Anatomical Adaptations of Halophytes Within the Southern Pannonian Plain Region

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Abstract

Halophytes have developed different types of adaptive strategies, which enable them to survive in salt stress and physiological drought conditions common to saline habitats. Anatomical adaptations at cellular, tissue, or organ level play a crucial role in the development of a wide range of salt tolerance. Succulence is one of the most prevalent adaptations, along with an increased cell volume, well-developed water storage parenchyma, Kranz leaf anatomy, strong lignification, successive cambia formation, formation of salt secretory structures, tracheoidioblasts and bulliform cells. Halophytes found in dry saline habitats tend to develop xerohalomorphous anatomical adaptations, which affect mostly epidermal, vascular, and mechanical tissue. The present investigation focuses on the diverse mechanisms employed by halophytes inhabiting continental halobiomes of the southern Pannonian plain region.

Keywords

Halophyte anatomy · Kranz anatomy · Pannonian halophytes · Salt glands · Succulence

1 Introduction

Halophytic species that occur naturally in saline habitats develop special strategies in order to survive complex and demanding conditions characterizing saline environments. More than 500 genera contain species capable of surviving on extremely salinated soils (Flowers et al. 1986). Among dicotyledonous species, family Chenopodiaceae has the highest number (>300) of halophytic species, followed by Asteraceae with over 50 species. Most (>100) of the monocotyledonous halophytic species belong to Poaceae family, followed by Cyperaceae. Halophytes are uncommon among higher plants, and not all species inhabiting saline environments can be classified as halophytes. Only about 0.25% of angiosperms, which survive and complete their life cycle in at least 200mM of salt, can be considered halophytes in narrow sense (Flowers et al. 2010). For some species, classified as glycophytes, just 25 mM of NaCl can be toxic, while extreme halophytes can tolerate salt concentrations as high as 500–1000 mM. Halophytes have different types of adaptive strategies, developed gradually during their evolutionary process. Owing to this complex adaptation mechanism, these plants can thrive under salt stress conditions.

Dissolved soil salts affect plants in different ways. They bind soil moisture, thus making it unavailable to the plant roots but can also be toxic to plants due to exhibiting unfavorable ionic effects on the enzymatic system and cell membranes (Stevanovic and Jankovic 2001). The first mechanism is often referred to as "physiological drought," given that salts decrease the water potential of the soil solution and render plants unable to absorb water in sufficient quantities. Physiological drought can induce common xeromorphic traits to plants vegetating in different habitats (Grigore et al. 2014). If such conditions persist for a prolonged period, or if they are strongly expressed, they may lead to functional disorders, reduced growth, lower fertility, and lower biomass production or may even cause visible damages to affected plants.

To be able to absorb soil water and transport it to the aboveground organs, halophytes develop different mechanisms to modify osmotic potential of their tissues (Akcin et al. 2017). They generate lower cell water potential, which should decrease acropetally in the xylem, to maintain water uptake under osmotic stress and to extract water from the saline soil (Safiallah et al. 2017). When plant roots absorb NaCl from salty soil, root osmotic potential decreases, due to the increase in root ion concentration, which facilitates further absorption of soil water even under physiological drought conditions (Stevanovic and Jankovic 2001). Halophytes expend considerable energy on ion transport and redistribution of ions within the cell and between the cells and tissues, but also on synthesis of stress proteins and osmotically active organic compounds. Ions are usually accumulated in the vacuole, whereas in the cytoplasm they bond with different organic compounds. As a result of these processes, their harmful effect on enzymes and biomembranes is largely neutralized.

2 Anatomical Adaptations of Halophytes

Halophytic species exhibit a considerable diversity of structural and physiological reactions to salinity at cellular, tissue or organ level (Dickison 2000). Their adaptive responses can be broadly classified as avoidance- or tolerance-based, as plants either avoid the harmful effects of salty soils, or enhance their tolerance to unfavorable biochemical effects of excessive concentrations of different salt ions (Stevanovic and Jankovic 2001). Some plants are able to exclude certain ions from further transport at the root or shoot level and thus regulate the amount of salts in their organs. Specific anatomical barriers in the root along the water transport pathway in the form of thick-walled cortex parenchyma cells and endodermal cells with Casparian strips serve as a salt solution filtration system. Avoidance of the adverse effects of salt ions can be achieved by interrupting the ion transport from the soil to the root, or from the root to the aboveground organs. (Schulze et al. 2005).

If salt ions enter the plant body, several other mechanisms are activated in order to eliminate their harmful effects. One such mechanism is dilution, attained through the development of succulent structure, which lowers ion concentration in plant cells. Moreover, plants possess several mechanisms for elimination of surplus salt outside of the plant body, which involves through salt glands, bladders, or hydathodes. Significant salt quantities can be eliminated by shedding off older parts, leaves, or branches (Stevanovic and Jankovic 2001). Salt deposition, usually in vacuoles, is also one of the strategies that enhance plant's osmotic potential. It typically acts in synergy with synthesis of compatible solutes in cytoplasm (osmolytes) and protective proteins.

Halophytes develop specific morpho-anatomical and physiological features and modifications which help them survive saline conditions and minimize negative effects of salt stress (Waisel 1972; Akcin et al. 2015). The most obvious adaptations manifest in the organization of plant body as a whole, as well as in the morphology of vegetative organs. Leaf is the organ that reacts the most strongly to the environmental factors, especially those which induce stress (Dickison 2000). Leaf position, size, and arrangement along the stem are closely linked to the salt elimination mechanisms adopted by the plant. Many halophytes, most of which belong to Plantaginaceae, Plumbaginaceae, Brassicaceae, or Asteraceae, have leaves arranged in rosette (Rancic et al. 2019). As some of these plants have salt secretory glands, and some eliminate salt by rejecting the whole leaf, this arrangement hinders salt transport from the root to leaves, while facilitating easier and faster salt elimination from the plant body. Smaller leaves, or presence of thorns, similarly reduce water loss by limiting the evaporation surface.

