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ARTICLE

Micromorphology and leaf ecological anatomy of *Bassia* halophyte species (Amaranthaceae) from Iran

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ABSTRACT *Bassia* belongs to the family Chenopodiaceae, which is widely distributed in the world, especially in Irano-Turanian Region. According to the morphological similarities among the species of the genus, ecological implications of structural features were studied. In fact, understanding these relationships is of great importance in natural classification. We have studied the relationships of *Bassia* species using morphological, anatomical, and micro-morphological methods. The current results indicated that phenotypic plasticity and repetitive patterns were probably due to ecological adaptations, especially in decreasing the leaf surface by changing the inner structure. All species have a Kranz anatomy structure (Kochioid subtype), related to C_4 photosynthesis. The changes in cell size increasing the cell membrane thickness, the density of two-vascular systems, the increase of palisade to water storage parenchyma ratio and photosynthetic system. The leaf surface is covered with long highly dense hairs and microechinate ornamentation. Though the adaptation caused some morphological similarities, the variation was seen in other descriptive characteristics such as morphological and anatomical features especially in two synonym species of *B. turkestanica* and *B. pilosa*. Information about the similarity species is provided.

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KEY WORDS

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Kranz anatomy
phenotypic plasticity
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Introduction

Amaranthaceae is one of the biggest Caryophyllales family (Cuenoud et al. 2002), which is comprises 110 genera with 1700 species. They are predominantly found in arid to semi-arid, saline (Grigore 2012; Grigore et al. 2014), disturbed, and agricultural habitats of temperate and subtropical regions. Only a few genera present in the tropics, *e.g., Chenopodium*, *Halosarcia*, and *Suaeda*, but most species of the family are annuals or subshrubs. Herbaceous perennials, shrubs, small trees, and lianas are restricted to only a few genera (Kadereit et al. 2003). However, this family is very problematic from a taxonomical point of view and lots of attempts were recorded in order, to clarify its position within Caryophyllales, and especially the phylogenetic relationships between Amaranthaceae and Chenopodiaceae (Kadereit et al. 2003). Angiosperm Phylogeny Group II (2003) and APG III (2009) do not recognize Chenopodiaceae as a separate family from Amaranthaceae, instead only the latter is being maintained.

Amaranthaceae and Chenopodiaceae constitute the most diverse lineage (180 genera and 2500 species) of the Caryophyllales (Kadereit et al. 2003) and have been regarded for a long time as two closely related families (Brown 1810; Bentham and Hooker 1880; Baillon 1887; Volkens 1893; Ulbrich 1934; Aellen 1965-1968; Behnke 1976; Thorne 1976; Carolin 1975). Some authors refer Chenopodiaceae in their work as "*sensu stricto*", while others Amaranthaceae, but also including species that traditionally have been previously described and regarded as Chenopods. Other authors refer to Amaranthaceae/Chenopodiaceae-Achatocarpaceae clade belonging to the core Caryophyllales (Cuenoud et al. 2002), and in this way a part of the Centrospermae as traditionally circumscribed (Cronquist and Thorne 1994).

Hedge et al. (1997) described 44 genera in the maintained Chenopodiaceae from Flora Iranica. Chenopodiaceae species are very well-represented in the Flora Iranica area with more than one third of the global total of genera present (Hedge et al. 1997). Ecologically and economically, this family is very important; in very inhospitable environments such as deserts, semi deserts or salt marshes (maritime and inland). This species are often dominants in plant communities and are an important source of forage for grazing livestock (Hedge et al. 1997).

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Bassia in Flora of Iran was separated from genera of *Londesia*, *Bassia*, *Panderia*, and *Kochia* (Assadi et al. 2001; Akhani and Khoshravesh 2013). In addition to taxonomical comparisons between *Kochia*, *Bassia*, and *Chenolea*, they were separated based on anatomical and micromorphological differences (Turki et al. 2006). In a new classification based on phylogenic analyses and morphological studies, these two genera were introduced as one genus called *Bassia* based on the numerous similarities (Kadereit and Freitag 2011).

