Stem and fruit anatomical study of the *Anchusa* species (*Boraginaceae*) in Iran

Maryam Keshavarzi[1], Fatemeh Nasrollahi[1] & Masoud Sheidai[2]

[1] Biology dept., Faculty of Science, Alzahra University, Vanak, Tehran, Iran, e-mail: neshat112000@yahoo.com (corresponding author)

[2] Faculty of Biological Sciences, Shahid Beheshti University, Evin, Tehran, Iran.

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Abstract. With four species, *Anchusa* is one of the weedy genera of *Boraginaceae* in Iran. Its species are distributed in different habitats. Species delimitation is mainly based on the corolla lobes, corolla tube condition and fruit shape. In this study, the fruit and stem anatomical features of the *Anchusa* taxa have been considered, so as to evaluate the diagnostic value of these characters. A total of 23 qualitative and quantitative anatomical features in 13 accessions of *A. italica, A. strigosa, A. arvensis,* and *A. aegyptica* have been studied. Statistical multivariate methods (cluster and factor analysis) were applied by means of SPSS software ver. 19. In stem cross sections, the factor analysis revealed that two main factors have mostly caused the observed variation. These factors comprise the epidermis diameter, cortex diameter, palisade diameter, parenchyma diameter, the vessel-to-epidermis diameter, stem-to-epidermis diameter, rupture of pith, width of vessels, inner phloem diameter, stem diameter, and stem-to-vessel diameter. In fruit cross sections, the most important features are: sclerenchyma thickness, number of vascular bundles, shape of margins of the cross section, and general shape of the nutlet cross section. Species relationship is evaluated on the basis of anatomical studies. This is the first study of the *Anchusa* weedy species of Iran.

Key words: anatomy, *Anchusa*, cluster analysis, Iran

Introduction

Anchusa L. (*Boraginaceae*) is a large genus, with about 170 annual and perennial species in the temperate and subtemperate zone of the Old World (Akçin & al. 2010). Although robust hairs are the main diagnostic feature of *Boraginaceae*, studies based on the structural diversity of hairs and leaf anatomy are rare. Most literature descriptions in the last decade deal with the general features of *Boraginaceae*. Major investigations about leaf anatomy in *Boraginaceae* s.str. tribe *Borageineae* have been conducted by Selvi & Bigazzi in 2001. Some locally limited anatomical studies into the vegetative structure have been carried out for one or several species (Jodin 1903; Fell & Peck 1968; Selvi & Bigazzi 1998) and indicated great changes in the vegetative forms and leaf structure of this tribe.

The southern parts of the Balkan Peninsula are the main centre of diversification of this genus (Selvi & Bigazzi 2003). The *Anchusa* species are distributed in Europe, North and South of Africa and West Asia. These weedy plants (annual, biennial or perennial species) are covered with robust hairs and rough nodes (Judd & al. 1998). The leaves are ovate or lanceolate and sessile on top of the stem. Blue or white flowers aggregate in cymes. The fruit is composed of four nutlets with wrinkled surface. In Iran, *Anchusa* is composed of four species and five taxa (*A. italica* has two varieties, namely *italica* and *kurdica)*. The *Anchusa* species are distributed in northern, northwestern, central, northeast-

ern and southern parts of Iran. *A. italica* var. *italica* is the widest distributed (Khatamsaz 2002) species. The *Anchusa* species have ornamental, medicinal and edible functions in different parts of Europe and Asia. In Iran, these species are used as a substitution for the famous medicinal *Borago* plant (Zargari 1989). The roots of *Anchusa* have a special resinous component as a coloring agent. Some species are also considered as weeds in different cultivations (Judd & al. 1998). Riedl (1967) and Khatamsaz (2002) and other authors have cited different infragenus and infraspecies classifications in the *Anchusa* (Guşuleac 1927; Chater 1972; Greuter & al. 1984; Brummit 1992; Selvi & Bigazzi 1998).