In salt-sensitive plants, even relatively low-internal salt concentrations can have adverse consequences on growth (Schulze et al. 2005). Cell division and elongation are particularly affected, albeit in different ways. As salinity prevents plants from absorbing water, it inhibits growth due to reduced cell division, earlier cessation of developmental processes, and premature leaf yellowing and fallout (Stevanovic and Jankovic 2001). On the other hand, as high salt concentration does not inhibit cell expansion, plant size is reduced, whereas fleshiness increases proportionally with salt concentration. Moreover, salt ions have harmful effect on chloroplast structure and disable electron transport. Consequently, photosynthesis intensity declines, which hinders plant growth and development.

Anatomical adaptations play a crucial role in the adaptation of halophytic plants to salinity stress. Some anatomical features, especially those of the leaf, can be used in determining the degree of stress tolerance in different species (Hameed et al. 2012). Succulence is one of the most obvious and most common adaptations to saline conditions. Halophytes develop succulent stems or leaves, in which water storage parenchyma tissue predominates. Succulent stems are leafless, or leaves are fused with stem, like in *Salicornia* (Waisel 1972; Evert 2006).

In nonsucculent plants, increased salt concentration leads to greater mesophyll and palisade tissue thickness, as well as a larger diameter of spongy tissue cells, which tend to form additional layers (Longstreth and Nobel 1979). The number and size of leaf trichomes also increase (Malcolm et al. 2003). In some species, uniseriate hypodermis is present in leaves, serving as water storage tissue. In such cases, chlorenchyma tissue becomes less developed and discontinuous, with a reduced number of chloroplasts in bundle sheaths, which are typically less prominent (de Villiers et al. 1996).

In general, anatomical adaptations to salt stress include increased cell volume due to which leaf thickness increases, whereas vessel diameter decreases (Akcin et al. 2015, 2017; Rancic et al. 2019). Formation of narrower vessels improves water-use capacity and reduces risk of xylem embolism and cavitation in saline habitats (Polle and Chen 2015). Well-developed epidermal and sclerenchyma tissues in stems are mainly involved in water conservation, while extensive vascular tissue contributes to more efficient water transport under stress conditions. The presence of mucilage cells in stem cortical and pith parenchyma has been recorded in many species (Abd

Elhalim et al. 2016). Mucilage increases osmotic pressure, improves water absorption and retention, and ensures better tissue hydration. Tribe *Salicornieae* Dum. includes leafless species with succulent stems, which have developed several adaptations to salinity and drought, the most common of which are sunken stomata, vascular bundles surrounded by lignified fibers, anomalous secondary thickening producing significant amount of lignified tissue, scattered tracheoidioblasts, well-developed water storage parenchyma, suberified cells near stem pericycle and strongly developed root endodermis (Keshavarzi and Zare 2006; Milic et al. 2011). In *Salicornia freitagii* Yaprak and Yurdakulol, salinity stress induces xylem tissue thickness and root and stem vessel diameter reduction, along with increased stem thickness and greater stem water-storing tissue size, formation of tracheoidioblasts and successive cambia, and decreased leaf stomatal density (Akcin et al. 2017). Grigore et al. (2012) recorded several adaptations in Romanian halophytic species, including succulence, successive cambia formation, salt secretory structures, Kranz leaf anatomy, and bulliform cells.

Stomata of halophytic species are usually smaller and are located below the epidermal cells level (Waisel 1972; Hameed et al. 2009; Akcin et al. 2015, 2017; Rancic et al. 2019). Salinity decreases the number of stomata and tends to lower the stomatal index. When combined with thick epidermis and cuticle, these adaptations prevent water loss. Smaller stomata, or even their absence from adaxial surface, can control turgor pressure and prevent water loss through epidermis, and are thus an important determinant of physiological drought tolerance (Hameed et al. 2012).

Root anatomy seems to play a prominent role in the development of salt tolerance (Abd Elhalim et al. 2016). Formation of phellem on root's surface and suberization reduces water absorption and salt penetration. Moreover, presence of starch grains in phloem parenchyma cells could enhance osmotic adjustment in plants of saline habitats. Root endodermis is important in determent of apoplastic flow of ions (de Villiers et al. 1996). Endodermis and exodermis have a crucial protective role in limiting water loss from roots and controlling water radial flow. It is posited that increased aerenchyma is positively correlated with poor ventilation of saline soil.

Early lignification characterizes many halophytes (Saadeddin and Doddema 1986). Lignification of root secondary xylem supports the vessels and is commonly observed in plants growing on habitats with low water potential. Abd Elhalim et al. (2016) also suggested that embedding of secondary xylem vessels in lignified cells provides additional root rigidity, improves cavitation resistance, and protects water columns from embolism. In some representatives of Cyperaceae, excessive sclerification and aerenchyma, as well as highly developed bulliform cells, have been recorded (Hameed et al. 2012).

2.1 Halophytes Classification and Their Relation with Morphological and Anatomical Adaptations

Different groups of halophytes develop different adaptations to salinity, depending on their halophytic status, type of the habitat they inhabit and water regime and its availability (Stevanovic and Jankovic 2001). It is very difficult to provide only one classification of halophytes, due to the significant differences in their morpho-anatomical adaptations, habitat preferences and several classification criteria (salt concentration characterizing the habitat, ecological conditions, amount of water within the habitat, plant's responses to salinity, etc.). Different classifications point to various logical relations between halophytes and their environment (Grigore and Toma 2017). One specific species actually belongs to several groups of halophytes. In this work, rather than examining different classifications, focus is given to different types of halophytic species, whose anatomical adaptations will be emphasized later in this paper.

