Anatomical Study of Salicornieae Dumort. (Chenopodiaceae Vent.)
Native to Iran

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Abstract: Tribe Salicornieae (Salicornioideae, Chenopodiaceae) includes halophyte plants. Five genera (6 species) of this tribe are distributed in different habitats of Iran. Members of this tribe have reduced vegetative parts, scale like leaves and articulated stems. The variation of anatomical characters of the tribe was studied in Iran using fresh and herbarium materials. In this study quantitative and qualitative anatomical characters for 36 accessions of tribe Salicornieae were evaluated. Morphological variation is fairly high and partly overlapping so they are not sufficient to distinguish these taxa. Despite some uniformity in stem and root transverse sections and stomata type, some anatomical variation is distinct. In epidermis there are some differences in stomata index, width and length of guard cells and papilla features between taxa. In the stem transverse sections the width of aqueduct tissues and medullary zone, number of vascular bundles, shape of tracheid cells and papilla hairs show some variations. The great variability in anatomical characters of Salicornia species indicated that this taxon should be studied more carefully by molecular and histochemical methods further. Anatomical features of stem and epidermis are capable of resolving taxonomic problems of this tribe in Iran.

Key words: Anatomy, Salicornieae, Chenopodiaceae, Salicornia, Halocnemum, Halostachys, Arthrocnemum, Microcnemum, Iran

INTRODUCTION

The members of chenopodiaceae Vent. are important components of the flora and vegetation of the arid region of the world. A great number of the halophytes and xerohalophytes of the world belong to this family (Akhani and Gorbanli 1993, Akhani et al., 1997; Akhani 2003). Elements of tribe Salicornieae Dumort., (Salicornioideae Kostel., Chenopodiaceae), are characterized by their distinctive reduced succulent leaves, which may be modified to form an articulated, photosynthetic stem (DeFries, 1912; Hedge, 1997). These specialized plants generally have spike-like compound inflorescences, comprised of paired cymes of tiny flowers that are sessile within succulent free or fused bracts (Kuhn et al., 1993).

The Salicornieae are among the most salt-tolerant land plant and frequently occur in saline areas associated with coast lines, tidal floodways and salt lakes (Wilson, 1980; Davy et al., 2001). These halophytes are globally distributed, being found on every continent with the exclusion of Antarctic (Kuhn et al., 1993).

The aerial parts of the plant, notably in Salicornia L. are very plastic, there by making specific identification difficult. Many member of the family are distributed in saline soils. This plant have leaves which are often succulent or reduced, whilst in some instances, the stem is succulent and takes the function of carbon assimilation.

Reduced morphology of the Salicornieae limits the availability of easily recognized diagnostic character at the tribal and generic levels. Furthermore the Salicornieae exhibit considerable phenotypic variation at the population level (Wilson, 1980; Davy, 2001; Freitag et al., 2001) and taxonomic confusion is exacerbated by the occurrence of species complexes (Wilson, 1980) and polyploidy (Shepherd and Yan, 2003). The succulent vegetative morphology may also become modified when dried, limiting the use of herbarium material. As a result, the sub-familial and infra-generic relationship of the Salicornioideae are not fully clarified (Shepherd and Colmer, 2005). Apart from taxonomic difficulties in many genera of Chenopodiaceae there are some fascinating aspects regarding the diversity of adaptive strategies and ultra structure in many species. One of the adaptive strategies which are correlated with morphology, ecology and even taxonomy is the photosynthetic type. Two types of C₃ and C₄ are frequently found in Chenopodiaceae (Carolin et al., 1975). C₃ and C₄ photosynthesis seem to be adaptation to environmental factors and not closely correlated with life from...
MATERIALS AND METHODS

In this study 36 Accessions of different salty marshlands of Iran were collected (Table 1). All collected samples are deposited at Herbarium of Alzahra University. Stem and root anatomical characters were recorded from 5 genera currently reported from Iran: Arthrocnemum, Halocnemum, Halostachys, Microcnemum and Salicornia. Characters were scored from fresh materials. In this study we usually use fresh materials for cross section (Table 2). Section in the stem was taken from 5 cm terminal part and from 2 cm terminal of tap root. Sections were stained by Methyl blue and Carmen. Images were taken using a digital camera mounted on a DP12 Olympus light microscope.

