

## Anatomical features of *Atriplex halimus* L. to Saline Environments

<sup>1,2</sup>MAHI Z.\*, <sup>2</sup>DEDALDECHAMP F., <sup>4</sup>BELKHODJA M., <sup>3</sup>LEMOINE R.

<sup>1</sup>Department of Plant Biotechnology, University of Science and Technology of Oran Mohamed BOUDIAF (USTOMB), Oran. Algeria

<sup>2</sup>Laboratory of Paleontology and Stratigraphic Palaeoenvironment LPSP University Oran I. Oran, Algeria

<sup>3</sup>Ecology and biology of interactions CNRS-UMR 7267, PHYMOTS Molecular Physiology of sugar transport in plants) University of Poitiers. France

<sup>4</sup>Department of Biology, Faculty of Natural Sciences and Life, Laboratory of Experimental Biotoxicologie bioremediation and phytoremediation (BTE-BD-PR), University Oran I. Oran, Algeria

### ABSTRACT

In arid and semi-arid regions, the genus *Atriplex* has agronomic and ecological interest. Indeed, in addition to their good forage quality, they ensure fixation and soil enrichment. Halophytic plants are able to tolerate saline environments. They often show a diversity of structural and physiological adaptations that include salt bladders, salt glands or hairs and multiple rows of hypodermis (probable with accumulation and storage role). Related to higher salinity of soil, the halophytes react also by some adaptive, anatomical features. In order to elucidate the anatomical variability level, roots, stems and Leaves of one species of *Atriplex* were used: *Atriplex halimus* L. The results show anatomical differences that can be linked to her degrees of adaptation to external conditions.

**Key words:** halophytes, anatomy, bladder vegetative organs, *Atriplex*, salinity.

### I. INTRODUCTION

*Atriplex* species are members of the family Amaranthaceae and contain both C3 and C4 plants. More than 200 species of plants belong to the *Atriplex* genera and most of them are salt bushes and highly tolerance to salt and drought [1]. *Atriplex halimus* L. (Mediterranean saltbush) is a halophytic shrub that is widely distributed in arid and semi-arid regions around the Mediterranean basin. *Atriplex halimus* L. grows naturally throughout Macronesia, the Mediterranean basin and beyond into western Asia: including southern Portugal, France, southern and eastern Spain (and the Canary Islands), Italy, Greece, Malta, Turkey, Cyprus, Israel, Syria, Lebanon, Jordan, Tunisia, Morocco, Algeria, Libya, Egypt and Saudi Arabia [2, 3, 4, 5]. Throughout its distribution, *Atriplex halimus* L. is exposed to high light intensity and temperature and varying degrees of drought and salinity; it can also withstand sub-zero winter temperatures or soil contamination by trace elements. Some of its physiological and biochemical tolerance mechanism such as adjustment of plant water relations are common

to all or several of these environmental stresses, but others are specific to particular stresses. The importance of *Atriplex halimus* L. in the functioning of ecosystems is reflected in its promotion of soil biota, while it also acts as a food plant for mammals and arthropods. Its deep root system decreases soil erosion in arid zones, due to stabilization of the soil. The protein-rich shoot material of *Atriplex halimus* L. makes it an important fodder species for livestock, particularly sheep and goats. However, its low energy value means that it should be supplemented with carbohydrate-rich material, such as cereal straw. Potential new uses of this versatile plant species include the phytoremediation of soils contaminated by trace elements and the exploitation of its biomass as a source of renewable energy. Such applications, together with its continued use in low-intensity farming systems, should ensure that *Atriplex halimus* L. remains a vital plant species in low-rainfall regions. Due to its varied uses, particularly as livestock forage, it has been introduced elsewhere: for example, Oman, Iran, Iraq, Pakistan, South Africa, Chile, Argentina, New Zealand and the U.S.A. In countries closer to the Equator where it has been introduced, like

