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Anatomy of *Salvia limbata* in Relation to Altitudinal Gradient in West Azerbaijan (Iran)

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Abstract

This study was conducted to investigate the effect of altitudinal gradients on the anatomy of *Salvia limbata* C.A.Mey. (Lamiaceae). The *S. limbata* species were collected from their natural habitats from various regions of West Azerbaijan province in Iran. The thickness of cuticle, collenchymas, phloem, xylem and pith of stem and leaf thickness were measured. Moreover, width, length and number of stomata on both adaxial and abaxial epidermal surfaces were analyzed. Statistical analyses were done on a completely randomized design. The results indicated that by increasing altitude leaf thickness increased from 239.1 to 300.1 µm. Number of leaf epidermal cells was also increased in higher altitude on both upper (4.30 to 5.61) and lower (1.05 to 6.55) epidermal surfaces. The results confirmed that more stomata on both adaxial and abaxial epidermis in high altitude samples were associated with presence of narrower and longer stomata on their leaves. Significant differences in the cuticle, collenchyma, xylem, phloem and parenchymatous pith thickness in the stem were observed in all the six studied populations. At higher altitudes thicker cuticle, increased number of collenchyma and wider xylem, phloem and parenchymatous pith were detected. Plants in Mahlamlu region had the thickest studied parameters and plants in Kabudan Island region had the thinnest tissues.

Keywords: Anatomy, altitude, Lamiaceae, *Salvia limbata.*

Abbreviations: SI, Stomata Index; **ANOVA**, Analysis of Variance

Introduction

The genus Salvia with over 900 species is probably the largest number of the family Lamiaceae and is found in Central and South America (500 species), Western Asia (200 species) and Eastern Asia (100 species) (Walker and Sytsma, 2007). Some species are perennial, herbaceous, suffruticose, fruticose and sub-shrubby (Kaya et al., 2007). About 58 species of this genus are found in Iran of which 29% are endemic (Rechinger, 1982). Due to essential oils in leaf trichomes, *Salvia* leaves are applied for disinfection processes, controlling blood sugar and as an anti-spasm material. Moreover, DNA synthesis is decreased by chemical composition of leaves (Chiej, 1988, Nakiboğlu, 1993). The production of essential oils in plants and other secondary metabolites is constantly influenced by environmental factors (Gobbo-Neto and

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Lopes, 2007). Modifications in the leaf anatomical structures have been previously reported for some other plant species (Kofidis et al., 2007; Zarinkamar et al., 2011). Owing to greater similarity in morphological characters and the prevailing hybridization among *Salvia* species, high diversity in polyploidy levels, the presence of heterozygous individuals and the taxonomical, ecological and genomic complexity, the species boundaries have become blurred (Haque, 1983, Rechinger, 1982). There are limited information regarding anatomy of *Salvia* genus under different environmental conditions. Recent studies have been done on the anatomical structure of few *Salvia* species (Baran and Ozdemir, 2006, Kahraman et al., 2009, 2009a, 2010, 2010a, 2010b, Özdemir and Şenel, 2001). Morphological, anatomical and palynological characteristics of *Salvia halophile* were previously studied by Kaya et al. (2007). Furthermore, the anatomy, trichome morphology and palynology of *Salvia chrysophylla* have been investigated by Kahraman et al. (2010c). Polat et al. (2010) presented comparative analysis of the anatomical and ecological characteristics of three *Salvia* L. species (*S. argentea*, *S. aethiopsis* and *S. viridis*). Therefore, the purpose of this study was to investigate the anatomical changes of *Salvia limbata* in relation to other parallel variation under different altitudes in West Azerbaijan province of Iran.

