

A Study on the Leaf Surfaces Micro-Morphological Characteristics in the Chenopodiaceae

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Abstract: The aim of the present study is to determine the micro-morphological properties of the leaf surfaces of nine species belonging to the family Chenopodiaceae, using scanning electron microscopy (SEM). Leaf surfaces were noticed to vary considerably with respect to cuticle structural types, stomatal pores position, guard cells cuticular ledges and types of trichomes. Lamellate and lamellate-reticulate cuticular types were recognized. These cuticular folds undergo various patterns, depth and orientation in the various species examined. Sunken, ridged, leveled, raised or depressed stomatal pores were encountered, whereas, the guard cells ledges were poorly or well-developed. In *Suaeda vermiculata* the stomatal pores are sunken in high longitudinal folds rendering the ledges indistinct. Non-glandular unbranched trichomes were detected in *Bassia muricata*, and collapsed salt bladders were reported in *Atriplex leucoclada*. The results showed that the micro-morphological characters of the leaf-surfaces examined are considered to be adaptations in response to arid conditions.

Keywords: Cuticular Structures, Guard Cells Ledges, Trichomes, Pores Position, Aridity, SEM

Introduction

Plant surfaces micro- and nano-structures are diverse (Koch *et al.* 2008), and are evolved in response to their interaction with the environment (Barthlott *et al.* 2017). These diverse surfaces have led to highly adapted functional structures (Koch & Barthlott 2009). These functional structures of the plant surfaces provide an effective water transpiration barrier (Riederer & Muller 2006), provide mechanical stability to the leaves (Bargel *et al.* 2006), affect trapping efficiency and insect transport (Gorb *et al.* 2005, Gaume *et al.* 2002), affect light reflection or absorption of UV radiation (Pfundel *et al.* 2006), reduce the magnitude of particle adhesion (Koch *et al.* 2008), affect the spread area of herbicide droplets (Sanyal *et al.* 2006), act as a barrier against pathogens (Walter 2006), and affect surface wettability (hydrophobic and hydrophilic) leading to self cleaning properties, water repellent surfaces, capillary liquid transport, and drag reduction during moving in water (Koch & Barthlott 2009, Koch *et al.* 2008, Neinhuis & Barthlott 1997).

Aerial plant surfaces (stems, leaves, flowers and fruits) are covered with extra-cellular layer (the cuticle) and contain specialized structures such as trichomes, stomata, ethereal oil cells and hydropotes

(Carpenter 2006). Leaf surfaces perform vital functions to plants and vary enormously in their topography in different taxa (Maiti *et al.* 2016).

The leaf surfaces of various members of the family Chenopodiaceae were previously examined by scanning electron microscopes (SEM). Various members were examined to study their ecological adaptation in saline habitat (Wang *et al.* 2015, Shabala 2013, Labidi *et al.* 2010), or were investigated to examine their taxonomic significance (Klopper & van Wyk 2001, Sukhorukov 2012, Zhang & Zhu 2016). The spread area of herbicide droplets of *Chenopodium album* was analyzed by Sanyal *et al.* (2006), whereas the effect of emitted dust particles on the distribution of *Atriplex leucoclada* was investigated by Oran & Al Zoubi (2016).

The aim of the present investigation is to study the micro-morphological characteristics of the leaf surfaces of various members of the family Chenopodiaceae from Qassim Region, Saudi Arabia, to determine their structural properties and understand their adaptation to the surrounding environment.

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 Published at: <http://www.ijsciences.com/pub/issue/2018-12/>
 DOI: 10.18483/ijSci.1874; Online ISSN: 2305-3925; Print ISSN: 2410-4477



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Material and Methods

The present investigation was carried out to study the leaf-surfaces of nine species belonging to eight genera of the family Chenopodiaceae collected from Qassim Region (Saudi Arabia). *Anabasis setifera* Moq., *Cornulaca monacantha* Del., *Seidlitzia rosmarinus* Del., *Salsola imbricata* Forssk. and *Suaeda vermiculata* Forssk. Ex J.F. Dmel. are succulent species with cylindrical leaves, whereas *Atriplex leucoclada* Boiss., *Bassia muricata* (L.) Asch. in Schweinf., *Chenopodium ambrosioides* L. and *C. murale* L. are non-succulent species.

