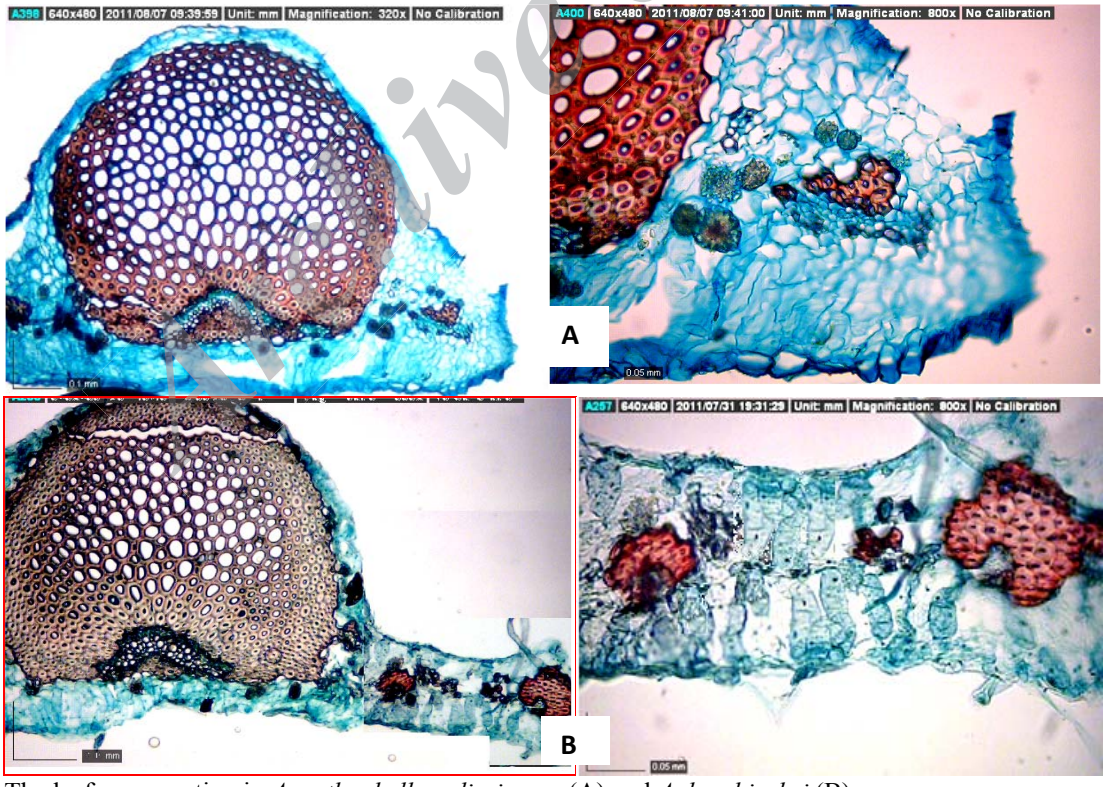

Fig. 6. The leaf cross section in *Acanthophyllum diezianum* (A) and *A. korshinskyi* (B).