Materials and Methods

Salvia limbata C.A.Mey. samples were collected from natural populations of West Azerbaijan in Iran in Jun 2014 at flowering stage (Table 1). Anatomical studies were carried out on specimens kept in glycerin and alcohol 96% (1:1) fixation materials. Transverse sections of the stem, the leaf, the central vein and the petiole were prepared manually and were stained with Carmine – Methyl Green & Safranin and Fast-Green (Johnson, 1940). Slides were viewed and photographed with light microscope (model BX40 Olympus, Japan) equipped with measuring lens. The stomata index was calculated according to the method described by Meidner and Mansfield (1968) by determining the number of stomata and epidermal cells in a unit area. SI = Number of stomata in a unit area X 100 / Number of stomata in a unit area + Number of epidermis cells in a unit area (Meidner and Mansfield, 1968). Stomata width and length of leaf were measured. Also, the thickness of cuticle, collenchymas, phloem, xylem and pith of stem and total leaf & cuticle thickness was estimated.

Statistical analysis

All analyses were done based on a completely randomized design. Values are expressed as Means ± SD of three replications. For statistical analysis, ANOVA and for comparing the means Duncan test were used ($P \leq 0.05$). Degree of correlation between anatomical characters and environmental data was analyzed using correlation analysis. All statistical analyses were performed using SPSS 23 software.

Table 1. Geographical characteristics of six *Salvia limbata* populations from West Azerbaijan.

Coordinates of Natural Site	Collection Sites	Altitude
37°48′ N 45°3′ E	Khoy: Garetappeh	1650
37°15′ N 45° 7′E	Oshnavieh: Agbolagh	1800
37°28′ N 45°37′ E	Kabudan Island	1275
39°3′ N 44°22′ E	Chaldran: Mahlamlu	2053
37°35′ N 44°52′E	Urmia: Marmisho	1700
46°6′ N 36°58′ E	Miandoab: Talkhab	1340

Results and discussion

Transverse sections taken from the stem, leaf, central vein and petiole of the plants were analyzed in details and the obtained results are outlined below:

Stem anatomical characteristics

Cross- sections from *Salvia limbata* stems have exhibited a mono layer epidermis covered by thick cuticle. The epidermis is composed of oval or rectangular cells. The stem is quadrangular single layered with glandular (most of them) and non-glandular trichomes on epidermis. Non-glandular hairs are unicellular or multicellular. While, glandular hairs are of two main types: capitate (unicellular head and 1-4 cell stalks) or peltate types. However, peltate hairs are rare. They have four central and eight peripheral cells. Peltate hairs are usually sinked toward the epidermis. Underneath the epidermis multilayered and angular collenchyma cells are located at the corners and there are 2-3 rows of them in the parts between the corners. The cortex tissue located under the collenchymas layer and consisted of 4-5 layers of oval or orbicular parenchymatous cells. The phloem (6-7 layers) is surrounded by sclerenchymatous fibers. The vascular bundles at the corners were larger than others (Figure 1).