RESULTS

Stem anatomy: All the salicornieae examined show basic stem structure, a well defined epidemis, more or less
Table 2: Evaluated anatomical characters and state of characters of tribe Salicornieae
1. Papilla: Present, Absent
2. Sunk stomata: Present, Absent
3. Sinuous wall in epidermis cell: Present, Absent
4. Crystal: Present, Absent
5. Crystal region: Present, Absent
6. Scattered vascular bundle: Present, Absent
7. External xylem: Present, Absent
8. Endodermis: Present, Absent
9. Cork: Present, Absent
10. Pericycle: Present, Absent
11. Medulla: Present, Absent
12. Tracheids: Present, Absent
13. Shape of Tracheids cell: Spinal, Stereides
14. Tracheids cell region: Aquatic tissue, Palisade tissue
15. Arrangement of vascular bundle: Collateral, Bicollateral
16. Number of vascular bundle: Four, six, eighth, more
17. Length of Tracheids Cell: 
18. Papilla length:

well defined chlorenchymatus zone, a zone of aqueous tissue within which occur vascular bundles and a central stele (Table 2).

Epidermis: In this study we found some kind of homogeneity in anatomical structure of epidermis in elements of tribe Salicornieae native to Iran. All of these taxa are glabrous. Stomata type is merely anemocytic which the guard cells are surrounded by certain number of cells that do not differ in size and shape from the other epidermal cells. There are pentagonal subsidiaries (Fig. 1). They are arranged with their long axes at right angles to the axis of the stem. The epidermis consists of single layer of thin-walled cells. This structure is same in all of sampled taxa but the epidermal cells show some differences between members of this tribe. Stomata index, width and length of guard cells show variations between these taxa.

We found in this tribe sunken stomata are present only in Halocnenum strobilaceum, Halostachys belangeriana and Arthrocnemum macrostachyum. The outer walls of epidermal cells are raised in to papilla. Length of these papilla shows variations between these species. Halostachys belangeriana shows papilla in the stem cross section and their length is varied between 0.04-0.06 mm. Papilla in Halocnenum strobilaceum are about 0.01 mm (Fig. 2). Studying S. europaea shows that cell wall in epidermis are wavy (Fig. 1, B). Other characters in this tribe as percent of crystal show variation. There are numerous crystals in epidermal cells (Fig. 1). This is evident in all species of this tribe.

Palisade tissue: Palisade tissue are two or three cell layered. Cells are thin-walled elongated at right angles to the epidermis, have rounded ends and are separated by numerous small intercellular spaces. There was not any variation in the thickenings of this tissue between different taxa. Palisade almost make one third of cortex in all transverse sections of taxa. We found respiratory cavities, lie beneath the stomata (Fig. 2).

The collateral bundles become completely surrounded by the fibers of the intermediate tissues. As this anomalous secondary growth proceeds, old stem shows a considerable thickening of lignified tissue in which the collateral vascular bundles are arranged in more or less regular concentric zones. Numerous vascular

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Fig. 1: Stem Epidermis of Salicornieae: (A) and (B) Salicornia europaea; (C) Halocnenum strobilaceum; (D) Arthrocnemum macrostachyum; c stands for crystal and p for papilla
Water-storing Parenchyma (aqueous tissue): Beside palisade tissue in the stem, we can see water-storing parenchyma. In this area the cell are large, thin-walled and colorless (Fig. 4). This zone occupies large part of stem and show little differentiation between genera. We can define these plant in two groups based on theirs water-storing parenchyma. In the first group water-storing parenchyma consist of one region with rounded parenchymatus cells. In second group this region consist of two parts which first part is intermediate of palisade tissue and water-storing parenchyma. Salicornia, Arthrocnemum and Microcnemum are in first group while Halocnemum and Halostachys are in the second group (Fig. 4).

First group consist of palisade like elongated cells which are achlorophilous and second group consist of spherical colorless cells. In all taxa we found numerous crystals. These crystals distributed in the epidermis and palisade tissue. We did not find any crystals in the stele of these plants. Crystals are made of calcium oxalate and are concentrated in palisade tissues and epidermis. In water-storing tissue and central stele there was not any crystals.

Another abnormal character in these plants is presence of vascular bundles in out side of stele. These vascular bundles are related to vascular network of leaf and usually replace between palisade and water-storing tissue. This character find in all members of this tribe. Another abnormal feature was the inner suberified cells which are originated from pericycle. These single or grouped suberified cells which are located near pericycle could be found in Salicornia, Halocnemum strobilaceum and Halostachys belangeriana (Fig. 5).