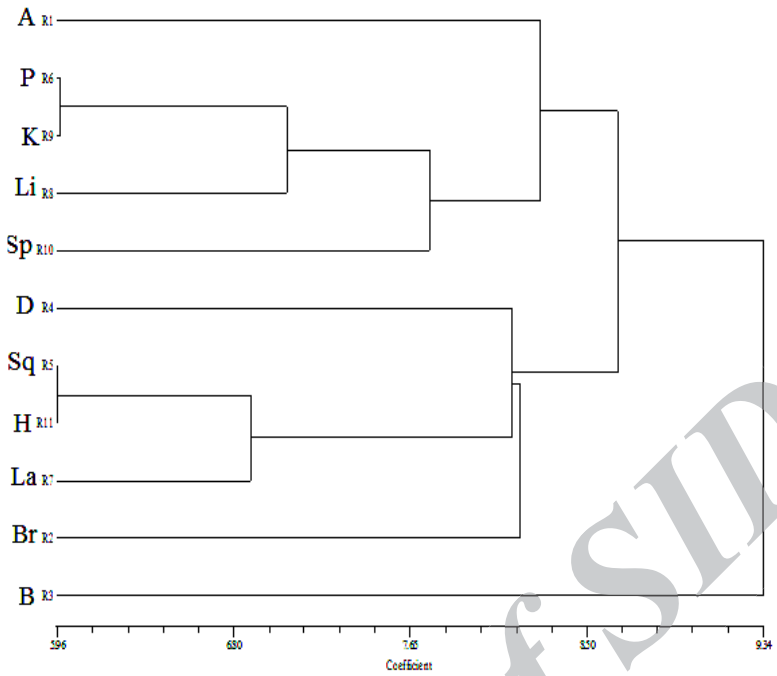

Fig. 7. Phenogram resulting from the UPGMA of *Acanthophyllum* sect. *Oligosperma* in Khorassan provinces. OTU'S represented by: A: *A. adenophorum*, P: *A. pachystegium*, K: *A. korshinskyi*, Li: *A. lilacinum*, Sp: *A. speciosum,* D: *A. diezianum*, Sq: *A. squarrosum*, H: *A. heratense*, La: *A. laxiusculum*, Br: *A. brevibracteatum*, B: *A. borsczowii*.

character is minimum in *A. laxiusculum*, *A. heratense*, *A. squarrosum,* and *A. pachystegium* (<0.02 mm).

The number of sclerenchymatous layers in peduncle. Sclerenchymatous layers are not observed in *A. borcszowii* peduncle while *A. pachystegium*, *A. brevibracteatum*, *A. diezianum*, *A. squarrosum* and *A. adenophorum*, have greatest amount of sclerenchymatous layers (4-7 layers).

The arrangment of mesophyll. This character is different among *Acanthophyllum* species and divided examined species into two groups as *A. diezianum*, *A. laxiusculum*, *A. squarrosum* and *A. heratense* had dorsi-ventral arrangment while *A. borsczowii*, *A. korshinskyi*, *A. pachystegium*, *A. speciosum*, *A. adenophorum* and *A. brevibracteatum* had isolateral arrangment (Fig. 6).

The number of sclerenchymatous layers in leaves. Sclerenchymatous thick walled cells layers are not observed in *A. borsczowii* while these layers difind in the greatest amount in *A. korshinskyi*, *A. adenophorum*, *A. brevibracteatum* and *A. pachystegium*.

Lamina size and druse crystal distribution. Lamina size in *A. borsczowii* is longer than 1 mm and druse crystal are seen densly in its lamina while in all other examined species lamina size is smaller than 0.5 mm.

Cluster analysis of micromorphological characters

The UPGMA of the OTU'S used in this study is shown in Fig 7. The arrangement of species in cluster was written in the left of cluster. The phenogram is divided into two clusters. *Acanthophyllum borsczowii* is separated from the rest of species in a single species cluster. In addition, the other cluster is divided into two branches. In the upper branch located *A. adenophorum*, *A. pachystegium*, *A. korshinskyi*, *A. lilacinum* and *A. speciosum*, in the lower branch *A. laxiusculum*, *A. heratense* and *A. squarrosum* located close together.

Principal component analysis of micromorphological characters. Only characters that have high eigenvalue on the first three principal component (r>0.5) and had the least correlation coefficient (r< 0.5) were selected to separate OTU's (Table 5). The first three components explain 94 % of the total character variation 64%, 23% and 7 % for the respective axes. In a plot of the first and second PCs (Fig. 8); the number of sclerenchyma thick walled layers in leaf (NSCL) character are isolated *A. korshinskyi* and *A. pachystegium* from the other taxa. Lenght of lamina (LELL), the size of greatest xylem elements in stem