Obligate halophytes require salty habitats for completing their life cycle and normal development due to the stimulating effect of salt on several of their biological processes (Grigore and Toma 2017). Facultative halophytes usually have less expressed structural changes. They sometimes escape to saline habitats, due to high competition with other species on nonsaline habitats, where they are at a disadvantage due to their fragility and more rapid water loss. Morpho-anatomical modifications are the most prominent in extreme or typical halophytes (euhalophytes) that obligatorily inhabit highly salinated soils (Dajic Stevanovic et al. 2019). These plants can withstand high salinity, which is necessary for their growth and stimulates dry mass production, and develop well-pronounced halomorphic structure. Mesohalophytes grow on saline, but also other soil types, and therefore have less expressed halomorphic adaptations. Glycophytes cannot survive on salinated soils and are not tolerant to salinity. As these species did not develop any mechanism to cope with salinity, even small amounts of salt can have toxic effects on them, as salinity inhibits their cell division, as well as growth. Most of the crop plants and cultivated species belong to this group.

Soil humidity is another important characteristic of saline environments, as it determines structural adaptations to specific combination of salinity and moisture. Le Houérou (1993) proposed halophyte classification based on the water regime of their habitats. Xerohalophytes are plants adapted to dry saline soils and can thrive underwater deficit regime. These plants have xeromorphic structure and are thus resistant to drought, but also to high salt concentrations. Hygrohalophytes inhabit wet, even flooded salty soils, and cannot withstand drought, while mesohalophytes are adapted to medium salinity and humidity. Hydrohalophytes are aquatic or semiaquatic plants, inhabiting salty water.

Grigore and Toma (2010) suggested new halophyte categories based on their anatomical adaptations, their relevance, ecological significance and general survival strategies. According to these authors, extreme halophytes are species occurring exclusively in saline environments, and thus possess the strongest anatomical adaptations. Irreversible extreme halophytes (like *Suaeda*, *Salicornia*, or *Halimione* species) belong to this group and have developed the strongest anatomical adaptations (such as succulent structure, successive cambia, well-developed water storage tissue, high lignin content, tracheoidioblasts, salt glands, or foliar Kranz anatomy), combinations of which are present in different halophytic species. Reversible extreme halophytes (such as some *Atriplex, Bassia,* or *Camphorosma* species) have less developed adaptations relative to the species from the previous group, because they are not strictly related to hypersaline environments. Mesohalophytes exhibit moderately expressed anatomical adaptations, such as well-developed endodermis, aerenchyma, xeromorphic features, or bulliform cells, since they can survive in many environments and are not restricted to salinized habitats. Therefore, salinity is not the major factor inducing anatomical changes in this group, which include some species of *Aster*, *Lepidium*, *Plantago*, or *Trifolium.* Rather, the effect of salinity is influenced by other environmental factors, such as drought, flooding, or humidity. Glycophytes are here also classified as plants that cannot normally grow in saline environments.

Anatomical adaptive characteristics should be considered for incorporation into salt-sensitive species using molecular engineering and transgenic techniques, and should become objects of breeding strategies in order to increase plants' capacity for salt tolerance (Hameed et al. 2009). Improving salt tolerance is especially important for breeding crop plants and cultivated species, which usually express low salt tolerance and are categorized as glycophytes. Increasing the salt tolerance of these plants would bring higher yields in areas with high salt content in the soil. Moreover, domestication of naturally salt-tolerant species as crop plants can have significant economic interest (Flowers et al. 2010). Species that accumulate significant amounts of salt may be used for phytoextraction, phytostabilization of salt-affected soils, and land remediation, potentially allowing nonhalophytes to establish or permitting cultivation of agricultural crops (Milic et al. 2013b). These plants may also accumulate different amounts of heavy metals, more in roots than in aboveground organs, which make them suitable for phytoremediation purposes (Milic et al. 2012).

2.2 Succulence

Some typical halophytes develop succulent structure of vegetative organs. Succulent plants contain in their stems (stem succulents) or leaves (leaf succulents) well-developed water storage parenchyma (Evert 2006). This tissue consists of large, chlorophyll-free cells, full of water. The cells have thin cell walls, sometimes mucilaginous, parietal cytoplasm and large vacuole with more or less mucilaginous content.

Increasing succulence is one of the means to avoid the toxic effect of salt (Abd Elhalim et al. 2016; Rancic et al. 2019). Succulent structure characterizing xeromorphic halophytes is a powerful tool for water retention and storage. It is a consequence of protoplast colloids swelling and volume increasement of water storage parenchyma cells, as a result of greater soluble salts absorption from the soil (Stevanovic and Jankovic 2001). By increasing the water content, succulence helps plants to control internal ion concentration and dilutes ion solution, which prevents reaching toxic ion levels (Dickison 2000). It also aids salt ion storage inside the plant body, due to the presence of large vacuoles in parenchyma cells, which is especially important for plants that have not developed glands as a salt removal strategy (Hameed et al. 2009; Akcin et al. 2017). Succulence also contributes in cellular turgescence and plant's erect position maintenance (Abd Elhalim et al. 2016; Grigore and Toma 2017). In the absence of salts, succulent shoots become thinner, with less developed cortex, pith, and vascular tissues. However, as salinity inhibits cell division, while stimulating cell growth, it leads to increased succulence, which can be an indicator of the maximum possible cell growth. Increased succulence is negatively correlated with leaf area per unit volume. Succulent leaf thickness increases due to an enlargement of mesophyll cells, those in the spongy tissue in particular.