Kenya and Ethiopia, the small photoperiod variation during the year means that it does not flower. [4] classified *Atriplex halimus* L. as a “euhalophyte”, able to withstand soil salinity levels equivalent to saturated paste EC values of 25-30 dS m<sup>-1</sup>. [6] demonstrated that the seed germination of a coastal population of *Atriplex halimus* L. was more salt-tolerant than that of a population from a non-saline site; complete inhibition occurred at 700 and 350 mM NaCl, respectively, which represents very-high tolerance in both cases. Thus, we hypothesized that these species has developed specific morpho-anatomical and physiological features which enable it to grow under saline- conditions. Numerous studies have suggested that *Atriplex* spp. can be comparable to alfalfa in nutritional quality, with the advantage that many *Atriplex* spp. are xerohalophytes, capable of growing under saline irrigation or in dryland conditions [7, 4, 8, 9, 10]. Despite initial pessimism about the production potential of halophytes, *Salicornia bigelovii*, *Atriplex lentiformis* and *Distichlis palmeri* show that euhalophytes can maintain high productivity of useful agricultural products up to a root-zone salinity of 70 g L<sup>-1</sup> TDS, double the salinity of seawater. [11] The objective of the present investigation was to uncover the anatomical features that aid in adaptation of salt tolerant of *Atriplex halimus* L. saline condition.

## II. METHODS AND MATERIAL

### A. Plant Material

The sample material subjected to our analysis is represented by leaves, stems and roots of *Atriplex halimus* L. Seeds of the specie were surface sterilized with sodium hypochlorite solution (1% available chlorine) for 10 min. After washing several times with distilled water, seeds were placed on wet filter paper soaked in distilled water in plastic cups in loam at 25°C for two weeks. Plants were germinated from seeds and grown in a soil mixture of loam and sand (1V2V) for 4 months in plastic cups in the University of Poitiers greenhouses [16-h light (25°C, 100  $\mu$ E m<sup>-2</sup>s<sup>-1</sup>)/8-h dark (20°C) cycle and 60% relative humidity] with Peter’s solution until the 5<sup>th</sup> month. The nutrient solution was applied twice a week. Salt treatment

was initiated by adding NaCl in the nutrient solution at concentrations of 0, 100 or 600 mM in a progressive way since 1 month.

To examine the roots, stems and leaves structure several segments (with length of 2–5 mm) were immediately fixed.

### B. Methods.

Sectioning of the samples was fixed into RWL (photonic microscope) and glutaraldehyde (electronic microscope MEB). Cross sections were performed using the ultra microtome technique. Histological observations and micrographs were performed with a ZEISS (AXIOPLAN) Light and electronic microscope (840 A de JEOL).

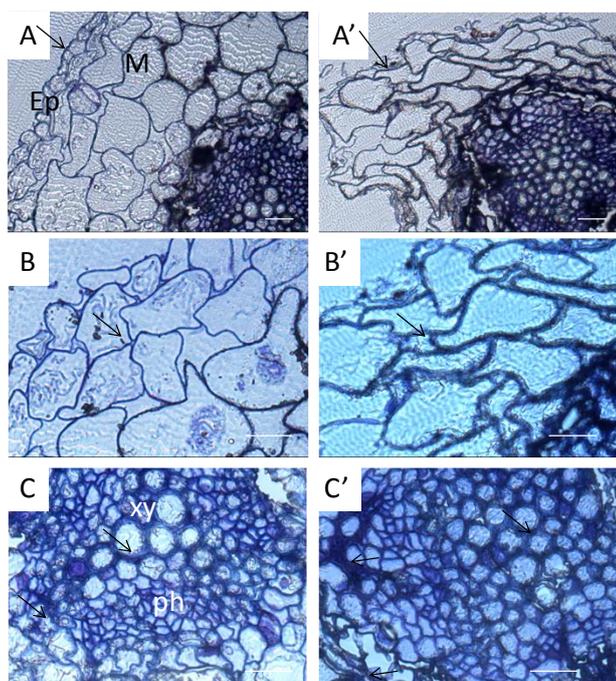
## III. RESULT AND DISCUSSION

### A. Root Anatomy

The examination of transverse sections of the young roots of the studied species (Fig. 1) revealed this specie have a more or less spherical form; They have the same general structure which consists of two separate parts, a considerably larger cortex and a central cylinder. Symmetry is axial. We meet from outside to the inside following tissues: epidermis, parenchyma Cortical or mesophyll, endoderm, pericycle (hardly visible), phloem xylem (protoxylem and metaxylem).

The comparison among the cuts controls and stressed roots brings up the following points:

- Deterioration of skin cells;
- Deformation of cortical parenchyma cells;
- Thickening at the cell walls of the endoderm
- Thickening of the walls of some cells in the endoderm near the phloem
- Decreased diameter of xylem vessels especially metaxylem.
- decrease the diameter of the cortex relative to the central cylinder.



**Figure 1 :** transversal sections in roots of *Atriplex halimus* L. controls (A, B, C) and stressed (A', B', C') stained with toluidine blue. A, A': Overview of a root (GX 20x10) B, B': detailed view in the cortex (GX 40 x10) C, C': detailed view in the central cylinder (GX 40x10). (Bar: 100; 100; 50; 50; 50; 50  $\mu$ m respectively) ) M: Mesophyl; ph: Phloem; Xy: Xylem Ep: Epidermis.