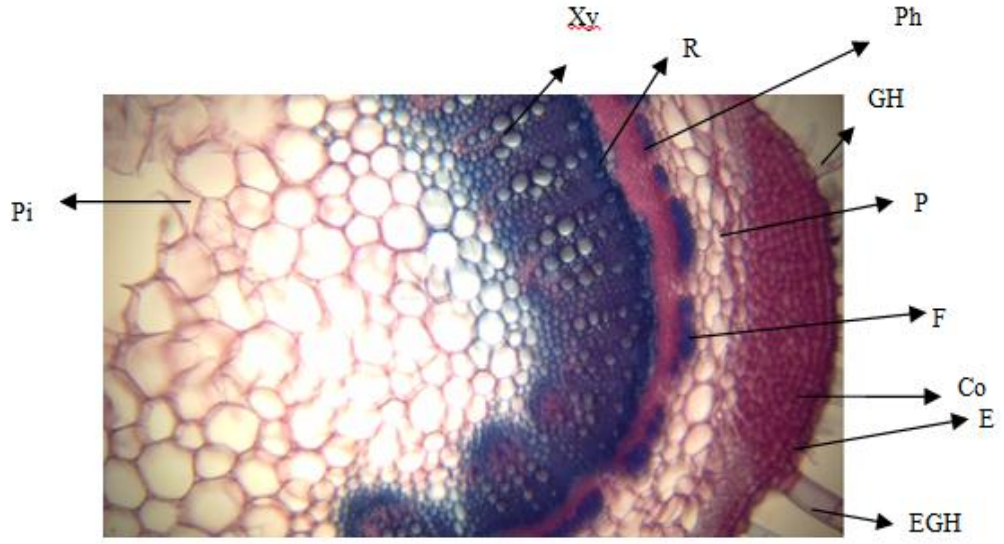

Fig. 1. Cross section of *Salvia limbata* stem (Co: Collenchyma, Xy: Xylem, E: Epiderm, F: Fiber, P: Parenchyma, Pi: Pith, Ph: Phloem, EGH: Eglandular hair, GH: Glandular hair, R: Ray) Bar =850 µm

Table 2. Anatomical measurements taken from the cross sections of *Salvia limbata* stem

Locality	Altitude (m)	MCT (µm)	MCOT (µm)	MPT (µm)	MXT (µm)	MPPT (µm)
Khoy: Garetappeh	1650	*48 ±0.05	*172.5 ± 0.05	*90.8± 0.05	*244.8± 0.05	*1449± 0.10
Oshnavieh: Agbolagh	1800	*75.7± 0.11	*195.1 ± 0.02	*97.9± 0.03	*351.4 ±0.07	*1609.9± 0.05
Kabudan Island	1275	*65.6 ±0.02	*156.0 ±0.20	*76.5± 0.04	*223.0± 0.10	*1300 ±0.05
Chaldran: Mahlamlu	2053	*80.1±0.01	*207.7 ±0.09	*102± 0.05	*473.5± 0.05	*2740.8 ±0.05
Urmia: Marmisho	1700	*74.8±0.005	*189.0 ± 0.12	*93.0± 0.10	*324.8 ±0.005	*1600±0.05
Miandoab: Talkhab	1340	*78.9±0.03	*160.9 ± 0.25	*85.3± 0.01	*241.0 ±0.05	*1538±0.007

*The difference between measured values was found to be significant at $p<0.05$ level.
MCT: Mean Cuticle Thickness, MCOT: Mean Collenchyma Thickness, MPT: Mean Phloem Thickness, MXT: Mean Xylem Thickness, MPPT: Mean Parenchymatous Pith Thickness.

The anatomical characteristics of Lamiaceae family include a quadrangular stem and a well-developed collenchyma, which supporting tissue at the corners of stem (Kahraman, 2010a). These features were seen in this study and showed that these rays consist of 1-6 line cells. The rays consist of 2-12 or more lines of cells in this family. Because the number of rays is different in every species, this can be used as an index to distinguish different species (Özdemir and Şenel, 1999).

There is a sclerenchymatous sheath on the phloem part. The pith is large and consists of parenchymatous cells with pith rays extending up to the pith from the cambium in the xylem tissue (Figure 1).

The stems of *S. aethiopsis* and *S. argentea* have sclerenchyma groups upon the phloem, but the herbaceous stem of *S. viridis* lacks the sclerenchymatous groups (Özdemir et al., 2009, Polat et al., 2010). Moreover, sclerenchymatous cells exist on the phloem of stem cross sections of the *S. sclarea* (Özdemir and Şenel, 1999) and *S. trichoclada* (Çobanoğlu et al., 1992).

Thickness of tissue in the stems was measured micrometrically and the results are given in Table 2. The six studied populations exhibited significant differences in the cuticle, the collenchyma, the xylem, the phloem and the parenchymatous pith thickness in the stem ($P \leq 0.05$, Table 6). These results showed that at higher altitudes the cuticle becomes thicker, the collenchyma increases and xylem, phloem and parenchymatous pith are wider. Mahlamlu region has the thickest studied parameters. Therefore, included the lowest (lower than 1340), medium (1340 to 1650 m) and highest (upper than 1700 m) for MCT, MCOT, MPT, MXT and MPPT. However, Kabudan Island region has the narrowest studied tissues (Table 2 and Figures 2 a-g).