Stomata of succulent leaves are less numerous, and are usually sunken, developing below the level of other epidermal cells (Grigore and Toma 2017). In order to reduce transpiration, the leaves often develop thick cuticle covering epidermal cells (Saadeddin and Doddema 1986). Gas exchange is less efficient: large substomatal chambers and intercellular spaces in palisade tissue are formed. Succulents have short pathway of photosynthates from assimilatory to vascular tissue, which leads to reduced phloem proportion.

Succulents exhibit special affinity for chloride ions, which takes precedence over all other anions. Na^+ is more effective than K^+, and Cl^- is more effective than SO_4^{2-} in promoting succulence (Milic et al. 2013b). Succulence most frequently occurs in members of the family Chenopodiaceae, in which several types have been recorded. The most salt-resistant plants are succulent salt accumulating halophytes, such as *Salicornia*, *Suaeda*, and *Salsola.*

2.3 Secretory Structures

The amount of salt which can be accumulated in the plant body is limited. Many halophytes have special secretory structures on the surface of their aboveground organs, with the main function of salt exudation from the plant body (Dickison 2000). This group of plants is called crynohalophytes and can develop on soils characterized by varying salt content. To maintain low salt content in their tissues, crynohalophytes excrete excess salt, thus preventing the plant from reaching toxic ion levels. This is an important adaptive and ecological strategy, since it enables regulation of ion concentration in plant organs. Ions are absorbed from the soil, transported via xylem, and eliminated unchanged from the plant by secretory structures. The chemical composition of the secreted salt strongly correlates with that of the soil salt. Available evidence indicates that NaCl is the most abundant salt secreted, comprising more than 66% of total secreted salts (Salama et al. 1999).

Vegetative organs of crynohalophytes do not have succulent structure, which means that salt secretion and succulence never coexist in the same plant species. Secretion occurs via salt glands and salt bladders (Evert 2006). Hydathodes can also excrete salt ions diluted in water, while smaller amounts of salts can be eliminated through the cuticle, or sent back to the root by their translocation to phloem.

Salt glands are the most important type of secretory structures, as they play a crucial role in salt elimination outside the cells and the plant body. They have been found in 11 dicot families, as well as in family Poaceae. Significant variability has been noted in their structure, number and arrangement of cells and secretion mechanism. They remove ions from underlying cells and actively secrete them on the leaf surface. No direct connection between salt glands and vascular bundles has been established. Poaceae possess the simplest glands, composed of a basal and a cap cell. The cap cell is covered with cuticle, which detaches from the outer cell wall under the pressure of excretes, and forms collecting chamber. Salty water is eliminated from the cell through the cuticle pores. Salt glands of dicotyledonous species have more complex structure. They are multicellular, composed of basal (collecting) and secretory cells. Salt ions are transported from mesophyll cells via the symplastic pathway all the way to the collecting cells. They transport ions to the upper secretory cells via plasmodesmata, which exude water with diluted salts through cuticle pores to the atmosphere. Such glands are present in plants of the family Plumbaginaceae (*Limonium*, *Plumbago*) (Grigore and Toma 2017). In plants that have not developed nonglandular trichomes, but live in arid environments, salt secretion on the leaf surface has an important ecological role. White salt coating is adaptation to drought, as it prevents water loss and reflects sunlight. Formation of glands begins early during leaf development and their differentiation ceases before other foliar tissues are formed. As glandular cells do not have central vacuole, they do not serve salt accumulation purpose.

Salt bladders (vesicular hairs, salt hairs) do not secrete salts directly to the plant exterior, which are consequently accumulated in vacuoles of their bladder cells. They are composed of unicellular or multicellular narrow stalk and a large, highly vacuolated bladder cell, connected via plasmodesmata (Salama et al. 1999; Evert 2006). All cells are covered with thick cuticle, which is thicker on stalk cells. Ions travel from xylem, via symplastic pathway of mesophyll cells, to the terminal bladder cell. There, they are released into the large central vacuole, where ion concentration is higher compared to that in other mesophyll cells. As ions are transported against the concentration gradient, this process leads to energy consumption (Grigore and Toma 2017). After accumulating maximum amount of salt ions in its vacuole, bladder cell collapses, stalk breaks, and salts deposit on the leaf surface. Again, a whitish coating is formed on the leaf surface, which protects the leaf from direct sunlight, reduces the amount of illumination, and protects the plant from the herbivores (de Villiers et al. 1996). Halophytes of the family Chenopodiaceae, such as *Atriplex* species, possess such salt hairs.

2.4 Tracheoidioblasts

Tracheoidioblasts are special, unusual structures, found in some species of Chenopodiaceae euhalophytes (Grigore and Toma 2017). They occur in stem succulents, always in the palisade cell region. They clearly differ in shape from palisade cells, as they are cylindrical, much larger and colorless and are present in a smaller number. Their position is almost always radial, parallel to palisade cells, usually under stomata. These cells typically have thin cell walls, although some lignification might occur. In the species of the tribe *Salicornieae*, they are of cylindrical shape, thick-walled, parallel with the palisade cells, rarely reach epidermis and are not connected to vascular tissue (Keshavarzi and Zare 2006).