Stele has a well differentiated pericycle. This is the only portion in this section which can be called stem. In this are embedded the collateral fibro-vascular bundles. Arrangement of vascular bundles is eu-stele. The xylems formed from cambium consist of thick-walled fibrous cells.
Fig. 4. Stem Transverse sections of Salicornieae: (A) *Salicornia europaea*, (B) and (C) *Halocnemum strobilaceum*, (D) *Halostachys belangeriana*. *aq* stands for aquatic tissue, *ca* for cambium, *co* for cork cells, *ph* for Phloem, *Pi* for pith, *Ex* for External xylem and *X* for xylem.

The phloem consists of thin-walled cells, forming, as usual, a cylinder outside of the cambium. No sieve-tubes could be detected among these cells. In addition to the phloem cylinder, there are phloem islands scattered about in the fibrous cells of the xylem in each case lying just outside the large vessels, from are separated by only a few fibrous cells. There are no differences in the structure of vascular bundle but the number of these bundles is different.

In vascular bundles there were cambial cells which made wood inward and phloem outward. Woods were almost fibrous inside. The thickness of phloem was less than that of wood. This secondary structure was very evident in *Halocnemum strobilaceum* and *Salicornia*. Pericycle and endodermis was not much differentiated.

The minimum number of vascular bundles is (4-6) in *Microcnemum*. We find 6-8 vascular bundles
in *Salicornia*, *Halocnemum* and *Halostachys*. The highest number was observed in *Arthrocnemum* (8-10).

The pith is a more or less cylindrical body of tissue in the center of the axis, enclosed by the vascular tissues. The pith consists of a rather uniform tissue, mainly parenchyma in which the cells are arranged usually loosely. In the ontogeny of the stem, the pith cells in the internodes of many species mature very early and stop growing, whereas the surrounding tissues are longitudinally and in circumference. Thus the pith may be torn apart and a hollow pith is formed.

In the examined taxa pith distinguished just in all cross sections of *Halocnemum strobilaceum* and occupy central of stem. Pith was observed only in *Salicornia A. macrostachyum M. coralloides* and *Halostachys belongeriana*. Pith was only evident in young plants and in other samples it was torn. There are not any difference between stem structure of *S. persica* and *S. europaea*.

**Root anatomy**: Root anatomy in the elements of this tribe is homogenous. Periderm is present in the outer layer of secondary vascular bundles. This part consists of suber outward and parenchyma inward. In this tribe primary xylem and phloem make some arcs in regular (spiral pattern) or concentric circles. The primary root is diarch and has a very well marked endodermis. The bundles of phloem are quite distinct and easily distinguished from the xylem. As in the majority of roots, the medulla becomes obliterated. The structures of old roots resemble that stems. Secondary xylem and phloem are developed from an extra-fascicular cambium but phloem islands in the root are larger than those in the stem and walls of fibrous cells are thinner. Observation of cross section enabled us to defined different seasons growth (Fig. 6).

**DISCUSSION**

In this study examined representatives of five genera of tribe salicornieae and documented anatomical features that were found to be at the genus level. Although some anatomical character may be useful potentially phylogenetically informative, none appear to be definitive at the species level.

Our results show that there are basic stem structure, a well defined epidermis, well defined chlorenchymatus zone with scattered vascular bundles and a central stele. There are some limited studies about Salicornieae in Iran. Isfahani (1998) studies *Salicornia* based on morphological and anatomical structure. She proposed a new species of *Salicornia* for Iran based on anatomical character (presence of papilla). Advanced study of her transverse sections and specimens, shows that she may confuse a population of *Halostachys belongeriana* due to a lot of morphological similarities as a new *Salicornia* species.

In the study of epidermis we found variation in Stomata index and guard cell size between genera and accessions. We couldn't find special correlation between these factors with geographical distributions, altitude and environment. The variation inside sympatric populations couldn't relate to ecological factors. Stomata index and the size of guard cells are usually related to the ploidy level so again we emphasize the need of further studies from cytological point of view.

Presence of papilla and their size are diagnostic characters between genera in this tribe. Based on these characters we can distinguish *Halocnemum, Halostachys* and *Arthrocnemum*.

Segmented stems occur in Salicornieae. The mesophyll is dorsi-ventral to centric or composed of palisade assimilatory tissue and water-storage cells.
Stomata are generally present on all parts of the leaf surface, the pores usually being oriented transversely to the median vein. Anomalous secondary thickening occurs in all species of this tribe. Anomalous secondary thickening rapidly producing a thick zone of lignified tissue in the secondary vascular bundles embedded. The cortical region is limited to a few cell-layers.