Leaf and central vein anatomical characteristics

Cross-sections of the lamina and surface sections of adaxial and abaxial epidermis of *S. limbata* have showed that both epidermises are covered with glandular and eglandular hairs and consisted of hexagonal cells with thick cuticles. The leaf is bifacial and epistomatic, with diacytic stoma type. Mesophyll (239.1 - 300.1 µm) comprises elongated palisade and isodiametric spongy parenchyma cells. Palisade parenchyma is 3-4 rowed under the adaxial epidermis. Spongy parenchyma consists of 2-3 layers in the abaxial epidermis. Angular collenchyma composed of 2-3 layers and located adjacent to the central vein. Furthermore, a single large vascular bundle exists in the center (Figures 2a-g and Table 3).

The species, having bifacial type of leaves has a diacytic type of stoma. Metcalfe and Chalk (1972) observed that mesophyll is completely parenchymatous and there is collenchyma both under and over the central vein in species of Salvia (Metcalf and Chalk, 1972). It has been observed that in *S. limbata*, mesophyll is parenchymatous and the central vein is surrounded by collenchyma cells (Metcalf and Chalk, 1972). In addition the number of palisade cells is high in the leaves of this species; this causes increase in the efficiency of light absorption by its leaves. These leaves have been defined as “sun leaves” by Shields (1950) and Kasaplıgil (1961).