There is no consensus in pertinent literature on their ecological and adaptive value. Some authors posit that they have air-storing function (thus referring to them as “air-storing cells”), even though they are not in direct contact with stomata, while others are of view that their role is merely supporting and mechanical. These structures have also been classified as isolated tracheids, as they are located outside vascular bundles. According to some authors, they might be involved in water conduction toward peripheral regions of the plant, or even serve as water storage structure. Saadeddin and Doddema (1986), for example, suggested that dew from the plant’s surface can be transported down the water potential gradient, via tracheoidioblasts, toward water storage tissue cells. Spiral cell wall thickenings provide mechanical support to the cells and prevent their collapse. More recently, Akcin et al. (2017) stated that, in *Salicornia freitagii*, tracheoidioblasts are involved in water level maintenance inside the plant. However, since they occur in succulent plants, which already have well-developed water storage tissue, their role in water storage is debatable. Moreover, they do not seem to be connected to vascular tissues. Mechanical tissues in succulent organs are not prominent, which leads to the conclusion tracheoidioblasts most likely play a mechanical role, especially if they have lignified cell walls. If the plants inhabit frequently flooded habitats, the air-storage function of tracheoidioblasts could also be considered.

2.5 Successive Cambia

Many halophytic species, especially those of the family Chenopodiaceae, have several concentric rings of cambium in their roots or stems (Grigore and Toma 2017). However, this characteristic is also shared by the species unaffected by salt stress and is therefore not typically considered as an important plant adaptation to salinity. Secondary structure of root or stem is attained through the activity of the first cambial ring, as the second cambial ring is formed at a deeper level, out of the pericycle cells. It produces new vascular elements, but also certain amount of parenchymatic tissue. Several new cambial rings can be subsequently formed via phloem parenchyma cell dedifferentiation. All rings remain active during the plant growth stage. Successive cambia do not always form complete rings, but rather patches of cambial cells: consequently, additional phloem and xylem are characterized by radial rows of parenchyma cells inserted within vascular elements. Newly initiated successive cambia in stems of *Suaeda* species lead to the formation of narrower vessels, which provide limited contribution to water conduction, but may play a crucial role during dry periods in saline environments (Shelke et al. 2019).

In terms of adaptations to salinity, additional cambial rings provide supplemental parenchymatic tissue, which alternates with vascular tissue, and can aid in water storage. Increased storage capacity seems to be the most important advantage of plants with this adaptation to salt stress. Moreover, greater organ thickness, more voluminous roots in particular, due to the increased parenchyma tissue volume, contributes to dilution of absorbed salts. It also hinders salt transportation to the aboveground organs.

Four successive vascular cambia have been observed in roots of *Atriplex semibaccata* R. Br. (de Villiers et al. 1996). However, relative proportion of vascular tissue decreases with increased salinity, causing disruption in vascular tissue arrangement. Successive cambia have also been reported in *Salicornia freitagii*, *S. europaea* L., *Suaeda salsa* L. (Pall.), and *Atriplex tatarica* Aellen (Akcin et al. 2017).

2.6 Kranz Anatomy

Plants have developed diverse physiological and biochemical mechanisms, in order to survive under high salinity conditions (Akcin et al. 2015). These processes are tightly connected with and dependent on morphological and anatomical characteristics. Photosynthesis, for example, would be critically compromised on extremely saline habitats in the absence of specific adaptations. Salinity induces photosynthetic rate reduction, due to reduced stomatal conductance or inhibition of biochemical processes (de Villiers et al. 1996).

C_4 photosynthesis evolved from C_3 type, improves photosynthetic efficiency, and minimizes the water loss in dry environments, which is an extremely important adaptation characterizing halophytes (Kadereit et al. 2014). C_4 plants have higher water-use efficiency and are capable of maximizing carbon gain per unit of water loss (Safiallah et al. 2017). The ratio of the size of palisade tissue cells and water storage tissue is a very important feature in C_4 plants. Biochemical processes of light and dark photosynthesis phase are separated in C_4 photosynthetic plants due to the presence of a special leaf structure, denoted as Kranz leaf anatomy (Grigore and Toma 2017). Its main characteristic is the existence of two separate photosynthetic parenchyma tissues in the leaf. The first one is mesophyll chlorenchyma tissue (photosynthetic carbon assimilative – PCA), where atmospheric CO_2 is assimilated and transformed into C_4 acids. The second one comprises of bundle sheath cells (photosynthetic carbon reductive – PCR) to which C_4 acids are transported. In these cells, C_4 acids become decarboxylated, and the obtained CO_2 returns to mesophyll cells to be assimilated. The processes in bundle sheath cells do not cease even when stomata are closed, which increases productivity during unfavorable conditions (Rancic et al. 2019). Chlorenchyma tissue surrounds vascular tissues in the form of ring and creates specific Kranz type of leaf anatomy. Central part of the leaf is thus occupied by vascular, as well as water storage parenchyma tissue.

Family Chenopodiaceae has the largest number of C_4 species (Voznesenskaya et al. 2007). Not all species having Kranz anatomy are halophytic, since this is an adaptation of plants not only to increased salinity, but also to dry and hot environments. Several types of Kranz anatomy have been described, mostly on the basis of differences in position and amount of water storage tissue, position and orientation of vascular bundles, presence of hypodermis, and arrangement of chlorenchyma and its relation to vascular bundles (Kadereit et al. 2014). Fourteen types are described in extant literature, the most common of which are atriplicoid, kochioid, suaedoid, and salsoloid. In the atriplicoid type, chlorenchyma cells form continuous sheath around the vascular bundles but are sometimes absent from the abaxial side. Kochioid type is characterized by chlorenchyma tissue adjacent to xylem of peripheral bundles. Continuous sheaths are never formed. Both suaedoid and salsoloid types have continuous rings of inner chlorenchyma at the peripheral part of the leaf. In suaedoid type, vascular bundles occupy central position and are embedded in water storage tissue, without having direct contact with chlorenchyma. In salsoloid type, some smaller vascular bundles are positioned beneath internal chlorenchyma, while large vascular bundles are more centrally situated in water storage parenchyma.