Hedge et al. (1997) described 5 species of *Bassia*, and separated this genus from *Kochia,* where 4 species are described in Flora Iranica, despite other authors reduced *Kochia* to synonymy under *Bassia* (Scott 1978). Nevertheless, they are closely related, being distinguished almost solely by the presence of wings on the fruiting perianth segments of *Kochia*, and spines or hooks in *Bassia.*

Many halophytes of the world belong to this above-mentioned lineage (Akhani et al. 1997). *Kochia* and *Bassia* of the family are so similar in small flowers, hairy leaves and wing perianth. *Kochia* has wing perianth during the maturity, while *Bassia* has spiny perianth (Chu and Sanderson 2008). *Panderia pilosa* with small urceolate (ornamentation) perianth and pilose hairs distribute in the Central Asia (Haber and Semaan 2007).

Moquin-Tandon (1849) recognized *Panderia* and *Kochia* genera, while Ulbrich (1934) added *Bassia* genus to the two mentioned genera; Scott (1978) maintained only *Panderia* and *Bassia* (including *Kochia*), Kühn et al. (1993) recognized *Panderia, Bassia* (including *Kochia*), while Kadereit and Freitag (2011) refer only to *Bassia* (including *Kochia* and *Panderia*).

The aim of this study is to discuss the ecological significance of micromorphology and anatomy of investigating species in relation to habitats, where since they have been collected.

Materials and Methods

Data and collection of plant material

Fresh plant material has been sampled from natural saline habitats of Iran. Majority of Iran is covered by Irano-Turanian Flora. We collected five taxa of the genus *Bassia* (Chenopodiaceae/Amaranthaceae) from Tehran, Garmsar, and Semnan areas of Iran based on the main source Flora of Iran, published by Research Institute of Flores and Rangland (Assadi et al. 2001; Hatami and Khosravi 2013). The species growth and development soil and the surface are usually dry and majority of these provinces visible to consist of essential element Na, Cl, K, Mg, in solonchak clay, loam and silt in Loes Zohary (1973).

Table 1. Collection data of *Bassia* with voucher number and province information studied. Investigated taxa, with their localities and correspondent voucher number.

Taxa	Locality	Voucher number (IAUGH)
Bassia scorparia (L.) A.J. Scott (= *Kochia scoparia* (L.) Schrad.) Freitag & G. Kadereit s.l. (2011) (= *Kochia scoparia*)	Tehran: Tehran-Karaj exp. Way, Vard Abad, Safiallah 1320 m	5893
Bassia stellaris (Moq.) Freitag & G. Kadereit s.l. (2011) (= Ko*chia stellaris*)	Garmsar: km 7 from Garmsar to Varamin, Safiullah, 1000 m	5894
Bassia prostrata (L.) A.J. Scott (= *Kochia prostrata* (L.) Schrad.) Freitag & G. Kadereit s.l. (2011) (= *Kochia prostrata*)	Tehran, Firoozkouh Road, 13th km to Pol-e-Sefid, Safiullah, 1000 m	5895
Bassia pilosa (Fisch. & C.A. Mey.) Freitag & G. Kadereit s.l. (2011) (= *Panderia pilosa* Fisch. & C.A. Mey., including *P. turkestanica* Iljin)	Tehran: northern east of Firoozkouh to Pol-e- Sefid, Safiullah, 2000 m	5896
Bassia turkestanica (Fisch. & C.A. Mey.) Freitag & G. Kadereit s.l. (2011) (= *Panderia pilosa* Fisch. & C.A. Mey., including *P. turkestanica* Iljin)	Semnan: 32 km from western part of Semnan, next to Lasjerd, Safiullah 1400 m	5897

Bassia scoparia, B. pilosa, B. turkestanica, B. stellaris, and *B. prostrata* were studied after being collected and taxonomically identified (Table 1). The specimens were deposited in the herbarium of the Islamic Azad University, Garmsar Branch (IAUGH). Examples of soil profiles from species have been collected are depicted in Figure 1.

Micromorphology

For scanning electron microscope (SEM) analysis, leaves were prepared as follows: the leaf surfaces were mounted on stubs and attached with sticky tabs. They were covered with gold. SEM analysis was used to study the morphological characteristics of the hairs on the leaf surface and observed using a standard described method (Akhani and Khoshravesh 2013).