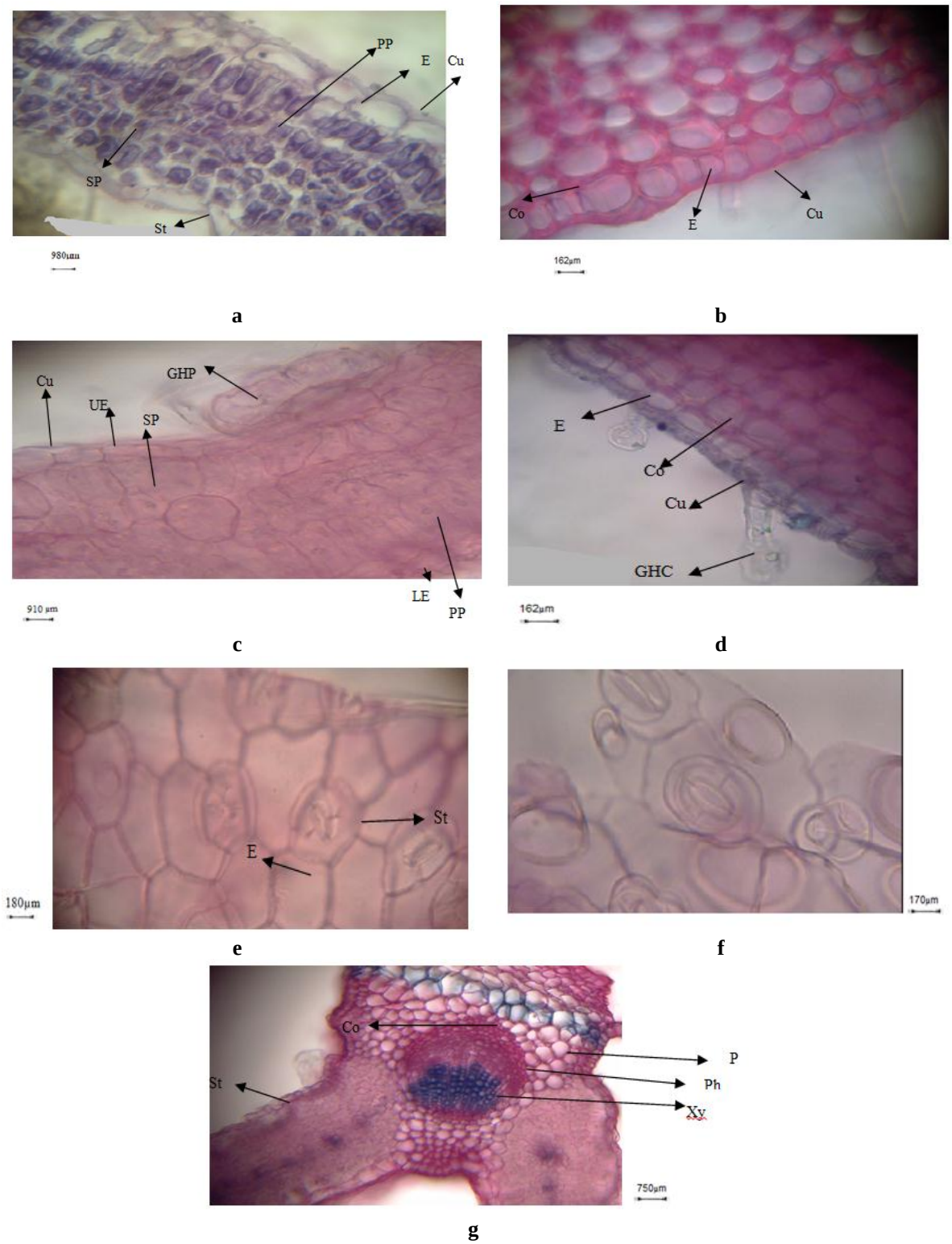

Fig. 2. a-g: Cross - sections of the *Salvia limbata* leaves and stems from regions with the highest: Mahlamlu region (a: cross – section of leaf, d: cross – section of stem and f (surface section of leaf) and of the lowest altitude: Kabudan Island region (c: cross – section of leaf, b: cross – section of stem, and e: surface section of leaf). g: Cross- section of leaf and central vein of *S. limbata.* (EGH: Eglandular hair, E: Epidermis, St: Stoma, GHP: glandular hair from Peltate type, GHC: Glandular hair from Capitate or Labiatae type, PP: Palisade Parenchyma, SP: Spongy Parenchyma, LE: Lower Epidermis, UE: Upper Epidermis, Co: Collenchyma, Cu: Cuticle)

Environmental factors potentially alter by changing in altitude level. For instance, the amounts of precipitation and radiation increase with an increase in altitude, furthermore wind, daily temperature differences, cloudiness and humidity can also be influenced. On the other hand, evaporation and mean temperature can decrease and vegetative phases and pedogenesis processes are shortened with an increase in altitude (Koçman, 1989). Alterations induced by altitude effect, influence plant anatomical characteristics.

The thickness of different leaf tissues at studied altitudes are given in Table 3. Total leaf and cuticle thickness increased in higher altitude (Table 3). A comparision leaf anatomy of 40 species of the genus *Calamagrostis* adans, from Poaceae family has revealed that environmental factors and altitudinal changes result in differences in the number of vascular bundles, epidermal papilla, number and distribution of stomata, arrangement and distribution of sclerenchyma cells (Escalona, 1991).

The increase in cuticle thickness is a common adaptive response to growing in environments with high radiation levels, such as the ones that survive at higher altitudes (Shepherd and Griffiths, 2006; Jacobs et al., 2007).

In this study we showed that in general the number of stomata and epidermal cells increases in higher altitude. Numbers of epidermal cells were also increased by increasing the altitude level on both upper and lower surfaces of the leaves (Tables 4 and 5). The highest number of stomata on the upper epidermis of the leaf was achieved in high altitude samples such as those from Mahlamlu region (2.44 ± 0.04). Notably, the number of stomata on the lower epidermis of the leaf reduced in all samples grown in high altitude level.

In plants like *Clinopodium vulgare* (Kofidis et al., 2007), *Origanum vulgare* (Kofidis et al., 2003), *Sedum atratum* (Codignola et al., 1987), *Miscanthus* spp. (Kao and Chang, 2001), *Picea erasifolia* (Qiang et al., 1986) stomatal density has been shown to be positively influenced by altitude level. Hypothetically, increase in stomatal density at high altitudes could be attributed to the higher solar intensity and reduced CO_2 concentration (Frukawa, 1997, Apel, 1989). Moreover, the low stomatal density at low altitudes presumably reflects the arid conditions (higher temperature and low humidity) available in the hillsides (Kofidis et al., 2003).