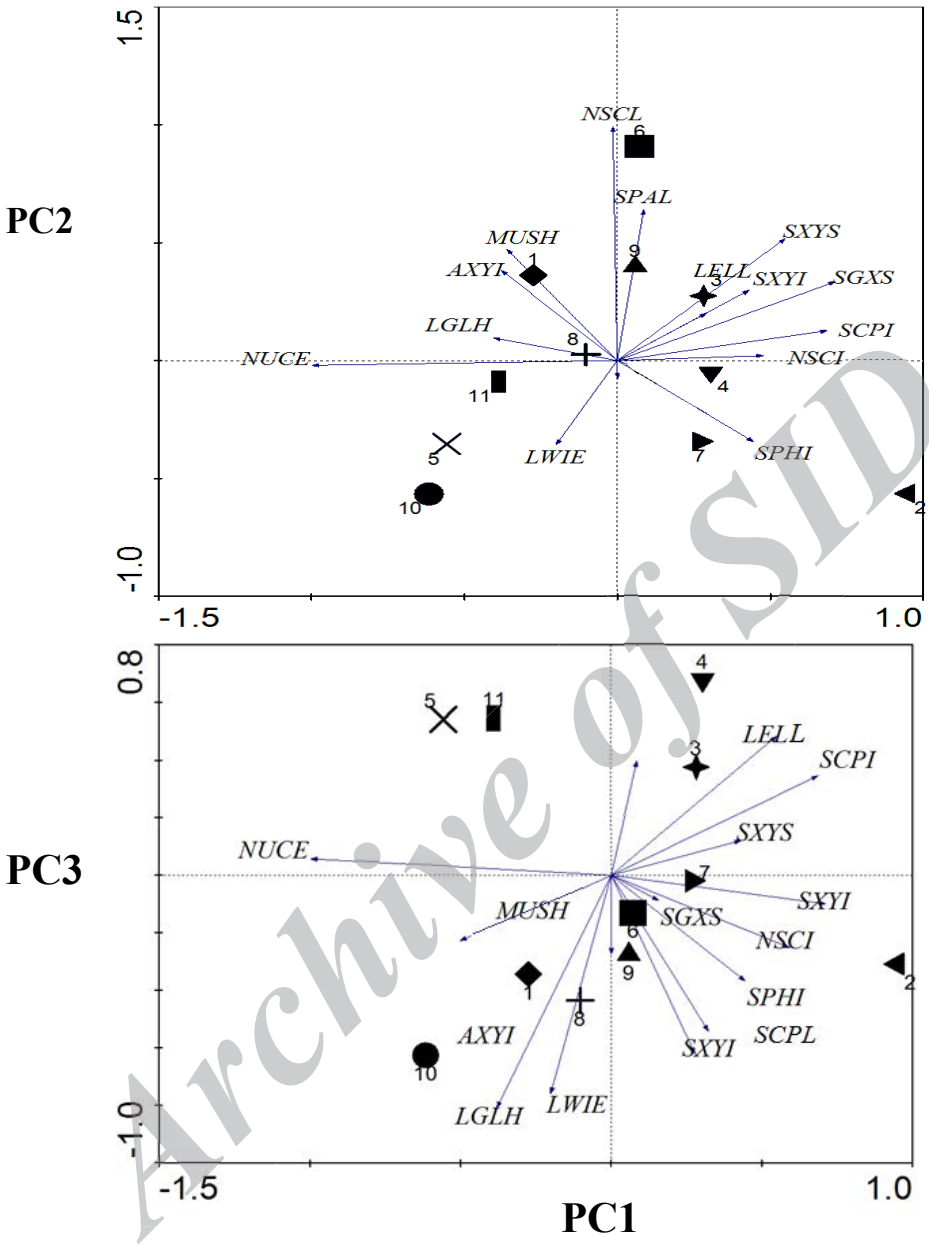

Fig. 8. Principal Component Analysis- Scatter diagram of specimens and characters from 11 species of *Acanthophyllum* sect. *Oligosperma* in Khorassan provinces. 1: *A. adenophorum*, 2: *A. brevibracteatum*, 3: *A. borsczowii*, 4: *A. diezianum*, 5: *A. heratense*, 6: *A. pachystegium*, 7: *A. laxiusculum*, 8: *A. lilacinum*, 9: *A. korshinskyi*, 10: *A. speciosum*, 11: *A. squarrosum*.

(SXYS) and the size of the greatest xylem elements in peduncle (SXYI) are the characters that separated *A. borsczowii*. *Acanthophyllum heratense* and *A. squarrosum* are isolated from the rest by length/width of epidermal cells (LWIE); the number of epidermal cells (NUCE) separates *A. speciosum*. Long glandular hairs (LGLH) character differentiates *A. lilacinum*. Multicellular simple hairs (MUSH) and arrangement of xylem elements (AXYI) are the characters that excluded *A. adenophorum*. *A. laxiusculum* is distinguished by size of phloem in peduncle (SPHI) and the number of sclerenchymatous layers in peduncle (NSCI) character exluded *A. diezianum* from the other taxa.