Then photos were taken using electron microscope Model Philips-XL30 and specialized terminology used to recognize the structure morphologically Payne (1978).

Anatomy

For light microscopy (LM) study, small sections of leaves

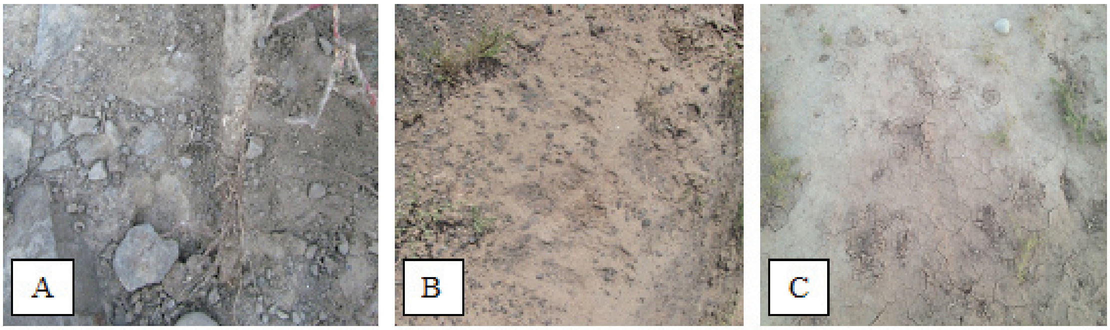

Figure 1. Soil samples from natural habitats of Iran. A: Tehran, Firoozkouh. B: Semnan, Lasjerd. C: Garmsar.

were used. Cross sections were collected from fresh leaves and were fixed in ethanol and glycerol (Purvis et al. 1966). Sections were cut by free hand. After sectioning and staining (Peterson et al. 1986), the samples permanent slides were studied by Olympus-BH2 light microscope. Both qualitative and quantitative methods were used in this investigation.

Results

Morphology and taxonomical features

Morphological observations in *Bassia* indicated traits such as simple, alternate and hairy leaves with parallel veined, 5-correlated perianth, spike inflorescence, pantadrous (5 stamen), curvate embryo and ovate seed. These traits are plesiomorphic ones, that show the near similarities and the relatives. The perianth ornamentation and wing, spiny or appendix perianth are apomorphic traits that are not important in the taxonomy of the species. With these characteristics of species morphology, the evolution could be followed by the weather changes and environment adaptation. In these species, the adaptation occurred with decreasing the leaf surface with much hair, while in some species, simple and long hairs were seen. *B. pilosa* is a decumbent-procumbent/prosrate plant with urceolate perianth and ornamentation. The main difference in comparison with *B. turkestanica* and the biggest differences among *B. scoparia, B. prostrata* and *B. stellaris* were found in quantitative characteristics, while in qualitative, *B. prostrata* was same with *B. pilosa* in decumbent vegetative form, but was different with other ones as a perennial plant (Table 2). The species were in red, purple, yellow, orange and green.

Light microscopy and modification of anatomical structure

The comparison of important anatomical characters of species reveals, that some structural changes of leaves were histologically decreased, while in some species increasing the cell size, cell wall thickness, vascular bundle dense area (phloem and xylem) and higher palisade parenchyma density compared with water storage parenchyma were observed. The palisade parenchyma cells were long and vertical or radiant to the vascular bundles. The development of mesophyll, that can be involved in increased photosynthetic activities is accompanied by reduced leaf area. Large bundle sheath (BS) cells are quite visible. These Kranz cells form bundle sheaths related to C_4 photosynthesis.

Usually, in all investigated species, the water storage tissue occupies the central part of the lamina, and its periphery, vascular bundles are located; the biggest bundles generally protrude toward the centre of the lamina.