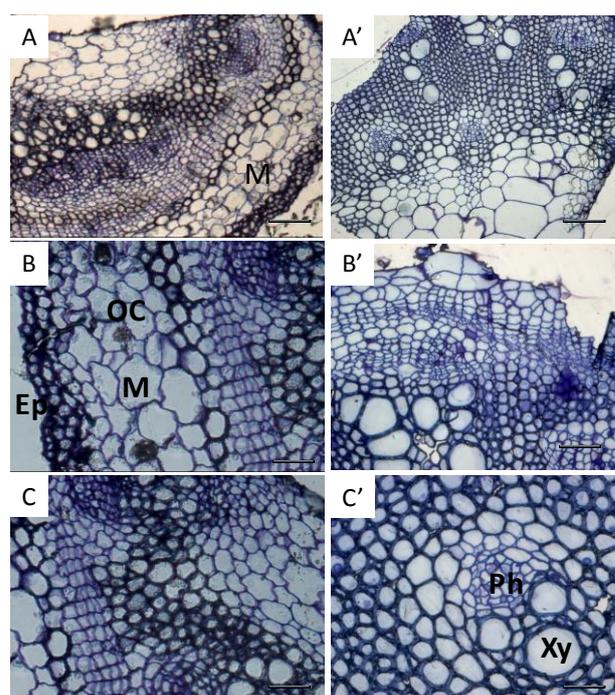
According to [12], the roots of the salt desert plants reduce their cortex to achieve a short distance between the epidermis and the central cylinder. However, the cortex of *Atriplex halimus* L. and *Atriplex canescens* (Pursh) Nutt. is wider than that observed by [13] in *Atriplex tatarica*, *Suaeda maritima* and *Canphorosma annua*.

A study on the structural and functional acclimatization of two ecotypes of *Cyperus* Rottb. (FW "sensitive" and BW "resistant") to salt stress conditions [14] show that gaps formed by the parenchyma aérifère roots are more important in the ecotype FW sensitive than in the resistant ecotype BW. In addition, the BW ecotype large cortical cells, endoderm a thick barrier, high sclérification vessels and a wide meta xylem, contributes to the effective conduction of water and solutes under stress salinity combined with congestion and contributing substantially to a better survival of this ecotype under severe stress environments. However, the last character quoted by these authors is not verified in *Atriplex halimus* L. we think that given the difficulty of

choosing identical roots to compare the two sections might not have exactly the same age. In another study [15] the xylem vessel diameter for *Nitraria retusa* and *Atriplex halimus* L. roots was reduced significantly only at the highest salinity levels (800 mM NaCl). For our study this reduction is noticed at 600 mM.

## B. Stem Anatomy

This report will insist mostly on some histo-anatomical features which we consider to be more revealing on the adaptation of the plants to the soil salinity, as a major ambient factor which acts upon halophytes. Anyway, that does not mean that the other features are not relevant for one species or another, but just the fact that subsystems of the biologic system - the plant - have different values in clarifying the whole structure. Therefore, as a consequence of our investigation (**Fig. 2**), we could observe the typical secondary structure in *Atriplex halimus* L. stem.



**Figure 2 :** transversal sections in stems of *Atriplex halimus* L. controls (A, B, C) and stressed (A', B', C') stained with toluidine blue. A, A': Overview of a stem (B, B': detailed view in the cortex C, C': detailed view in the vascular bundle. (Bar= 100; 50; 50; 50; 25 and 10  $\mu$ m respectively) M: Mesophyll; ph: Phloem; Xy: Xylem Ep: Epidermis; OC: Calcium Oxalate

The successive cambia affects the axial vegetative organs (the root and the stem) of halophytes species (*Atriplex littoralis* L., *Atriplex prostrata* Boucher and *Petrosimonia triandra* (Pallas) Simonkai. studied by [16] and the old root of 12 *Amaranthus* taxa (Amaranthaceae) [17].