2.7 The Xeromorphic Nature of Adaptations in Halophytes

Soil salinity and soil moisture exert formative effect on halophytes' anatomical structure (Grigore and Toma 2007). In the absence of water, salt accumulates in the cells, which may be deleterious to plant survival. High soil salinity induces osmotic stress, because sodium and chloride ions are biologically aggressive osmolytes due to their small diameters, high surface charge densities and strong water affinity (Schulze et al. 2005). Stress caused by salinity thus manifests as both dehydration and ionic stress, due to which plants subjected to physiological drought develop xeromorphic adaptations in order to maintain optimal water balance. Some authors consider that halophytes should be regarded as a special form of xerophytes, since many adaptations found in halophytes also occur in xerophytes and vice versa and have classified such species in a separate group, assigning them to xerohalophytes or haloxerophytes (Grigore et al. 2014; Grigore and Toma 2017). For these plants, which have to survive under joint salt and drought stress, managing water uptake, transport, and storage is crucial for survival. Such plants must possess higher osmotic potential relative to that of the soil, which is achieved through salt retention within cells, mostly vacuole (Schulze et al. 2005).

Morphological, but also anatomical changes, in the leaves of xerohalophytes play an important role in water loss prevention. Changes in the structure and thickness of dermal tissue are among the most common xeromorphic adaptations of halophytic species. Xerohalophytes usually develop thick epidermis, covered with thick cuticle, or with densely distributed protecting trichomes (Rancic et al. 2019). These adaptations lower the transpiration rate and protect the plant from heating and insolation, thereby preventing excessive water loss.

In most of the monocot species, mainly those of family Poaceae, bulliform cells are present in leaf epidermis (Evert 2006). They are not a specific adaptation of halophytes, but bring some advantages to plants growing under salt stress. These cells are much larger compared to the other epidermal cells, contain large amount of water and are organized in bands along the leaf. The cells have thin radial walls, although outer walls may be as thick as the walls of adjacent epidermal cells. Besides their function in water storage, it is hypothesized that due to changes in turgor, these cells play a role in the hygroscopic opening and closing of leaves. During dry periods, they lose water faster than the other epidermal cells, as well as lose turgor, decrease in size, and induce leaf rolling. In rolled leaves, stomata stay hidden and protected, and transpiration rate declines. Consequently, bulliform cells have a crucial role in prevention of water loss through transpiration and leaf overheating, while also increasing water-storing capacity (Hameed et al. 2009, 2012). Leaf rolling is a feature common to many xerophytic species of families Poaceae, Juncaceae, and Cyperaceae, but is also found in halophytic species exposed to both drought and salinity stress (Grigore et al. 2010).

In xerohalomorphic leaves, number of stomata is usually reduced, or stomata are of significantly smaller size compared to plants favoring habitats characterized by high moisture. They are often sunken, or covered with protective trichomes, with relatively large stomatal chamber. This allows plants to regulate water loss intensity and reduce the transpiration rate. Presence of calcium increases resistance to salinity and reduces its effect on stomatal conductance due to which transpiration reduction becomes less important. Calcium also induces the formation of crystal druses, which are calcium oxalate crystals, also involved in salt stress tolerance by aiding in ionic balance maintenance (Abd Elhalim et al. 2016; Safiallah et al. 2017).

Well-developed sclerenchyma tissue is another very common xeromorphic characteristic in halophytes. Sclerenchyma also occurs in the form of sclereid idioblasts of different sizes and shapes, as in the leaf mesophyll of *Limonium* species (Zoric et al. 2013). Low water amount induces low turgor pressure in plant cells, which compromises plant's mechanical strength. Well-developed sclerenchyma and presence of sclereids give plants additional strength and help in maintaining upright position. Xerohalophytes are also characterized by a highly developed lignified vascular tissue, which provides good additional vascular support (Grigore and Toma 2007; Abd Elhalim et al. 2016).

Development of succulent structure is also considered a xerohalomorphic feature. Presence of well-developed water storage tissue helps plant to cope not only with salinity, but also with lack of water. According to Abd Elhalim et al. (2016), succulence, presence of trichomes and crystals, sunken stomata, well-developed sclerenchyma tissue, and formation of cylindrical, unifacial leaves are morpho-anatomical adaptations to drought, salinity, and heat stress.

3 Adaptations of Halophytes in the Southern Pannonian Plain Area (Northern Serbia)

Two types of saline habitats occur in Europe: coastal (maritime) and inland (continental) salines, both composed of specific halophytic plant communities (Dajic Stevanovic et al. 2019). Vegetation of inland salines is herbaceous, developed on soils containing sodium salts originating from soil sediments, saline underground water, or accumulation of salts. Vegetation is composed of a relatively small number of species, and floristic diversity is negatively correlated with soil salinity. Inland salines are distributed across arid and semiarid areas with low precipitation (Miljković 1972). In such conditions, evaporation rate exceeds that of precipitation, which leads to accumulation and deposition of inorganic salts. During summer, owing to greater aridity and evaporation, the groundwater, which contains dissolved salts, moves upward, and the salts accumulate in the topsoil (Dajic Stevanovic et al. 2019). Besides climate conditions, relief, hydrology, presence of underground water and irrigation also affect saline formation.

European inland salines are located mostly in Pannonian Basin and their area decreases toward Southern Europe (Dajic Stevanovic et al. 2019). They form discontinuous patches and occur in the Balkans and Southeastern Europe in the form of isolated saline sites. Habitat fragmentation increases their fragility, while agricultural practices, eutrophication, and ruderalization may lead to their endangerment, resulting in extinction of rare halophytic species and spreading of invasive ones. Several sources of salt have been identified in the Pannonian part of Serbia (Miljković 1972). Ground and underground water originating from salty, maritime sedimentary rocks are considered as the main source of salt in the Pannonian plain soils. Sodium salts stem from decomposition of soil sediments, saline underground water, or accumulation of salts in soil sinks (Dajic Stevanovic et al. 2019).