B. prostrata was observed with more decrement in leaf surface, higher diameter of palisade parenchyma, while *B. turkestanica* was found to have an increase in leaf surface and less a decrease in the diameter of palisade parenchyma cells. *B. stellaris* is distinct with a very big irregular water storage parenchyma. In addition, numerous calcium oxalate crystals were noticed (Fig. 2A), more abundant in the palisade tissue cells and fewer in the water storage parenchyma. In studying the number of *Bassia* species, we recognized smaller size in leaf surface, more palisade cells in *B. pilosa*, the calcium oxalate crystals are fewer, but larger (Fig. 2E), especially located towards water parenchyma at the border to vascular bundles. In *B. turkestanica* (Fig. 2C), the calcium oxalate crystals seem to have a very precise localization, at the border of palisade and water storage tissues, thus having the occurrence of a regulate position.

The ratio between the length of palisade cells and development of water storage tissue, seem to be a very important trait in C_4 plants (Table 2).

Based on survey C_4 leaf anatomy, two subtypes of C_4 kochioid anatomy were recognized in the studied species as followed: 1.) *B. prostrata* subtype 2.) *B. scorparia* subtype include *B. pilosa*, *B. scoparia*, *B. turkestanica*, and *Bassia stellaris.*

Table 2. Comparison characters (morphological, anatomical and SEM of hair) in species of *Bassia*.

	B. scoparia	*B. prostrata*	*B. stellaris*	*B. pilosa*	*B. turkestanica*
Leaf phyllotaxis	Alternate	Alternate	Alternate	Alternate	Alternate
Leaf shape	Linear or lanceolate/ Acut apex	Linear or lanceolate/ Acut apex	Linear or lanceolate/ Acut apex	Linear or lanceolate/ obtuse apex	Linear or elliplic/ Acut or obtuse apex
Leaf length	50 ± 60	4 ± 30	4 ± 12	5 ± 6	3 ± 8
Leaf width	8 ± 10	1 ± 1.5	1 ± 1.5	1 ± 2.5	1 ± 3
Perianth ornamentation	-	-	-	+	-
Presence of hypodermis	-	+	-	-	-
Water storage parenchyma length (μm)	14.43 ± 5.99	14.95 ± 10.34	43.12 ± 12.99	30.61 ± 7.24	27.17 ± 4.56
Palisade parenchyma length (μm)	14.50 ± 6.11	23.57 ± 19.52	19.94 ± 7.08	17.62 ± 10.58	8.72 ± 8.34
Number of vascular bundles	12	18	17	8	22
Number of palisade cells	130	290	292	137	107
Number of water storage cells	55	37	35	50	57
Size of leaf surface (μm)	347/02	153/53	143/41	155/34	216/66
Hair ornamentation	Microechinate	Microechinate	Microechinate	-/seldom	Microechinate
Hair surface	Striate/ sulcate	Striate/sulcate	Striate/sulcate	Striate/sulcate	Striate/sulcate

SEM and leaf hairs morphology

Each studied species has hair possesses of similar shape, ornamentation and agglomeration. Most likely, the hair coverings keep a warm and dry microclimate of leaves, taking in considerations the ecological characteristics, where these species vegetate. In such conditions, hair increment (increase) is one of the morphological structural adaptations. The observed trichomes have high density and size in all species, and they have long hair, because of the ecological condition. Species investigated in the present study; simple, sulcate, striated hairs with small and microechinate/aculate ornamentation. The hair apex was sharp in the head (acute), but prominent at the end and on the base cuneate (Fig. 3F).

SEM micrographs of *Bassia* hair were compared and it showed that the hair in *B. pilosa* differs from other species and is non-ornamentation. Diagnostic character microechinate was found in *B. scoparia*, *B. turkestanica*, *Bassia stellaris* and *B. prostrata* (Fig. 3).

Discussion

Different organs showed relationships and structure correlations in many ways.

Morphological, micromorphological and anatomical features are important characteristics, that can be involved in taxonomical diagnosis, as well as in explaining ecological conditions, where halophytic species vegetate. *Bassia* genus in Flora of Iran has been divided into genera, including *Panderia*, *Kochia*, *Bassia*, and *Londesia* described by Assadi et al. (2001). According to Turki et al. (2006), *Kochia* and *Bassia* species were separated based on their morphology, anatomy and micromorphology. Based on morphological observations of the species, numerous similarities can be noticed, because of ecological and environmental conditions show a convergent evolution. Another important finding (Kadereit and Freitag 2011), that morphological studies showed a relationship and a convergence with phylogenetic analysis and suggested that there is a natural classification to distinct intra species and hence, *Panderia* and *Kochia* genera could be reclassified into genus as *Bassia*.