Morphological and anatomical studies of this genus are not so extensive (Selvi & Bigazzi 1998; Selvi & Bigazzi 2000a,b) and have mainly focused on the leaves features (Nyauwame & Gill 1990; Selvi & Bigazzi 2001). Akçin & al. (2010) have studied the anatomy of some native *Anchusa* species of Turkey and have mentioned the epidermis, parenchyma and colenchyma of *A. italica* var. *italica*. Several studies have dealt with the interspecific and intraspecific variation in this genus across the world (Selvi & Bigazzi 1998; Akçin & al. 2010).

Due to the relative stability of some anatomical traits related to environmental factors, the anatomical results are important in the systematic of *Anchusa* (Guşuleac 1927; Selvi & Bigazzi 1998). In this study, different accessions of *Anchusa* have been collected from different parts of Iran and anatomical studies of the stem and fruit cross sections were performed, so as to evaluate the affinities and relationships of the taxa.

Material and methods

Stem sections of 13 populations and fruit sections of five populations from four species of *Anchusa* collected from different parts of Iran were studied out (Table 1 and 2). Fresh material of the *Anchusa* species and herbarium specimens were used in the study. Voucher specimens were deposited in the Herbarium of Alzahra University (AUH). A total of seven qualitative and 12 quantitative features were selected for stem anatomy (Table 3). The materials for anatomical studies were fixed in FAA and preserved in 70 % ethanol. Sections of stem and fruit were taken manually, cleaned with sodium hypochlorite, stained with methyl green and carmen-vest. An Olympus BX51 light microscope and DP12 digital camera were used for the examination of slides, photography and drawing. In order to detect significant differences in the studied characters among the various studied species, an analysis of variance (ANOVA) was performed. To reveal the species relationships, cluster analysis and principal component analysis (PCA) were applied. For multivariate analysis, the mean of the quantitative characters was used, while the qualitative characters were coded as binary/multistate characters. Standardized variables were used for the multivariate statistical analysis. Average taxonomic distances and squared Euclidean distances were applied as dissimilarity coefficient in the cluster analysis of anatomical data. In order to determine the most variable anatomical characters among the studied species, factor analysis based on principal components analysis was performed. SPSS ver. 19 software was used for statistical analysis.

Table 1. Collection data on populations used in stem section.

Species	Origin	Voucher No	Collector
A. italica var. *italica*	Tehran, Vanak	AUH 1 3910	Nasrollahi
A. italica var. *italic*	Tehran, Sadat Abad	AUH 13913	Nasrollahi
A. italica var. *italic*	Hamedan, Barfjin, Emamzadeh Koh	AUH 13909	Keshavarzi
A. italica var. *kurdica*	Kurdistan, Sanandaj to Saghez	AUH 139051	Abassian
A. italica var. *kurdica*	Kurdistan, Sanandaj, Abidar Mt.	AUH 13902	Nasrollahi
A. italica var. *kurdica*	Chaharmahal Bakhtiari, Dasht Joshaghan	AUH 13701	Rahimpour
A. arvensis subsp. *orientalis*	Alborz, SW Karaj, Mardabad	AUH 13671	Soudi
A. arvensis subsp. *orientalis*	Kermanshah to Sanandaj	AUH 13908	Abassian
A. arvensis subsp. *orientalis*	Tehran, Vanak	AUH 13672	Khalili
A. strigosa	Alborz, Baghestan Mt.	AUH 13903	Mosaferi
A. strigosa	Alborz, Rejaii Shahr, Baghestan	AUH 13904	Mosaferi
A. strigosa	Quazvin, Mohamadiyeh 15 km to Quazvin	AUH 13905	Nasrollahi
A. aegyptiaca	Bushehr, Genaveh Port	AUH 13901	–

Table 2. Collection data on populations used in fruit section.