Saline soils in the Pannonian part of Serbia are of solonchaks, solonetz, and solod type (Fig. 1) (Miljkovic 1972). Their evolution is based on three main processes. Solonchaks are formed during the process of salinization. They are characterized by saline water floods in the spring, and dry summers during which white soil crusts are formed at the soil surface (Dajic Stevanovic et al. 2019). They usually form mosaics and represent relatively small area in Europe. Solonetz are characterized by high Na^+ ion concentration and are formed during the process of alkalization (Miljkovic 1972). In spring, these soils are saturated, and sometimes shallowly flooded, by soluble carbonates (Dajic Stevanovic et al. 2019). During summer they become dry, whereby cracks form at the surface. Among the other soluble salts, sodium and potassium carbonates dominate in this soil type. Solods are soils formed by desalinization and dealkalization of saline soils.

Eco-anatomical investigations of halophytes of continental salines in the Pannonian part of Serbia began in the 1980s and have continued until the present day (Janjatovic et al. 1989, 1990, 1991, 1992, 1995; Kneževic et al. 1996, 1998; Polic et al. 2009; Milic et al. 2011, 2012, 2013a, b; Zoric et al. 2013). These investigations have provided valuable information on the floristic and vegetation

Fig. 1 Autumn aspect of some saline habitats in the Pannonian part of Serbia. (**a**) Slano Kopovo; (**b**) Rusanda lake; (**c**) Okanj. (Photo by D. Milic and J. Lukovic)

composition of salines, based on detailed analyses from ecological perspective. Several species, mostly euhalophytes, have been recorded to date and their anatomy is adequately described. The anatomy of their vegetative organs was analyzed using light and scanning electron microscopy. Plant nomenclature is given according to the valid checklist (The Plant List 2013).

3.1 *Salicornia europaea* L.

Salicornia species are among the most salt-tolerant land plants. They have aphyllous, succulent stems, and the adaxial side of the leaves is fused with the stem (Keshavarzi and Zare 2006; Milic et al. 2011). Although they lack easily recognized taxonomic characters due to their reduced morphology, they do express considerable phenotypic variation at the population level.

Several adaptations to high salinity have been developed in this species. The succulent shoots have rounded cross-section, with single-layered epidermis, covered with rugose cuticle (Fig. 2) (Milic et al. 2011). The outer part of shoot cortex is differentiated into 2–3 layered palisade tissue, comprising of elongated, densely arranged cells, under which water storage parenchyma is located. A circle of leaf vascular bundles is present between palisade and water storage tissue. Tracheoidioblasts, with characteristic spiral thickenings, protrude into the palisade tissue. Uniseriate endodermal cells lack Casparian strips and occasionally contain starch grains. Vascular bundles, with well-developed sclerenchyma, are arranged in a circle, beneath single-layered pericycle. More separate fibers are present in the pericyclic zone. In the shoot center, small cavity occurs, surrounded by pith parenchyma.

These plants exhibit very low water potential under normal growth conditions, which allows them to absorb water from very salty soils or seawater (Ellison et al. 1993). Within the shoots, regions with significantly decreased xylem vessels diameter have been detected, which hinder fluid flow and are thus considered as adaptations to water stress. In *Salicornia*, vessel diameter decreases only locally, and the vessel is equally wide on both sides of the constriction, which might be the consequence of axillary bud development.

Fig. 2 Cross-sections of *Salicornia europaea.* (**a**) Shoot; (**b**) endodermis and pericyclic fibers; (**c**) tracheoidioblasts

3.2 *Suaeda maritima* (L.) Dumort

Suaeda maritima is a succulent saltmarsh euhalophytic species, tolerant to high levels of salinity, found in Europe on sea coasts and inland salines (Polic et al. 2009). Its stem has single-layered epidermis. Cortex is composed of parenchyma cells, as well as chlorenchyma and collenchyma strands, which radially alternate (Fig. 3). Central cylinder has well-developed sclerenchyma and vascular bundles arranged in a circle. However, plant maintains its upright position primarily due to the cell turgor, rather than mechanical tissue. Pith parenchyma is composed of large cells.

Leaves have typical halomorphic structure. Cross-sections are rounded to elliptical, with large epidermal cells and slightly sunken paracytic stomata. Isolateral leaves are composed of subepidermal palisade tissue comprising of 2–3 rows of elongated cells, and central water storage tissue. The main vascular bundle is centrally positioned, while other bundles become progressively smaller toward the leaf tip. Succulent leaves of this species have large mesophyll cells in which the vacuoles, where Na+ is accumulated, occupy 77% of the area (Hajibagheri et al. 1984). This species has austrobassioid type of leaf anatomy, since it has less than 30% of water storage tissue and no special chlorenchymatous sheath (Polic et al. 2009).

3.3 *Salsola soda* L.