Leaf-segments of mature fully expanded leaves of approximately 15 mm² taken halfway between the base and apex of the adaxial surfaces of the lamina were examined. These leaf segments were mounted on aluminum stubs with double-coated carbon conductive tape, with no attempt to remove foreign matters from the surfaces. The segments were then coated with Platinum via (Jeol- 1300) sputter, and examined with (Jeol JSM 5500) scanning electron microscope (SEM). Leaf surfaces were studied with at least three replications for each species and were photographed at X500 and X1000 magnifications.

The terminology used to describe cuticular structures follow Holloway (1982) and Prum *et al.* (2012).

Results

Leaf-surfaces of nine species belonging to eight genera of the family Chenopodiaceae from Qassim Region, Saudi Arabia were examined under scanning electron microscope (SEM). These leaf-surfaces showed a considerable variation in cuticle structure types, stomata characteristics and types of trichomes (Figs. 1 and 2).

a. Cuticular structures types:

The leaf-surfaces of the nine species examined were characterized by the presence of thick cuticular layer. According to differences in their micro-morphological characteristics, two types were recognized viz., lamellate and lamellate-reticulate cuticular types. Cuticular folds recognized as high or low, wrinkled or longitudinal (Table 1).

b. Stomata characteristics:

Stomatal pore position and guard cells cuticular ledges vary considerably among the nine species examined. The stomatal pores were noticed to be sunken, slightly depressed, leveled with the surface, slightly raised or ridged. The guard cells possess conspicuous or prominent wavy broad cuticular ledges (Table 2).

c. Types of trichomes:

Non-glandular uniseriate recurved short hairs with acuminate apices were detected in *Bassia muricata*, whereas collapsed salt bladders were reported in *Atriplex leucoclada*.

Table 1: Cuticular structures of the species examined (L = lamellate, L-R = lamellate-reticulate).

Taxa	Type	Cuticular folds		Folds wrinkled	Folds longitudinal
		High	Low		
<i>Anabasis setifera</i>	L	+		+	
<i>Atriplex leucoclada</i>	L-R		+	+	
<i>Bassia muricata</i>	L-R		+	+	
<i>Chenopodium ambrosioides</i>	L	+		+	
<i>Chenopodium murale</i>	L-R		+		+
<i>Cornulaca monacantha</i>	L-R	+		+	
<i>Salsola imbricata</i>	L	+		+	
<i>Seidlitzia rosmarinus</i>	L-R		+		+
<i>Suaeda vermiculata</i>	L	+			+

Table 2: Stomatal characteristics of the species examined (T = with thin poorly developed irregular borders, B = with well-developed broad wavy borders).

Taxa	Stomatal pores position					Guard cells ledges	
	Sunken	Ridged	Leveled	Raised	Depressed	T	B
<i>Anabasis setifera</i>		+				+	
<i>Atriplex leucoclada</i>					+	+	
<i>Bassia muricata</i>			+			+	
<i>Chenopodium ambrosioides</i>				+		+	
<i>Chenopodium murale</i>			+				+

<i>Cornulaca monacantha</i>					+	+	
<i>Salsola imbricata</i>					+	+	
<i>Seidlitzia rosmarinus</i>			+				+
<i>Suaeda vermiculata</i>	+						