Table 5. Eigen vectors of the characters used on the first three axes in the PCA.

N	NAME	AX1	AX2	AX3	AX4
1	MUSH	0.5833	-0.037	0.0831	0.0052
3	LGLH	0.5148	-0.2294	0.005	0.7133
4	AXYI	0.5457	-0.2162	0.3366	0.7491
8	NUCE	0.3274	-0.9442	0.0042	-0.0359
9	LWIE	0.9909	0.1293	-0.0336	-0.0156
10	SXYS	-0.0084	0.5253	0.3243	-0.224
12	SGXS	0.5606	0.3005	0.0164	-0.0071
13	SCPI	-0.4968	0.5469	0.174	-0.2294
14	SPAI	-0.2518	0.5479	0.0886	0.2556
15	NSCI	0.5708	0.7156	-0.1937	-0.1455
16	SPHI	0.0107	0.5748	-0.4096	0.3501
17	SXYI	0.5156	0.4294	-0.2917	-0.2049
22	NSCL	0.1791	0.0709	0.9737	-0.1205
25	LELL	0.1392	0.5612	0.1306	-0.3428

DISCUSSION

The present study matches with the results of Schiman-Czeika (1988) and Shishkin (1936) who reported different type of trichomes in *Acanthophyllum* species. Schiman-Czeika (1988), in Flora Iranica, described *A. borsczowii* as a glabrous plant but our result is in agreement with Bidi (2007) who reported unicellular simple hairs on *A. borsczowii*. The basic type of stomata is diacytic in Caryophyllaceae family according to Metcalfe and Chalk (1983) while in *A. borsczowii* the anomocytic type of stomata was observed. Some other stomata types such as anisocytic and anomocytic were observed in several species (Table 3). This study is also in agreement with Jafari & al. (2008) who reported crenate and entire subsidiary cell walls in some species of *Silene*. Similarly, anisocytic and anomocytic types of stomata were reported in some *Silene* species by Sahreen et al. (2010).

In *Acanthophyllum* species pollen grains are fairly uniform with radially symmetrical and pantoporate grains, although, our results showed differences in pollen size, distance between two pori and number of pores but these variation were not remarkable and are not important characters for taxonomy of *Acanthophyllum* species.

Stem anatomy was somewhat simillar in the examined taxa, but stem cross section shape and size, the number of sclerenchymatous layers are taxonomically significant to identify species.

In this study calcium oxalate crystals are observed both in endodermis and pith that are dissent with Metcalfe and Chalk (1950). They reported that crystals in Caryophyllaceae are placed only in endodermis. However, it is well known that it is an environmentally influenced anatomical character and we cannot use it as a strong taxonomic character for grouping species (Kilic 2009). The number of sclerenchymatous layers difined in the greatest amount in *A. borsczowii*, it should be mentioned that the stem in this species contrary to the other species of the section except for *A. elatius* Bunge ex Boiss. is vertical.

The arrangment of xylem in peduncle and the arrangment of mesophyll in leaf were qualitative characters that taxonomically significant to separating *Acanthophyllum* species. The second character was removed in the PCA analysis due to the high correlation coefficient on the PCA analysis. Examined species have been divided to three groups by the arrangment of xylem elements character in peduncle; these species are well separated by morphological characters too.

The results of the cluster analysis (Fig. 7) indicated that *A. laxiusculum*, *A. squarrosum* and *A. heratense* are linked together that are in agreement with Basiri & al. (2011) taxonomic results. They concluded that the species *A. heratense* and *A. laxiusculum* are as synonyms. With respect to the proximity of *A. heratense* habitat to *A. laxiusculum* and *A. squarrosum*, molecular study and a ditailed morphological study on

these three species in the field are suggested.

The results showed endomorphic characters in *Acanthophyllum* species are only useful to separate the species that are morphologically apart, as the species *A. adenophorum*, *A. pachystegium* and *A. lilacinum* that are morphologically similar having more or less irregular epidermal cells with wavy walls, glandular and eglandular hairs, cluster or radial chain pore with cluster elements, leaf with isolateral mesophyl and also generally greater sclerenchymatous layers while *A. laxiusculum*, *A. heratense* and *A. squarrosum* have rectangular epidermal cells with entire walls, eglandular hairs, radial chain pore or radial chain pore with cluster elements and dorsi-ventral arrangement in leaf mesophyll. *Acanthophyllum borsczowii* with vertical stem, ovate-lanceolate leaves, anomocytic stomata, polygonal epidermal cells, solitary xylem element arrangement and rectangular stem cross section was different from all examined species in this genus.