Adaptations made in morphology and anatomy due to facultative conditions among the species and the ecology. These include hairy cover on the perianth, leaf, and stem, yellow color of the leaves of some species, tiny flowers and photosynthetic system.

Grigore et al. (2012, 2014) described anatomical adaptive features in many halophyte species, including Chenopodiaceae, and the clarity of the adaptive structures in leaves, stems, and roots. Leaf anatomy in the studied species had C_4 kochioid photosynthetic system and dense mesophyll, which was due to dry and warm ecological conditions. They found that not all *Bassia* species have a C_4 photosynthetic pathway, despite similar ecological conditions; thus, *Bassia hyssopifolia* has the C_4 kochioid subtype, while *B. sedoides* and *B. hirsuta* were found to be C_3 species, based on the leaf anatomy. For all investigated species, *B. prostrata* presents a hypodermis, like other C_4 halophyte species (Grigore et al. 2014).

The presence of water storage parenchyma is very impor-

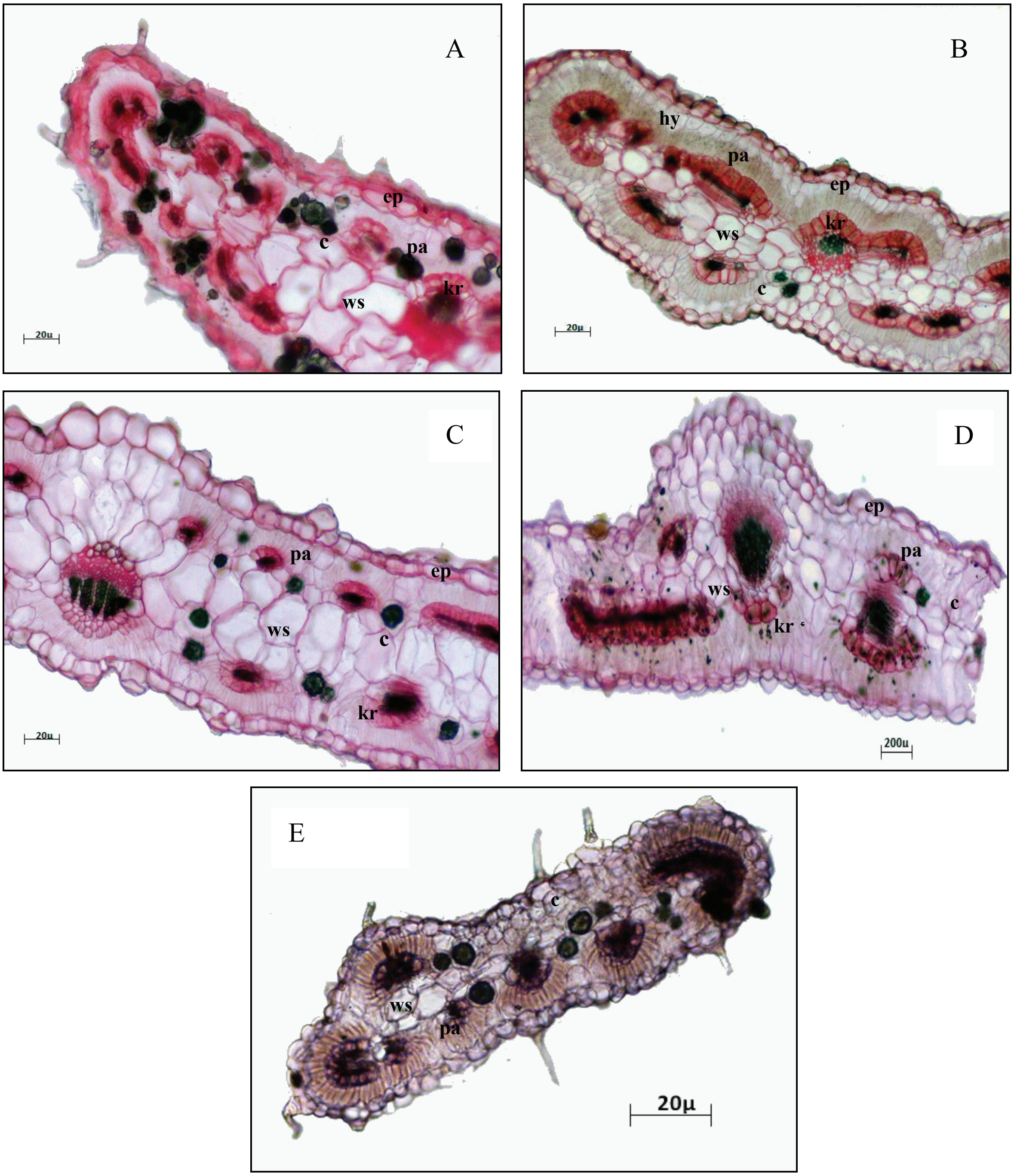

Figure 2. Cross sections of C_4 leaf in *Bassia stellaris* (A), *B. prostrata* (B), *B. turkestanica* (C), *B. scoparia* (D), and *B. pilosa* (E). c: crystal; ep: epidermis; kr: Kranz cells; hy: hypodermis; ws: water storage cell; pa: palisade cell.

tant in the direction of different metabolic processes related to C_4 photosynthesis; in addition, water deposit in the lamina of such species is explained by the ecological conditions, where these species grow, in environments dominated by dry and saline conditions. Bundle sheath cells surround the vascular bundles of the kochioid type of Kranz anatomy. In some individuals of *B. stellaris,* big water storage parenchyma was seen irregularly. According to observations, which made by (Payankov et al. 2010; Muhadit et al. 2007; Kaderedit et al. 2003) studies in phylogenetic and anatomical aspects, these