Species	Origin	Voucher No	Collector
A. italica var. *italica*	Tehran, Sadat Abad	AUH 13913	Nasrollahi
A. italica var. *kurdica*	Kurdistan, Sanandaj to Saghez	AUH 139051	Abassian
A. arvensis subsp. *orientalis*	Tehran, Vanak	AUH 13672	Khalili
A. strigosa	Alborz, Baghestan Mt.	AUH 13903	Mosaferi
A. aegyptiaca	Bushehr, Genaveh Port	AUH 13901	–

Table 3. Stem anatomy features in the *Anchusa* species of Iran.

Characters	Code	State of character
Epidermis diameter	e.d	–
Scalar parenchyma diameter	Sca.pa	–
Collenchymas diameter	Co.d	–
Parenchyma diameter	pa.d	–
Cortex diameter	s.o.d	–
Width of vessels	l.o.v	–
Inner phloem diameter	Ph.d	–
Stem diameter	s.d	–
Stem to vessel diameter	s.v	–
Vessel to epidermis diameter	v.e	–
Stem to epidermis diameter	s.e	–
Length of norm of hair	l.n.h	–
Cambium	ca	Distinct (1), indistinct (2)
Rupture of pith	Pi.rup	present (1), absent (2)
Ray of pith	pi.ray	present (1), absent (2)
Presence of storage material in pith	St.pi	present (1), absent (2)
Presence of storage material in parenchyma	St.pa	present (1), absent (2)
Shape of stem cross section	Sh.st	Round (1), oval (2), pear-shaped (3), polygonal (4)
Shape of margin of stem cross section	Sh.ma	Smooth (1), wavy (2), deep prominence and notch (3)

Results

Stem section. Methods of numerical taxonomy were used to evaluate the species relationships. Cluster analysis based on morphological features revealed the accession relationships. A dendrogram by WARD method shows two main clusters (Fig. 1). The first cluster comprises two subclusters containing populations of *A. aegyptiaca* DC. and both varieties of *A. italica* Retz. The main second cluster comprises of two subsets, the first with populations of *A. arvensis* M.Bieb. and the second with populations of *A. strigosa* [Soland].

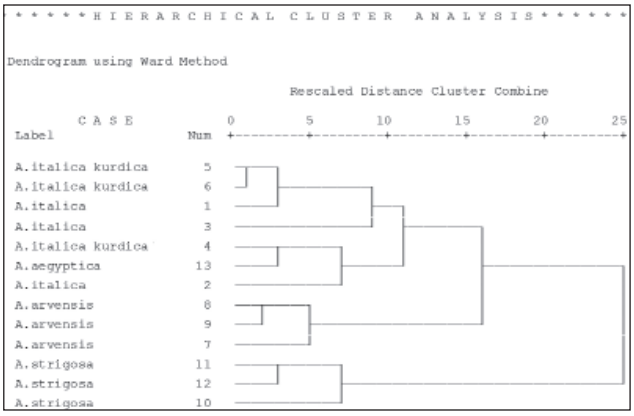

Fig. 1. WARD dendrogram based on stem anatomical traits of four *Anchusa* species.

In order to determine the most variable characters among the studied species, a factor analysis based on PCA was performed, revealing that the first two factors comprise over 52.82 % of the total variation. In the first factor, with about 28.99 % of total variation, the features of epidermis diameter, cortex diameter, scalar parenchyma diameter, parenchyma diameter, vessel to epidermis diameter, stem-to-epidermis diameter have shown greater correlation. In the second factor, with about 23.83 % of total variation, the rupture of pith, width of vessels, inner phloem diameter, stem diameter, and stem-to-vessel diameter have shown greater correlation (Table 4).

Table 4. Factor analysis results based on the stem anatomical characteristics of the *Anchusa* populations of Iran.

Characters	1 factor	2 factor
Epidermis diameter	96/0	–
Cortex diameter	90/0	–
Scalar parenchyma diameter	88/0	–
Parenchyma diameter	87/0	–
Vessel to epidermis diameter	69/0	–
Stem to epidermis diameter	61/0	–
Rupture of pith	–	88/0
Width of vessels	–	85/0
Inner phloem diameter	–	72/0
Stem diameter	–	72/0
Stem to vessel diameter	–	63/0

A PCA graph based on the two main factors (Fig. 2) reveals relationship between the studied species and is in concordance with the cluster analysis.