REFERENCES

Basiri, Sh., Bidi, B., Assadi, M., & Rahimi Nejad, M. 2011: A taxonomic study of *Acanthophyllum* C. A. Mey. (Caryophyllaceae) in Iran. -Iranian Journal of Bot. 17 (1): 24-39.

Bidi, B. 2007: Taxonomic study of *Acanthophyllum* C. A. Mey. sect. Oligosperma in Iran. -MSc thesis, Isfahan University. Isfahan.

Bokhari, M. H. 1987: Recent Trends in Angiosperm Taxonomy. Mod. Trends. -Pl. Sci. Res. Pak. -Department of Botany, University of Peshawar, Peshawar. pp. 248 – 252.

Erdtman, G. 1960: The acetolysis method, a revised description. -Svensk Bot. Tidskr. 54: 561-564.

Fior, S., Karis, P. O., Gabriele, C., Minuto, L. & Sala, F. 2006: Molecular Phylogeny of the Caryophyllaceae (Caryophyllales) Inferred From Chloroplast Matk and Nuclear RDNA ITS Sequences. -American Journal of Botany. 93(3): 399–411.

Ghaffari, M. 2004: Cytotaxonomy of some species of Acanthophyllum (Caryophyllaceae) from Iran. -Biologia, Bratislava. 59 (1): 53-60.

Ghaffari, M., 2002: Biosystematic study of some Acanthophyllum species. -PhD thesis, University of Tehran.

Jafari, A., Zokai, M & Fathi, Z. 2008: A biosystematical investigation on *Silene* L. species in North East of Iran. -Asi. J. Pl. Sci. 7(4): 394-398.

Kilic, S. 2009: Anatomical and pollen characters in the Genus *Silene* L. (Caryophyllaceae) from Turkey. -Botany Research Journals. 2 (2-4): 34-44.

Legendre, P. & Legendre, L. 1998: Numerical Ecology. -Elsevier, Amsterdam. pp. 47–48.

Metcalfe, C. R & Chalk, L. 1983: Anatomy of the Dicotyledons, Wood Structure and conclusion of the general introduction. -Oxford. Claredon Press.

Metcalfe, C. R. & Chalk, L. 1950: Anatomy of the Dicotyledons. Oxford. -Claredon Press. vol 1: 147-152.

Perveen, A & Qaiser, M. 2006: Pollen Flora of Pakistan. Zygophyllaceae. -Pak. J. Bot. 38 (2): 255-232.

Punt, W., Blackmore, S., Nilsson, S & LE Thomas, A. 1994: Glossary of Pollen and Spore Terminology. -LPP Contributions Series. LPP Foundation, Utrecht, vol. 1: 1–71.

Rohlf, F. J. 1998: NTSYS, numerical taxonomy and multivariateanalysis system. -New York: Exeter Software.

Sahreen, S., Ajab Khan, M., Rashid Khan, M & Ali Khan, R. 2010: Leaf epidermal anatomy of the genus *Silene* (Caryophyllaceae) from Pakistan. -Biological Diversity and Conservation. 3(1): 93-102.

Schiman-Czeika, H. 1988: Acanthophyllum in Rechinger, K. H. (ed.). Flora Iranica no. 163: 253-330. -Akademische Druck-u, Verlagsanstalt Graz-Austria.

Schweingruber, F. H. 2007: Stem anatomy of Caryophyllaceae. -Flora. 202 (4): 281-292.

Shishkin, B. K. 1936: Acanthophyllum in Komarov, V. L. (ed.) Flora of USSR, vol. 6: 781-802. -Moskva & Leningrad.

Takhtajan, A. L. 1986: Floristic Regions of the World. -University of California Press, Berkley, USA.

Ter Braak, C. 1988: CANOCO, an extension of DECORANA to analyze species-environment relationship. -Vegetatio. 75 (3):159–160.