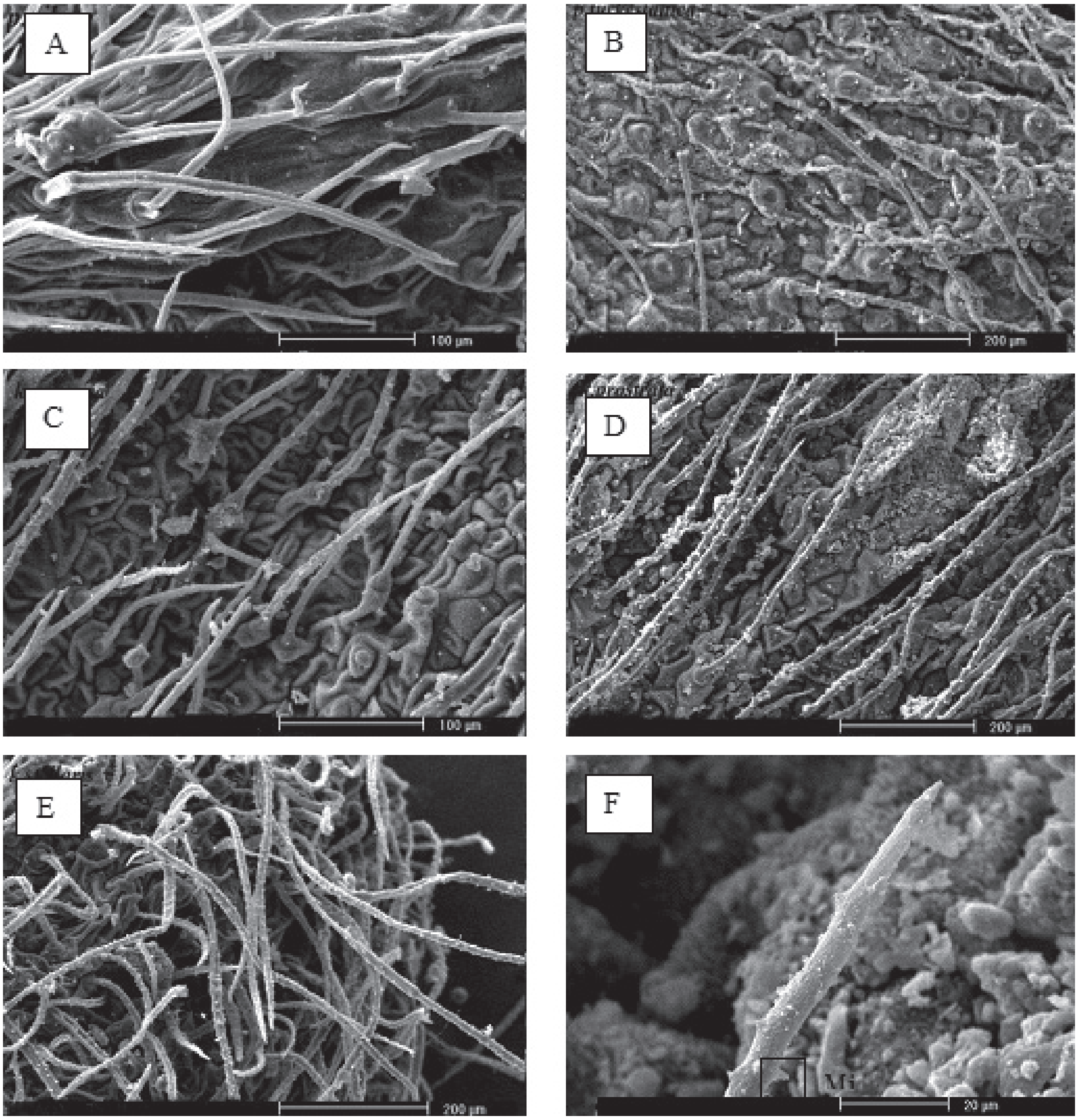

Figure 3. SEM micrographs of leaf hairs of *B. pilosa* (A), *B. turkestanica* (B), *B. scoparia* (C), *B. prostrata* (D), and *B. stellaris* (E). Microechinate (F).

similarities are due to convergence relationships. However, these adaptations could be different in some species. Freitag and Kadereit (2014) described two different types of Kranz anatomy kochioid and Atriplicoid in *Bassia* species. The Kranz cells form arcs along the xylem of peripheral bundles (Carolin et al. 1975; Jacobs 2001).

All investigated *Bassia* species have a C_4 photosynthetic pathway, based on the diagnosis of foliar anatomy. In the result of the study, C_3-C_4 does not quickly convert to C_4 in a single step. The tropism of palisade parenchyma around the bundle sheath is the most important development cause of the bundle sheath (Harold and Hatters Ley 1989). The result of this study indicated different types of the species based on C_4 kochioid photosynthetic system that the types are described based on the order. So, C_3 plesiomorphic conserved and C_4 apomorphic in this genus.

Based on the observed structure, it indicates that the most significant difference is between *B. turkestanica* and *B. pilosa*. Even some differences in perianth ornamentation were seen in the morphology of the vegetative form.