Special types of stem structure occur in succulent plants of the Salicornia type. Mesophyll often is exhibiting specialized structure in correlation with the halophytic condition. This structure is consisting of various arrangements of assimilatory and water-storage tissue. Assimilatory tissue occupying a narrow zone beneath, but some times separated from, the epidermis by a hypoderm, the remainder of the organ being occupied by a cylinder of aqueous tissue with thin walled vascular cylindrical assimilatory organs whether these are morphologically leaves or stems in such genera as Salicornia. In the mesophyll of the leaves of the abnormal plant the assimilatory tissue was confined to the abaxial side and the cortex of the stem consisted of the downward continuation of the free parts of the leaves. This theory that the primary cortex of the axis is homologous with leaf tissue was first expressed by De Frain (1912) following a morphological and anatomical study of normal Salicornia plants and by Cooke (1912) after making observation on S. australis.

Cork in these plant arising internally to the pericyclic fiber strands in the Salicornioideae. A thick layer of cork, composed of polygonal cell, noted in old stems of Salicornia. Outer part of the primary cortex some time differentiated as palisade tissue in species of Arthrocnemum Moq., Salsoa L. sclerosed cells some time showing spiral striations, occur in the palisade chlorenchyma of Salicornia. A secondary aerenchymatous cortex at the base of the main stem, in the upper part of the main root and in the rhizome of perennial species of Salicornia is recorded by De Frain (1912). Vessels small spiral thickening observed or reported in some species Halocnemum M. Bieb, Halostachys Moq. Included (inter-xylary) phloem typical: usually of the concentric type, with the strands of phloem linked by broad, irregular bands of conjunctive parenchyma, but sometimes tending toward the foraminite type reported by Pfeiffer to be present in Arthrocnemum, Halocnemum and Salicornia. Primary vascular structure is diarch in Salicorniaceae. Vascular system exhibiting the same type of anomalous secondary thickening as that in the stem, even in species with stems which do not grow in thickness sufficiently to exhibit this feature. Concentric rings of bundles recorded in species of Salicorniaceae (Metcalf and Chalk, 1989).

A theory which might account for these phloem islands is this: The formation of the large vessels consumes time. While these are forming the cells each side of the group of vessels, growing more quickly. Grow over enclosing a small patch of cambium. This gives rise to the phloem and several small fibrous cells, the latter separating the phloem from the vessels. The phloem islands consist of thin-walled cells, which show great uniformity in length. In this plant the secondary wood consist chiefly of fibrous cells. These have thickened walls (Cook, 1912).

The function of the stereides needs no discussion for they are obviously part of the mechanical support of the plant. De Frain observation considered the tracheids as water storing elements and abundant in the foliar sheath at the nodal region and in the reproductive shoots our results are accordance with his view. The function of air-storage is similar to air-storing tracheids as it is referred by Ganong and Duval Jouve in Salicornia Europaea.

In the reduced leaves of salicornia, broad short tracheid-like cells are found among the palisade cell (Fahn, 1963, Fahn and Arzoo, 1959). The function of this cell has been variously interpreted by different workers. Duval-Jouve (1868) stated that they are filled with air. These cells transport water to the peripheral layers. Other investigators (De Frain, 1912) believe that these tracheid-like cells (tracheoids) have a water-storing function.

Our results show that there is a kind of homogeneity in the root structure of this tribe in Iran although there is a vast variation in stem structure. Characters as papilla, number of vascular bundles, water storage parenchyma, medullary parenchyma, the suture cell walls of the epidermis, Stomata index and guard cell size were important diagnostic features in this tribe. By this character set we can almost distinguish different genera of this tribe in Iran. These are not efficient in separation of two species of Salicornia in Iran. To have a better knowledge of phylogenetic relationships in this tribe, a lot of diagnostic and reliable characters are needed.

A rapid radiation could account for lack of diagnostic characters between genera, but dose not explain the considerable variation, expressed at lower levels (Shepherd and Clomer, 2005).

The heterogeneity of Iranian salt lakes and plates, which exhibit differences in soil type, salinity level, area, depth and daily radiation (day long) provide infinite habitat variability (Asiri, 1998; Akhani and Gobballi, 1993).

Species complexes and hybridization are reported and polyploidy is also evident with diploids, triploids, tetraploids reported within Salicornia species (Sayedcrassi, 1994).
All these factors may influence diversity at the population level among Iranian saliciniae.
Due to the wide distribution of steppe region and salty soils in Iran and the wide range of salt tolerance of members of Saliciniae, it seems that they are good choices for desert improving and management and also as fodder supply. In traditional medicine of Iran, Salicornia has also an edible and officinal importance.

ACKNOWLEDGMENTS

We are grateful to Dr. Phil Davidson, Dr. Paul Wilson and Farzaneh Zoie for their help and co-operations.

REFERENCES