Fruit section. Fruit sections of both varieties of *A. italica* are studied. Sclerenchyma tissue thickness in *A. italica* var. *italica* is 155.02 μm and in *A. italica* var. *kurdica* is 151/18 μm. Vascular bundles in *A. italica* var. *italica* are 35 and in *A. italica* var. *kurdica* are 15.

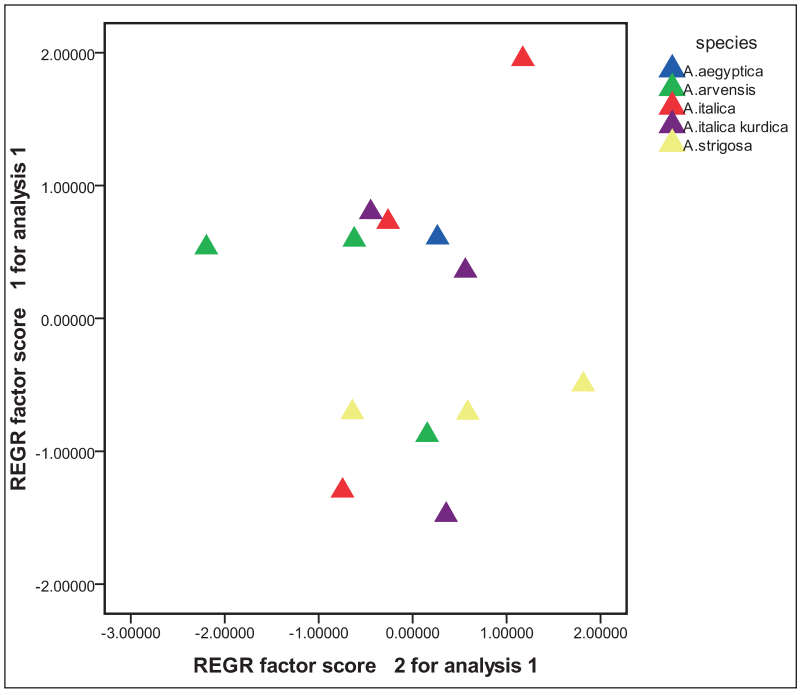

Fig. 2. NPCA ordination of the *Anchusa* species based on stem anatomical characteristics.

These two varieties are similar to each other in the general shape of cross section and nutlet margins. Both are ovate and the margin is deeply notched (Figs 8 and 9). Sclerenchyma tissue thickness in *A. strigosa* is 127.89 μm and there are 24 vascular bundles. General shape of the cross section is ovate and the margin is slightly wavy (Fig. 10). Fruit section of *A. arvensis* subsp. *orientalis* is asymmetric and winged. Nutlets of the other species are erect or suberect, but the nutlet of *A. arvensis* subsp. *orientalis* is curved. Thus the difference in cross sections seems normal. Margin of the fruit section is quite wavy. This species shows the lowest sclerenchyma tissue thickness (106.78 μm) and 13 vascular bundles (Fig. 11). Sclerenchyma tissue thickness in *A. aegyptiaca* is 244.64 μm and that is the maximum sclerenchyma thickness. Vascular bundles are 25. General shape of the cross section is round and the margin of fruit section is quite wavy (Fig. 12).