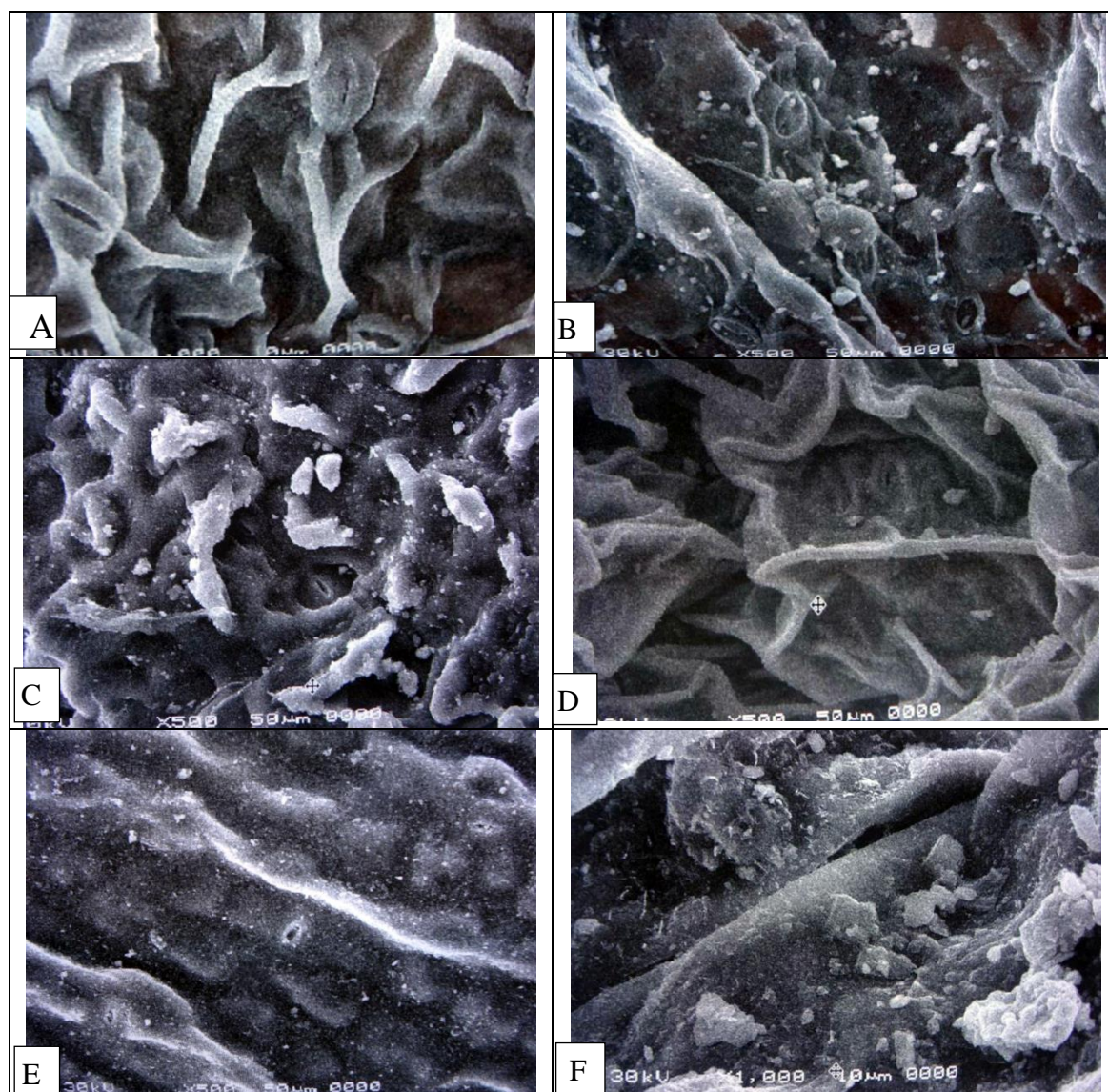


Fig. 1: Scanning electron micrographs of leaf surfaces:

- A:** *Chenopodium ambrosioides* X1000, with wrinkled, high cuticular folds and slightly raised stomatal pores.
B: *C. murale* X500, with lamellate-reticulate cuticular structures and broad well-developed stomatal ledges.
C: *Cornulaca monacantha* X 500, with high wrinkled cuticular folds and slightly depressed stomatal pores.
D: *Salsola imbricata* X 500, with high wrinkled cuticular folds and slightly depressed stomatal pores.
E: *Seidlitzia rosmarinus* X 500, with lamellate-reticulate cuticular structures and stomatal pores leveled with the epidermis.
F: *Suaeda vermiculata* X 1000, with stomatal pores sunken in high longitudinal folds.