The stomata length was higher in both adaxial and abaxial epidermis of high altitude samples grown in Agbolagh and Mahlamlu regions. Stomata length in Agbolagh region was 279.3 ± 0.02 µm and 285.6 ± 0.01 µm on adaxial and abaxial epidermis. The longest stomata were observed in adaxial (288.9 ± 0.02 µm) and abaxial (290 ± 0.02 µm) epidermis for Mahlamlu region. The widest stoma was observed in the adaxial (209.1± 0.02 µm) and abaxial epidermises (209.7±0 .01µm) of the Kabudan Island region.

Table 3. Leaf and cuticle thickness of *Salvia limbata* from different altitudes.

Locality	Altitude (m)	Mean total cuticle thickness (µm)	Mean total leaf thickness (µm)
Kabudan Island	1275	*20.9±0.14	*239.1± 0.02
Miandoab: Talkhab	1340	*24.5±0.14	*255.6 ± 0.01
Khoy: Garetappeh	1650	*25.5±0.4	*269.4 ± 0.02
Urmia: Marmisho	1700	*27±0.83	*281.7 ± 0.03
Oshnavieh: Agbolagh	1800	*27.3±0.13	*282.3 ± 0.0
Chaldran: Mahlamlu	2053	*37±0.13	*300.1 ± 0. 14

*The difference between measured values was found to be significant at $p<0.05$ level.

Increasing altitude from 1275 to 2053 m above sea level led to an increase in the stomata number in both upper and lower epidermal tissues. These results showed more stomata exist on both lower and upper epidermises of high altitude grown samples, however the stomata in those altitudes are narrower and longer.

Table 4. Stomatal features in adaxial epidermal cells of *Salvia limbata* collected from different altitudes.

Locality	Altitude (m)	MNS1	MNE1	MSL1 (µm)	MSW1 (µm)
Kabudan Island	1275	*1.51± 0.14	*4.3 ±0.01	*246.6± 0.02	*209.1± 0.02
Miandoab: Talkhab	1340	*1.65± 0.005	*4.99± 0.01	*255.3± 0.01	*199.2± 0.01
Khoy: Garetappeh	1650	*1.8 ±0.005	*5.31± 0.2	*260.7 ±0.01	*195 ± 0.01
Urmia: Marmisho	1700	*1.98 ±0.01	*5.5 ±0.10	*270.9 ±0.05	*189.3± 0.20
Oshnavieh: Agbolagh	1800	*2.15± 0.005	*5.53± 0.02	*279.3± 0.02	*192 ± 0.005
Chaldran: Mahlamlu	2053	*2.44 ±0.04	*5.61±0.01	*288.9 ±0.02	*180.6 ± 0.06

MNS: Mean Number of Stomata, MNE: Mean Number of Epidermal Cells, MSL: Mean Stomata Length, MSW: Mean Stomata Width

*The difference between measured values was found to be significant at $p<0.05$ level.

Table 5. Stomata features in abaxial epidermal cells of *Salvia limbta* collected from different altitudes.

Locality	Altitude (m)	MNS2	MNE2	MSL2 (µm)	MSW2 (µm)
Kabudan Island	1275	*1.2± 0.07	*1.05± 0.07	*239.1± 0.26	*209.7±0 .01
Miandoab: Talkhab	1340	*1.52 ±0.13	*4.06± 0.05	*258.6± 0.02	*186.9 ±0.05
Khoy: Garetappeh	1650	*2.4 ±0.10	*4.58± 0.07	*265.2± 0.01	*184.2± 0.01
Urmia: Marmisho	1700	*2.59± 0.08	*5.53± 0.05	*267.2± 0.03	*174.6 ±0.04
Oshnavieh: Agbolagh	1800	*2.6± 0.08	*5.78± 0.07	*285.6 ±0.01	*165.9 ±0.03
Chaldran: Mahlamlu	2053	*4.58± 0.07	*6.55 ±0.09	*290 ±0.02	*154.5± 0.01

MNS: Mean Number of Stomata, MNE: Mean Number of Epidermal Cells, MSL: Mean Stomata Length, MSW: Mean Stomata Width

*The difference between measured values was found to be significant at $p<0.05$ level.