Grigore et al. (2014) have reported the presence of these crystals of calcium oxalate in many halophytic Chenopodiaceae. They assumed that beyond the possibility of being a common feature of the Chenopodiaceae family, the organic acids and the druses of calcium oxalate could be correlated with the metabolism of halophytes (Grigore et al. 2014). It is known that the uptake of cations by plants is balanced partly by the absorption of inorganic anions and partly by the internal synthesis of organic anions (Waisel 1972). When the nitrates and the sulphates are assimilated or when the cations are absorbed excessively, a balancing with the help of organic anions in the growth processes is needed. In the case of many species of halophytes, the balance is made with the contribution of organic acids. Such organic acids can be excessively produced especially in the case of *Atriplex* species. The plants with such a metabolism can contain significant druses of calcium oxalate deposits. Hence, there are data suggesting that the oxalate synthesis is connected to the ionic balance. The druses of calcium oxalate can be considered as an effort to maintain the ionic balance (Franceschi and Horner 1980). The chemical leaf analyses of many halophytes species have shown that oxalic acid was present in notable quantities, appropriate to a high level of calcium oxalate (Osmond 1967).

For instance, the calcium oxalate in *Atriplex halimus* is the predominant compound of the total organic acid content. The content of this acid diminished only, when it was cultivated in weak ionic solutions (Mozafar et al. 1970). The same principle is applicable to other species, when the hydric potential of the tissues is low (Zolhevitch and Koretskaya 1959).