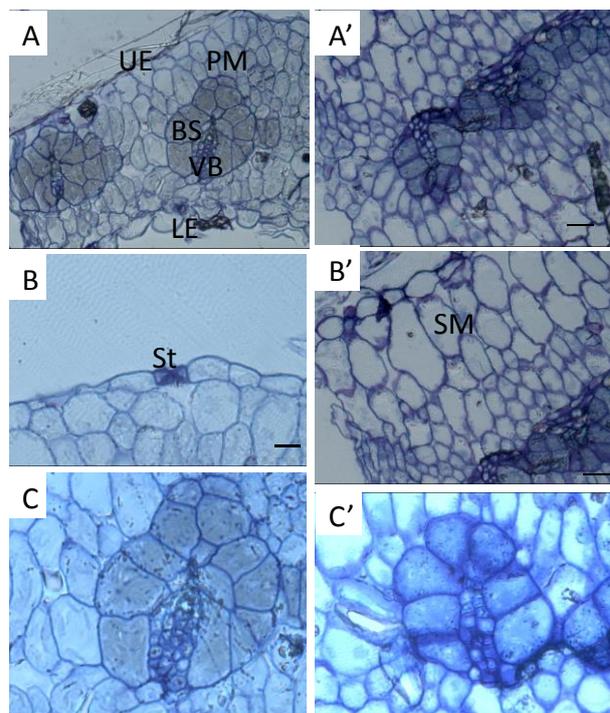
According [15], the cuticle and epidermis thickness of xero-halophytic species were unaffected by salinity but our finding show that epidermis is altered at 600 mM and the cell divided into four are visible at the periphery of the section (Fig. 2 B').

### C. Leaf Anatomy

Concerning the leaves, there is a thickness (Fig. 3 B') of the cortical parenchyma with development of spongy parenchyma with particular form as has been observed for roots.

The number of conductive beams forms a continuous sheath (Fig. 3 A' C').

*Atriplex halimus* L. possesses the C<sub>4</sub> photosynthetic pathway [18, 19], in which CO<sub>2</sub> is incorporated into phosphoenolpyruvate (PEP) to form oxaloacetate through the action of PEP carboxylase. Accordingly, its leaves have the “Kranz” anatomy with a layer of bundle sheath cells surrounding each vascular bundle and radially-arranged palisade cells, although the bundle sheath is open [18]. Our observations are in agreement with the latter feature (Fig. 3C C'). C<sub>4</sub> plants represent about 5% of Earth's plant biomass and 3% of its known plant species [20]. Despite this scarcity, they account for about 30% of terrestrial carbon fixation [21]. Increasing the proportion of C<sub>4</sub> plants on earth could assist bio sequestration of CO<sub>2</sub> and represent an important climate change avoidance strategy. Present-day C<sub>4</sub> plants are concentrated in the tropics and subtropics (below latitudes of 45°) where the high air temperature contributes to higher possible levels of oxygenase activity by RuBisCO, which increases rates of photorespiration in C<sub>3</sub> plants.



**Figure 3 :** Transversal sections in leaves of *Atriplex halimus* L. controls (A, B, C) and stressed (A', B', C') stained with toluidine blue. A, A': Overview of a leaf (GX 20x10) B, B' detailed view in the lamina (GX 40 x10) C, C': detailed view in the vascular bundle (G X40x10). (Bar= 100; 50; 50; 20 20 μm respectively). BS bundle sheath, LE lower epidermis, UP upper epidermis, PM palisade mesophyll, SM spongy mesophyll, VB vascular bundle

C<sub>4</sub> photosynthesis is normally associated with the compartmentation of photosynthesis between mesophyll (M) and bundle sheath (BS) cells. The mechanisms regulating the differential accumulation of photosynthesis proteins in these specialized cells are fundamental to our understanding of how C<sub>4</sub> photosynthesis operates. Cell specific accumulation of proteins in M or BS can be mediated by post transcriptional processes and translational efficiency as well as by differences in transcription. Individual genes are likely regulated at multiple levels. Although *cis*-elements have been associated with cell-specific expression in C<sub>4</sub> leaves, there has been little progress in identifying *trans*-factors. When C<sub>4</sub> photosynthesis genes from C<sub>4</sub> species are placed in closely related C<sub>3</sub> species, they are often expressed in a manner faithful to the C<sub>4</sub> cycle. Next-generation sequencing and comprehensive analysis of the extent to which genes from C<sub>4</sub> species are expressed in M or BS cells of C<sub>3</sub> plants should provide insight into how the C<sub>4</sub> pathway is regulated