Table 6. Analysis of variance for anatomical parameters in *Salvia limbata*.

Mean squares

Source of variation	df	MCOT	MPPT	MPT	MXT	MCT	ML	MNS1	MNE1	MSL1	MSW1
Salvia accessions	5	4.900	4.370	1.000	44.60	0.940	1.750	0.34	0.73	0.820	0.36
error	12	0.020	0.000	0.003	0.004	0.003	0.004	0.00	0.00	0.001	0.001
Total	17										
F		225.857*	0*	296.14*	1.100*	787.98*	46766.02*	1.04*	370.49*	850.72*	370.49*

Mean squares

Source of variation	df	MNS2	MNE2	MSL2	MSW2
Salvia accessions	5	4.26	1.34	1.45	1.20
error	12	0.01	0.05	0.012	0.001
Total	17				
F		297.16*	2.29*	1.10*	923.20*

*: significant at $p<0.05$

Holland and Richardson (2009) stated that guard cell length is generally increased with altitude elevation for many species such as *Betula papyrifera* var. cordifolia, *Sorbus americana*, *Cornus canadensis* and *Dryopteris carthusiana*. Our results was consistent with those of published studies conducted on *Origanum onites* L. (Günüz and Özörgücü, 1999). They investigated changes occurring in the anatomy of *Origanum onites* (Lamiaceae) by increasing in altitude level. They showed an increase in the vascular tissues size in higher altitudinal level. Moreover, the number and size of stomata were also increased with increasing altitude level.

Petiole anatomy

In cross - sections taken from the petiole of *S. limbata* it has been observed that epidermal cells shape on both surfaces were oval or rectangular. There were numerous glandular and eglandular hairs on epidermal cells. Most of them were glandular. There were several layers of collenchyma cells under the epidermis. Parenchymatous cortex was present under collenchymas cells (Figure 3).

Metcalfe and Chalk (1972) pointed out that in the Lamiaceae family the structure of the vascular bundles in petiole is an important taxonomic feature. In our study there were five broad vascular bundles in the middle of *S. limbata* petiole and also three small vascular bundles in each of the petiole wings (Figure 3). Vascular bundles were collateral type. In most of the anatomical studies on Salvia, it has been found that the order of petiole vascular bundles is different in various species. Cırıg and Seçmen (1990) have pointed out that there are three vascular bundles in petiole of *S. kronunburgii*, two of which are on the sides, and the third in the center of petiole (Cırıg and Seçmen, 1990). *S. ballsiana* (Kahraman, 2010b) has a broad vascular bundle in its middle part of the petiole and four or six small bundles on its wings, and *S. macrochlamys* (Kahraman, 2010a) has a single large vascular bundle in the center of the petiole and two small bundles on its wings.

Nakipoğlu & Oğuz (1990), has investigated seven species of *Salvia* and divided vascular bundles of petiole in two types: species with and without basal leaves. *S. limbata* is a species with basal leaves; the order of vascular bundles of petiole is the same as the two types mentioned above (Nakıpoğlu and Oğuz, 1990). The sclerenchymatous tissue is well developed outside of the phloem and the xylem. These findings are consistent with other published on *S. limbata* (Kahraman and Dogan, 2010), *S. trichoclada* (Çobanoğlu et al., 1992) and *S. hypargeia* (Kandemir, 2003).

The sclerenchymatous tissue is well developed outside of the phloem and xylem. These findings are in accordance with those reported in *S. limbata* (Kahraman and Dogan, 2010), *S. trichoclada* (Çobanoğlu et al., 1992) and *S. hypargeia* (Kandemir, 2003).