Osmond (1963) states that calcium oxalate was present in foliar cells as an antagonist for sodium and potassium.

The ecology of C_4 halophytic species is well-documented and supported (Ehleringer et al. 1997; Sage and Monson 1999; Tipple and Pagani 2007; Sage 2004). C_4 species form a particularly high proportion of the herbaceous flora of saline environments, even in cool temperate regions (Long and Mason 1983). Apparently, the inherently higher water use efficiency of C_4 species would have two theoretical advantages in saline environments (Long 1999). First, saline soils have a soil water potential of around 2.5 MPa; to extract water, the halophytes must generate a lower water potential, even though this exceeds limits that can apparently be tolerated by many mesophytic vascular plants. Transpiration must be minimal, and the higher water use efficiency of C_4 species would confer the advantage of maximizing carbon gain per unit of water lost. On the other side, plant mineral content is inversely correlated to water use efficiency as an assumed result of increased passive uptake with increased transpiration. For a halophyte, increased transpiration increases the energy needed to exclude Na^+ and Cl^- (Long and Mason 1983).

It has been suggested that halophytes are a special case among xerophytes (Wiessner 1899; Henslow 1895; Schimper 1903; Kearney 1904; Warming 1909; Clements 1920; McDougall 1941; Grigore and Toma 2010). This implies the occurrence of some mechanisms serving to protect the water reserves of the plant in periods of drought or high potential evapotranspiration when soil water potential fall. A cost of xeromorphy increased resistance to the diffusion of CO_2 to the mesophyll, because of the low leaf intercellular pressure, necessary to saturate C_4 photosynthesis, this cost is minimized in C_4 species.

Despite the fact, that C_4 species represent only about 8000 of the estimated 250000 to 300000 land plant species (Sage et al. 1999), they are major components of biomass that cover more than 35% of the Earth's land surface area. These species are dominant in tropical and subtropical grassland and savanna, warm temperate grassland and savanna, arid steppe, beach dunes, salt marshes, salt desert, hot deserts and semi deserts.

C_4 also represents an important ecological strategy in certain desert shrubs, most notably species of *Atriplex*, particularly in saline soils (Keeley and Rundel 2003). In these species, the key adaptation is the ability to maintain growth under high summer temperatures and drought conditions at a time when C_3 species are dormant. The maximal rates of photosynthesis in these desert C_4 species are generally no higher than that of concurring C_3 species, but the water use efficiency is far greater. In addition, C_4 plants have higher nitrogen use efficiency.

Some studies certify the close relationship between C_4 photosynthesis and extreme habitats such as deserts and salinized areas. Thus, Wang (2007) identified among species vegetating in the deserts of China that, 36.5% of the Chenopodiaceae species were found with C_4 photosynthesis, which was about 48% of the total C_4 species. These taxa were predominantly members of the genera *Anabasis, Atriplex, Kochia, Salsola,* and *Suaeda*.

Qualitative and quantitative differences in morphological and anatomical studies are also due to microevolution differences that are affected by ecologic and soil conditions. Akhani and Khoshravesh (2013) suggested that the hairy surfaces of the fruit of two species of *Bassia* are together with spinulose ornamentation. Our results indicated microechinate or spinulose ornamentation on the leaf surface. Having ornamentation such as microechinate on the hairy surface of the fruit and leave of the species. Morphological, anatomical and micromorphological observations indicated differences in the vegetative form, the ornamentation on the perianth surface and hair and photosynthesis subtype of C_4.

Conclusions

High morphological similarities exist among the species of Chenopodiaceae that are obvious in *Bassia* mixed genus. In addition to the phenotypic levels, they were also seen in anatomical and micromorphological structure levels. Qualitative and quantitative differences among *B. stellaris, B. scoparia* and *B. prostrata* are few, but significant differences were seen in anatomical, micromorphological and morphological aspects of the synonym species, *B. pilosa* and *B. turkestanicum*. Based on this, the two above mentioned species should be revised in the future. For better understanding of classification, also need phylogeny and cytogenetic study.

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