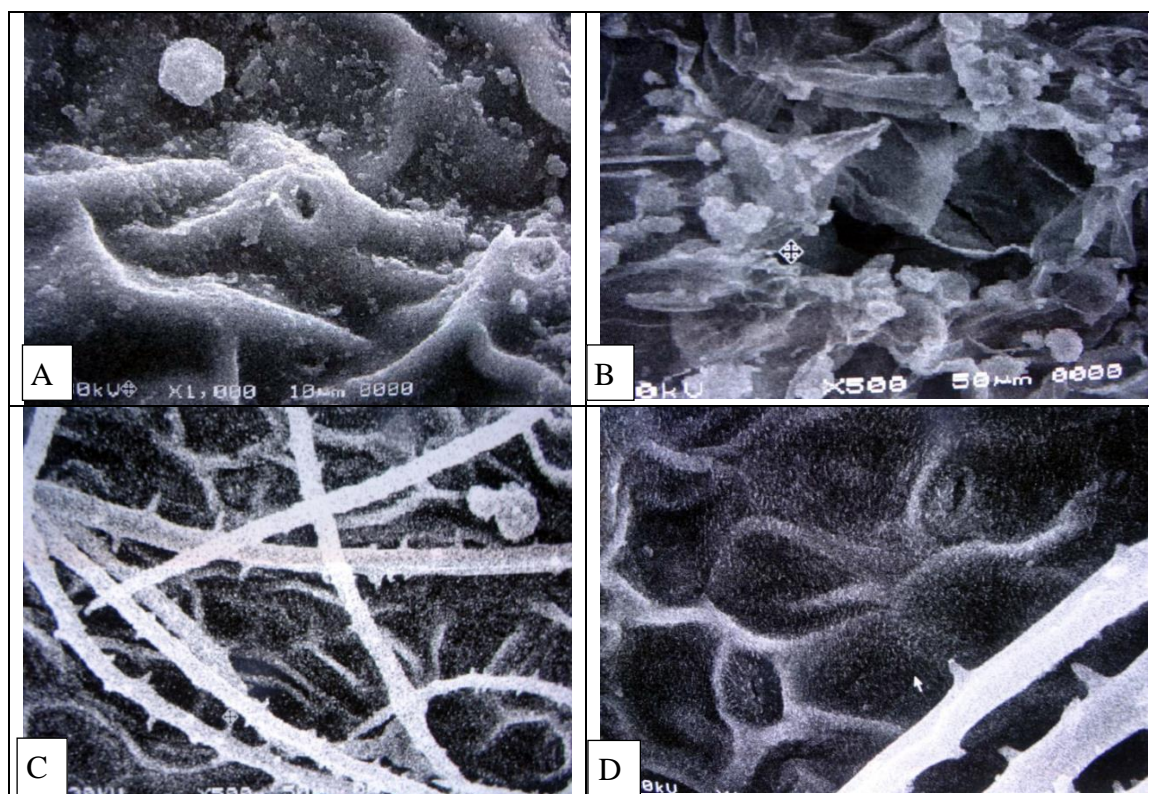


Fig. 2: Scanning electron micrographs of leaf surfaces

A: *Anabasis setifera* X 1000, with high wrinkled cuticular folds and ridged stomatal pores.

B: *Atriplex leucoclada* X500, with collapsed salt bladders and depressed stomatal pores.