Özdemir and Senel (1999) reported five vascular bundles in petiole of *S. sclarea*, two of which are in the center of the petiole and the others are on the sides. Among studies conducted in different altitudes no clear difference in petiole anatomical structure has been reported.

Correlation coefficient analysis

Correlation analysis was performed to clarify the relations among parameters evaluated in this study (Table 7). As shown in table 7, studied parameters were significantly varied according to the different locations ($p<0.01$).

Fig. 3. Cross - section of *S. limbata* petiole
E: Epidermis, Co: Collenchyma, SVB: Small Vascular Bundle, BVB: Broad Vascular Bundle, P: Parenchymatic layer.
Bar =950 µm

Table 7. Correlation coefficient between pair wise traits studied on anatomical characteristics and altitude.

Traits	Correlation coefficient
Mean Cuticle Thickness (stem)	0.94*
Mean Collenchyma Thickness (stem)	0.99*
Mean Phloem Thickness (stem)	0.91*
Mean Xylem Thickness (stem)	0.88*
Mean Parenchymatous Pith Thickness (stem)	0.82*
Mean Total Leaf Thickness	0.97*
Mean Number of Stomata (upper leaf epidermis)	0.97*
Mean Number of Epidermal Cells (upper leaf epidermis)	0.87*
Mean Stomata Length (upper leaf epidermis)	0.96*
Mean Stomata Width (upper leaf epidermis)	-0.94*
Mean Number of Stomata (lower leaf epidermis)	0.95*
Mean Number of Epidermal Cells (lower leaf epidermis)	0.88*
Mean Stomata Length (lower leaf epidermis)	0.94*
Mean Stomata Width (lower leaf epidermis)	-0.93*

*: significant at $p<0.01$

There were significant positive correlation between elevation and mean collenchyma thickness (R^2=0.99), mean parenchymatous pith thickness (R^2=0.82) and mean stomata length data in adaxial (R^2=0.96) and abaxial epidermis (R^2=0.94), mean stem phloem (R^2=0.91) and xylem thickness (R^2=0.88), Mean number of stomata in adaxial (R^2=0.97) and abaxial epidermis (R^2=0.95), Mean number of epidermal cells in adaxial (R^2=0.87) and abaxial surface (R^2=0.88), mean total leaf (R^2=0.97) and cuticle (R^2=0.94) thickness. Moreover, there was a significant negative correlation between elevation and mean stomata width data in adaxial (-0.94) and abaxial (-0.93) epidermis (Table 7).

Vasic and Dubak (2012) analyzed anatomical characteristics of *Juniperus oxycedrus* (Cupressaceae) leaves from Kopaonik Mountain (Serbia) and determined the level of distinction between leaves taken from various altitudes (420-1420 m) by analyzing anatomical characters. The results of descriptive statistics of anatomical characters of red common juniper leaf have shown that mean values change with altitude level increasing; however, there is no obvious regular interaction between these changes (Kaya and Aksakal, 2007).

Leaf thickness in *Nepeta nuda* (Lamiaceae) apparently is not influenced by altitude. In general, significant correlation was not observed between altitude and leaf thickness.. Therefore, in some cases leaves taken from samples grown in higher altitude were found to be thicker than those of plants in lower altitude while converse results was obtained in some other plants (Kofidas and Bosabalidis, 2008).

Conclusion

This study has focused on the effect of different altitude gradients on *Salvia limbata* anatomy. Quantitative assessment of the cuticle, collenchymas, pith, phloem, xylem, stomata frequency, epidermal number and stomata length/ width shows that these characters vary according to the altitude changes. Results showed that at higher altitudes the cuticle becomes thicker, the collenchyma increases and the xylem, phloem and parenchymatous pith are wider. Furthermore, higher stomatal density were observed on both lower and upper epidermis of high altitude samples, however, they became narrower and longer. In addition, possible increase in stomatal density in high altitudes can be due to the higher solar intensity and lack of CO_2 concentration. The present study provides proper background knowledge for further research on impact of altitude on anatomical structure of *Salvia* species or other plants.

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