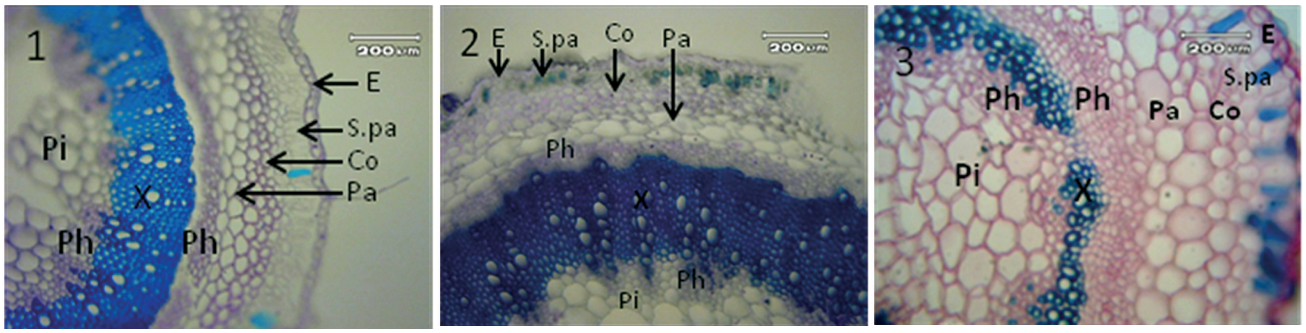

Fig. 3. Stem anatomy of *A. italica* var. *italica* in the populations of: 1) Hamedan, 2) Sadat Abad, 3) Vanak. E: epidermis, S.pa: scalar parenchyma, Co: collenchymas, Pa: parenchyma, Ph: phloem, X: xylem, Pi: pith, St.pa: presence of storage material in parenchyma, St.pi: presence of storage material in pith.

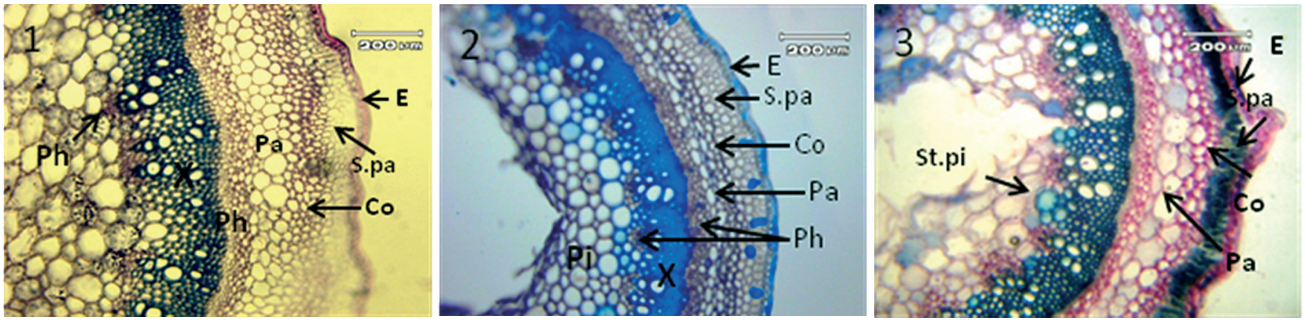

Fig. 4. Stem anatomy of *A. italica* var. *kurdica* in the populations of: 1) Chaharmahal Bakhtiari, 2) Abidar Mt., 3) Sanandaj to Saghez.

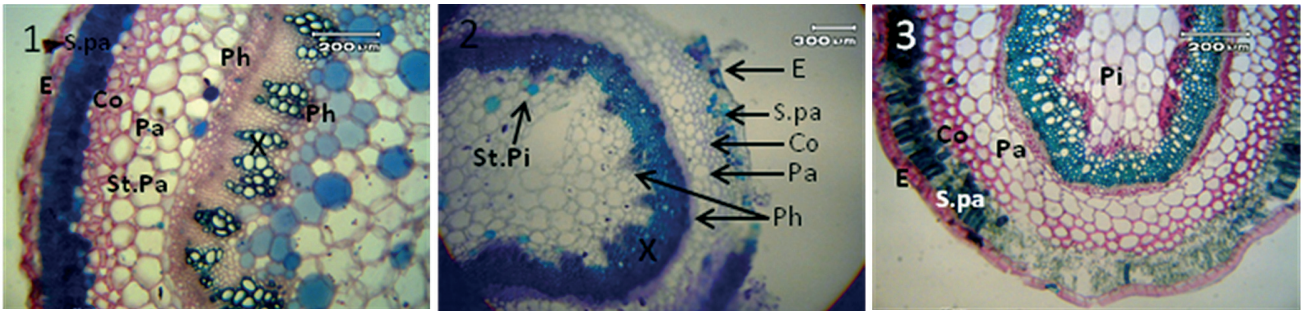

Fig. 5. Stem anatomy of *A. strigosa* in the populations of: 1) Quazvin, 2) Rejaii Shahr, 3) Baghestan Mt.