C-D: *Bassia muricata* X 500 and X 1000, with uniseriate unbranched trichomes and low wrinkled cuticular folds.

Discussion and Conclusions:

Leaf-surfaces of nine plant species were examined comparatively using (SEM) to evaluate their structural properties and understand their adaptation to the surrounding environment. These species vary considerably with respect to cuticular structure types, position of stomatal pores, presence of cuticular ledges and type of trichomes.

Zarinkamar (2007) in a survey of more than 470 dicotyledonous plant species belonging to 65 families showed that in the Chenopodiaceae, there is minor differences between the adaxial and the abaxial surfaces. This could be attributed to the nearly vertical position of the leaf laminas in the Chenopodiaceae in an attempt to avoid direct sunlight. In the light of the later study, leaf surfaces only on the adaxial surfaces were taken in consideration in the present study.

Stomatal pores within the species examined were not on the same level with the leaf surface. These stomatal pores were encountered to be sunken, slightly depressed, leveled, slightly raised or ridged. This diverse stomatal poral position represent various adaptations to environmental factors affecting the

rates of transpiration and photosynthesis (Larcher 2003).

Sunken stomata in the present study were encountered in *Suaeda vermiculata*. In the latter species, the stomata were not visible and are completely sunken between cuticular folds. Sunken stomata may also be buried in thick cuticle, in cavities formed by raised stomatal rims, in deep pits, between elevations of wax or under hairs (Waldhoff & Parolin 2010). The presence of sunken stomata were regarded by Jordan *et al.* (2008) as indication of increasing adaptation to aridity, as distinct xeromorphic feature (Lyshede 1979), and can prevent excessive transpiration losses (Jimenez *et al.* 2000).

The presence of ridged stomatal pores in *Anabasis setifera* is another unique leaf surface property within the species examined. These cuticular ridges around the pore opening may radiating (Pearce *et al.* 2005), wrinkled (van Wyk *et al.* 1982), curved (Akcin *et al.* 2012), with buttresses ridges (Waldhoff & Parolin 2010), with parallel ridges formed by the thickened periclinal walls (Renzaglia, *et al.* 2017), with striations between or across the ridges (Sun *et al.* 2010). These cuticular ridges are characteristics of

plants of arid zones and help to close the pores tightly in response to high vapour pressure deficit (Jenks 2002).

Cuticular ledges of the guard cells (poorly or well-developed) were encountered in the nine species examined. Both *Chenopodium murale* and *Seidlitzia rosmarinus* were noticed to possess stomatal pores with well-developed broad wavy borders, while the rest of species possess stomatal pores with thin poorly developed irregular borders. The presence of these cuticular ledges highlighted the unique properties of the guard cells. These guard cells are characterized by the presence of proline-rich protein, which lead to cell wall strength and facilitate the interaction with outer cuticle (Hunt *et al.* 2017). The presence of these cuticular ledges prevent the wide opening of the stomata pores and their rising above the surface of the epidermis (Pautov *et al.* 2017), and are considered to reduce transpiration (Hunt *et al.* 2017).

The leaf surfaces of the nine species examined, possess cuticular folds of various patterns, depths and orientations. Longitudinal cuticular folds were noticed in *Chenopodium murale*, *Seidlitzia rosmarinus* and *Suaeda vermiculata*, while the rest of species possess wrinkled cuticular folds. These folds were high in *Anabasis setifera*, *Chenopodium ambrosioides*, *Cornulaca monocantha*, *Salsola imbricata* and *Suaeda vermiculata*. On the other hand, the rest of the species possess low cuticular folds. The presence of cuticular folds on leaf surfaces, reduce the effective adhesive devices of insects in carnivorous (Bauer *et al.* 2018), and non-carnivorous plants (Prum *et al.* 2012). In addition, these cuticular folds help to increase the wettability of the surfaces of these plants (Bauer *et al.* 2013) which is highly needed in arid zones such as Qassim Region.

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