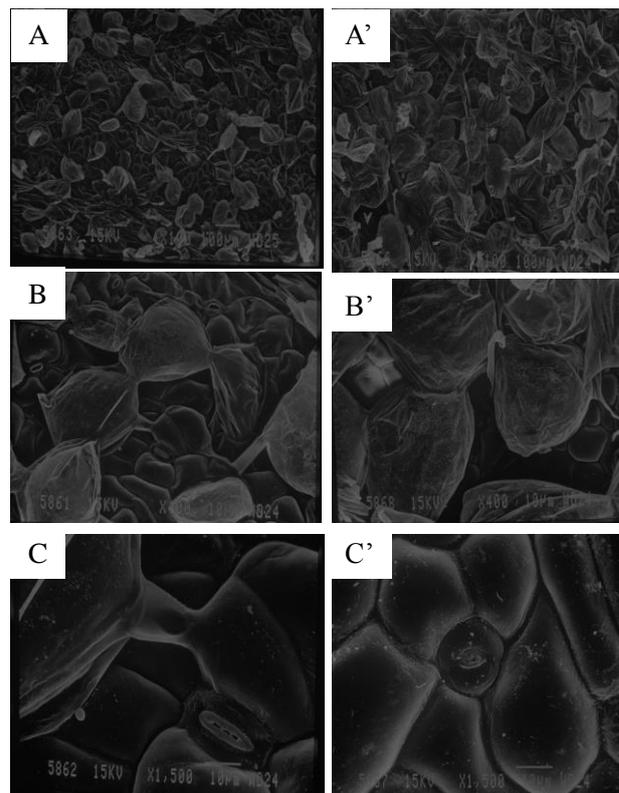
and evolved [22]. Today, plants using C4 photosynthesis are widespread and important components of major tropical and subtropical biomes, but the events that led to their evolution and success started billions of years ago (bya). A CO<sub>2</sub>-fixing enzyme evolved in the early Earth atmosphere with a tendency to confuse CO<sub>2</sub> and O<sub>2</sub> molecules. The descendants of early photosynthetic organisms coped with this property in the geological eras that followed through successive fixes, the latest of which is the addition of complex CO<sub>2</sub>-concentrating mechanisms such as C4 photosynthesis. This trait was assembled from bricks available in C3 ancestors, which were altered to fulfill their new role in C4 photosynthesis. The existence of C4-suitable bricks probably determined the lineages of plants that could make the transition to C4 photosynthesis, highlighting the power of contingency in evolution [23].

#### D. Bladder hairs (*Trichomes*)

Morphology of bladder hairs (**Fig. 4**) showed that young leaf lamina was covered with bladder hairs on the adaxial and abaxial surfaces. This observation suggests that bladder hairs are differentiated at the young leaf stage. Bladder hairs of *Atriplex* species are shown to be differentiated from epidermis of leaf primordium at the very early stages of leaf differentiation [24]. At later developmental stages bladder hairs eventually rupture, releasing the salt on to the surface of the leaf laminae [24].

The Fig. 4 show that lower epidermis of the leaves show more stressed vesicles with a larger volume (Fig.4 A', B', C'). The same changes are observed at the upper epidermis (Fig.5 A'). The two skins showed stomata closed to varying degrees (Fig.4 C, C'; Fig.5 B, B'). Remarkable connections are visible between the vesicles that are connected to the epidermal cells by a single stalk (Fig.4 B, B', C). According [25, 26] NaCl treatment increased the size of the trichome cells. Vesicular trichomes were observed on both the adaxial and abaxial surfaces of *Atriplex nummularia* leaves. The vesicular trichomes It is common to find vesicular trichomes covering the entire epidermis in species of *Atriplex* [27, 15]. However, the details of this morphology can vary among species. *Atriplex nummularia* is similar to *Atriplex triangularis* [28]. Microscopic observation of young leaves of *Atriplex gmelini* showed many bladder

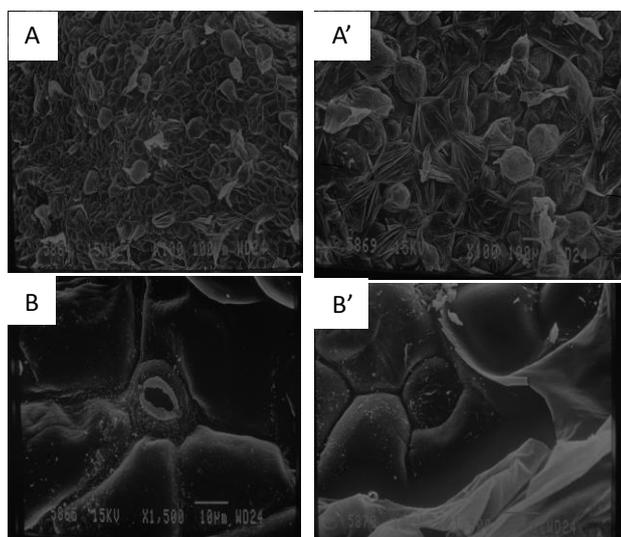
hairs on their surfaces, but their total number decreased along with leaf maturity. Sodium Green fluorescent approach revealed Na<sup>+</sup> accumulation in bladder cells of young leaves when *Atriplex gmelini* was grown at high salinity (250 mM NaCl). Due to fewer bladder hairs in mature leaves, Na<sup>+</sup> accumulation was mostly found in mesophyll cells of mature leaves under high salinity [29]. According to these authors, differential accumulation of glycine betaine and choline monoxygenase was observed in bladder hairs of *Atriplex gmelini* under high salinity.