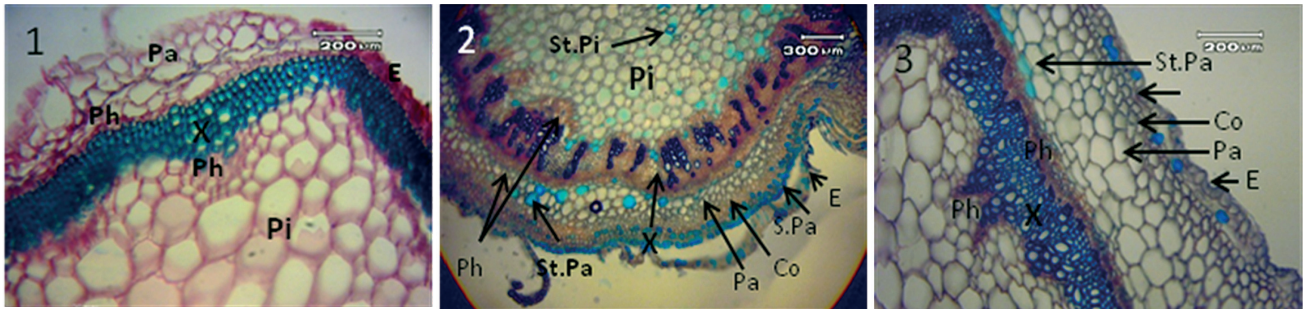

Fig. 6. Stem anatomy of *A. arvensis* in the populations of: 1) Vanak, 2) Kermanshah to Sanandaj, 3) Mardabad.

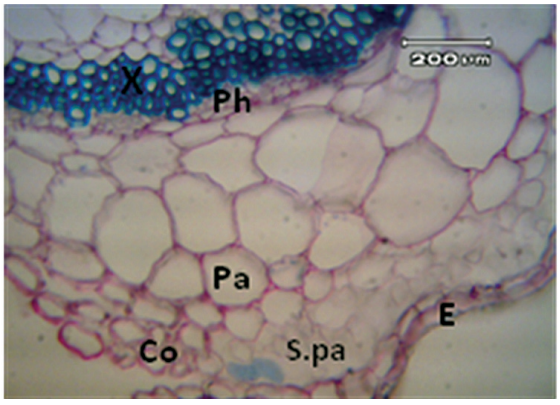

Fig. 7. Stem anatomy of *A. aegyptiaca* in Genaveh Port.

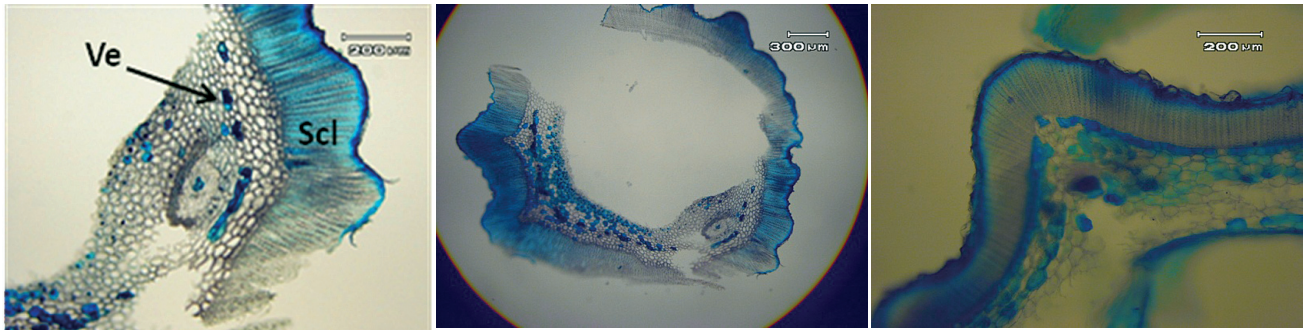

Fig. 8. Fruit anatomy of *A. italica* var. *italica*. Scl: sclerenchyma,

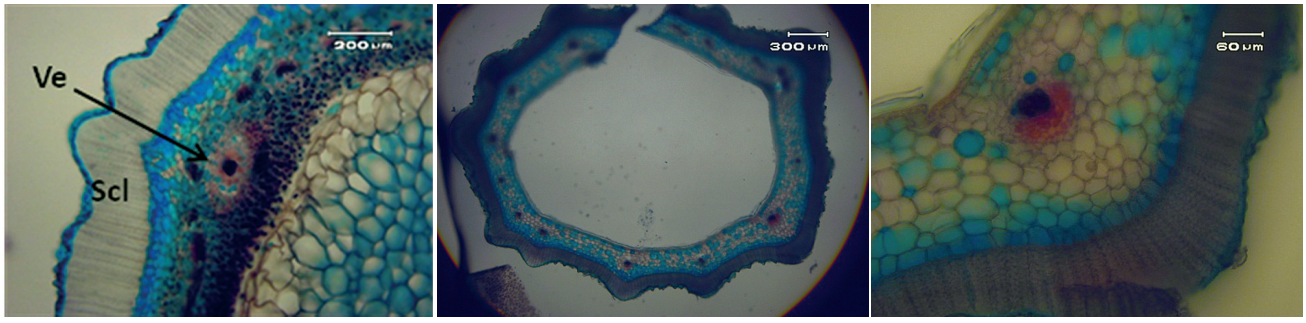

Fig. 9. Fruit anatomy of *A. italica* var. *kurdica.*

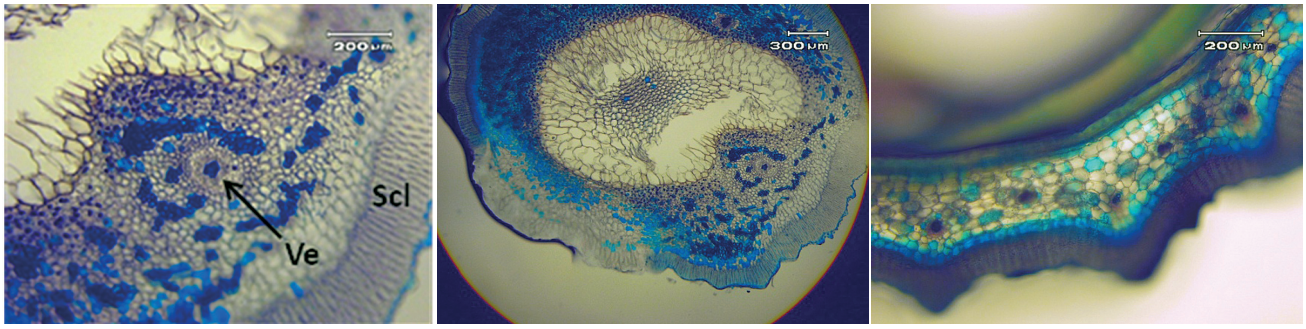

Fig. 10. Fruit anatomy of *A. strigosa.*

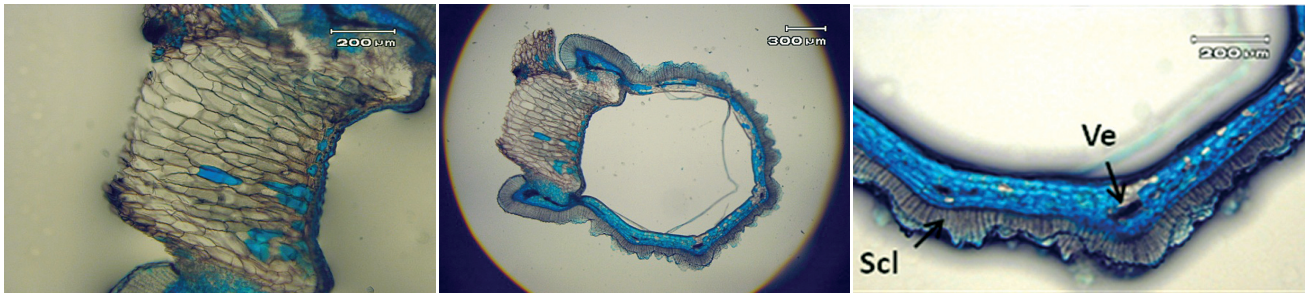

Fig. 11. Fruit anatomy of *A. arvensis* subsp. *orientalis.*

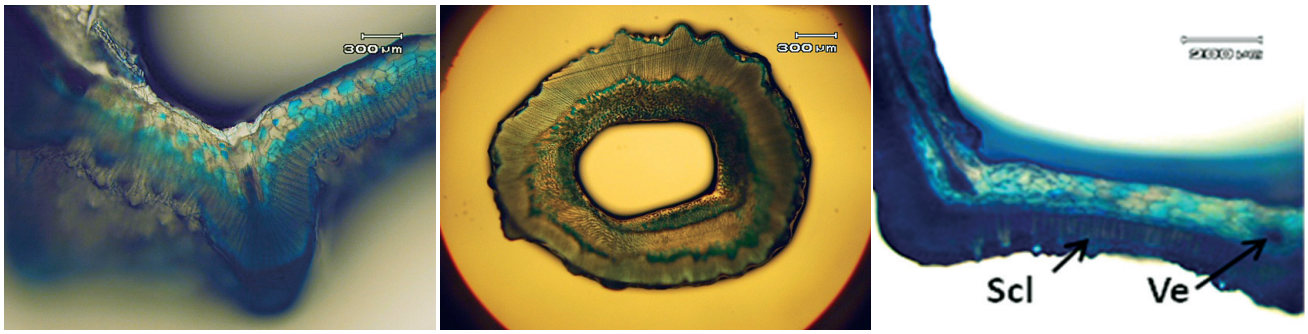

Fig. 12. Fruit anatomy of *A. aegyptiaca.*

Discussion

Owing to differences in the stem anatomical features, the *Anchusa* species are clearly distinguished. Our results have shown that populations of *A. aegyptiaca* are nested in the *A. italica* clades. Stem anatomical structure of the two varieties of *A. italica* and of *A. aegyptiaca* has shown more similaritieds. Diagnostic features are related mostly to the cortex and epidermis. Characters of the vascular bundles are of lesser importance. Akcin & al. (2010) have studied stem anatomy of some native species to Turkey and have mentioned the epidermis condition, collenchymas and parenchyma of cortex in *A. italica* var. *italica*. The results of the present study are consistent with the findings of that research.

Great morphological similarity has been observed between the two varieties of *A. italica* (var. *italica* and var. *kurdica*). Anatomical studies help to distinguish these two varieties so that all populations would compose a subclade. Statistical analysis of the data has shown close relationships between the species pair of *A. italica* and *A. aegyptiaca, A. strigosa* and *A. arvensis*. The results are not in concordance with our morphological observations (Nasrollahi & al. 2012), where *A. aegyptiaca* has gained a separate position, as in this study *A. aegyptiaca* is located between the clades of *A. italica*.

The fruit anatomical study has shown a difference in the sclerenchyma thickness, number of vascular bundles, general shape of the cross section and nutlet margins. The fruit cross sections of *A. arvensis* sub. *orientalis* is asymmetric and winged. Thus it becomes evident that the fruit anatomical features have diagnostic value in the *Anchusa* species in Iran.

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