**Figure 4 :** Scanning electron microscopy photographs showing leaves abaxial surface: control (A, B, and C) and stressed (A', B' and C') of *Atriplex halimus* L. (Different forms of vesicular or trichomes and stomata)

However, only limited experiments have been conducted with this plant, and therefore its Important anatomical feature of *Atriplex halimus* L. (and other *Atriplex* spp.), particularly in relation to stress tolerance, are the vesiculated hairs [19] or vesicular trichomes [30] present on the leaf surface. These living cells consist of balloon-like hairs or bladder cells [31], 80-200 mm in diameter and with a surface coating of a waxy material, attached to a stalk that is embedded in an epidermal cell.

The number of salt glands, the size of the bladders and the percentage of collapsed bladders depends on the amount of salt present during plant growth [32, 33]. Salts crystallize on the leaf when the bladders burst, a process which appears to be related to leaf age [34, 35]. Like other halophytes, *Atriplex halimus* L. accumulates the main component ions of salinity Na<sup>+</sup> [36, 37] and Cl<sup>-</sup> and other anions [38, 39] in its tissues, storing them in the vacuole. The vesiculated hairs on the leaf surface also act as a sink for salt [27]; according to [1], more than 50% of the Na<sup>+</sup> and Cl<sup>-</sup> accumulated are translocated to the hairs. At very-high external salt concentrations (300 mM NaCl), damage appears, regarding stomatal conductance [40] the root plasma membrane permeability, root hydraulic conductivity and chlorophyll content [41], photosynthesis [42, 43] and intracellular organelles [44, 45].



**Figure 5 :** Scanning electron microscopy photographs showing leaves adaxial surface: control (A, B and C) and stressed (A', B' and C') of *Atriplex halimus* L.

The latter species exhibits vesicular trichomes formed by a vesicular cell that binds to the epidermis through a single-celled stalk. In contrast, *Atriplex halimus* L. has a multicellular stalk. In adult trichomes of this species, the stalk can consist of up to three cells. *Atriplex* species accumulate the highest concentrations of salts in the vesicular trichomes. However, the stalk cell, unlike the vesicular cell, did not respond to differences in the level of water stress [46]. Because the stalk represents the connection between the epidermal and vesicular cells [47], changes in the structure of the stalk cell could

affect the plant's ability to transport salt from the epidermal cells to the vesicular cells and store it in the vesicular cells.

Total number of bladder hairs was observed to decrease with increased maturity of leaves and the distribution of bladder hairs becomes sparse as leaves develop and expand. This view is in line with a previous study on other *Atriplex* species [48]. However, the effect of NaCl treatment on distribution or concentration of the bladder hairs was not observed. Numerous halophytes possess leaf trichomes and salt glands that are able to remove large amounts of salt from the photosynthetically active tissues. In several cases, ion excretion is not specific for Na<sup>+</sup> or Cl<sup>-</sup> ions and high amounts of divalent cations, especially Ca<sup>++</sup>, were also found in those structures [49].

#### IV. CONCLUSION

At roots level, there is thickening of some cells of the central cylinder and the formation in the cortex of spongy parenchyma with particular form is very marked.

At stems level, no differences have been revealed between the control and the stressed plants but the presence of calcium oxalate in plant control, indicate that this Cristal is not an indicator of salt tolerance. Concerning the leaves, there is a thickness of the cortical parenchyma and the number of conductive beams forms a continuous sheath. Stressed leaves of *Atriplex halimus* L. show dominance of the vesicles to the upper face, an important size in the underside's vesicles and presence of small stomata.

All these differences between the control and the stressed plant in the anatomy level show that there are some adaptations that can be related to Na Cl stress and to the degree of adaptation of *Atriplex halimus* L.

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