Salsola soda is native to Eurasia and North Africa. It is endangered species in Serbia, limited to saline areas in the north, in Backa and Banat region (Milic et al. 2013a). Stem cross-sections are round to elliptical, with monolayered epidermis (Fig. 4). Single layers of collenchyma and chlorenchyma are located subepidermally, followed by several layers of cortex parenchyma cells. Vascular bundles are arranged in a circle in the central cylinder, surrounding compact pith parenchyma. Leaves have succulent halomorphic structure of Kranz type. Epidermis is single-layered, covered with thick cuticle, with paracytic, slightly sunken stomata on both leaf surfaces. Hypodermal cells contain chloroplasts and calcium oxalate crystals. Uniseriate palisade tissue is located between hypodermis and chlorenchymatous

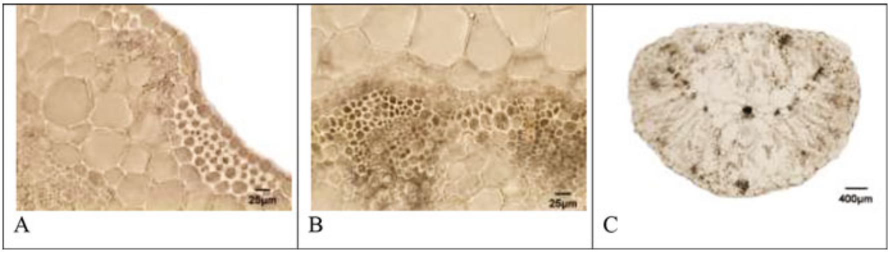

Fig. 3 Cross-sections of *Suaeda maritima*: (**a**) stem, primary cortex; (**b**) stem, vascular tissue; (**c**) leaf

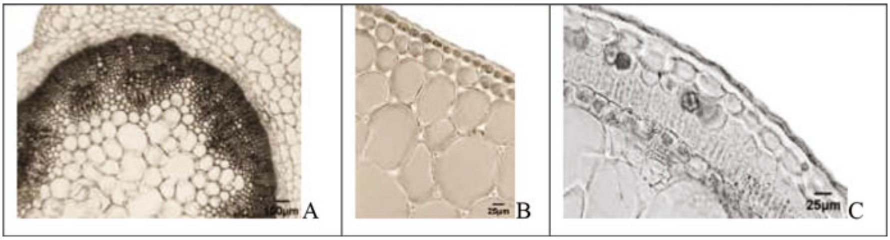

Fig. 4 Cross-sections of *Salsola soda*: (**a**) stem, central cylinder; (**b**) stem, cortex; (**c**) leaf

bundle sheath. Spongy tissue expands to water storage parenchyma, composed of large thin-walled cells, those adjacent to bundle sheath often containing crystals. The main vascular bundle is situated in the middle of the leaf, whereas peripheral bundles are located next to the bundle sheath.

The species has developed halomorphic and xeromorphic anatomical adaptations. Stem cortex parenchyma cells are generally large, and vascular bundles have well-developed sclerenchyma above them. Leaf epidermis has thick cuticle and slightly sunken stomata. Presence of crystals in hypodermis and well-developed water storage parenchyma are adaptations which aid in ion balance regulation. Kranz leaf anatomy is of salsoloid type, with continuous ring of chlorenchyma at the periphery, smaller vascular bundles beneath internal chlorenchyma and one vascular bundle centrally positioned in water storage parenchyma, which improves photosynthetic rate and minimizes water loss.

3.4 *Limonium gmelinii* (Willd.) O. Kuntze

Limonium gmelinii, its Pannonian endemic subspecies *hungaricum* (Klokov) Soo, inhabits continental, xerothermic halobiomes in Pannonian plain (Zoric et al. 2013). Stem epidermis is composed of isodiametric cells, covered by a thick cuticle (Fig. 5). The cortex consists of several layers of parenchymatic cells, those of hypodermic layer being more radially elongated, like palisade cells. Central cylinder begins with massive sclerenchyma ring, composed of thick-walled fibers. Vascular bundles form three rings. Small peripheral bundles are outside the sclerenchyma ring, or maintain contact with it via their xylems. The second ring is composed of larger bundles which are completely embedded in sclerenchyma. The third ring is more centrally positioned, and comprises the largest bundles, having phloem incorporated in sclerenchyma and xylem in pith parenchyma. Peripheral pith parenchyma cells have lignified walls.

Halomorphic adaptations are particularly pronounced in leaves, which have uniseriate epidermis, composed of isodiametric, relatively large cells with flat walls. Stomata of anisocytic type occur on both sides and are more numerous adaxially. The leaves have dorsiventral structure, with mesophyll comprising of 2–3 layers of palisade and 5–6 layers of spongy tissue cells. The main vein is prominent

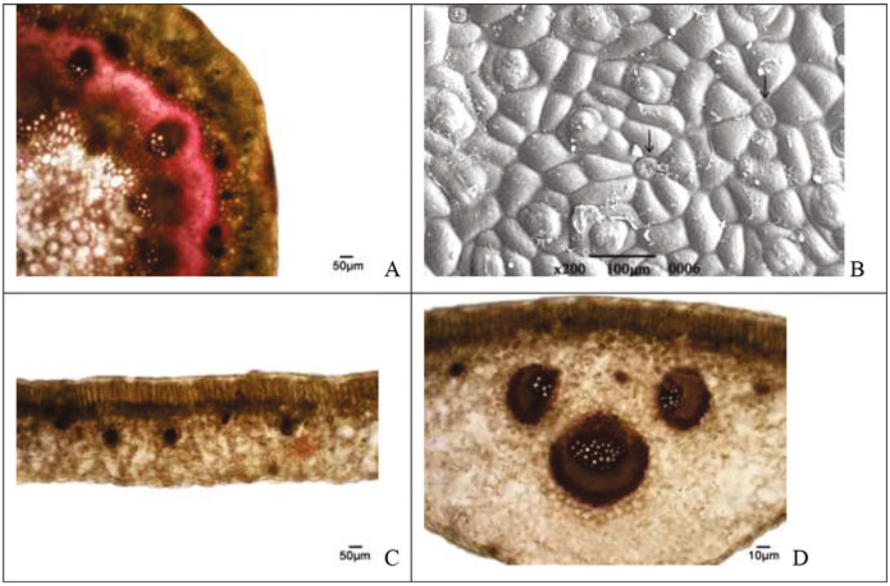

Fig. 5 Cross-sections of *Limonium gmelinii* subsp. *hungaricum*: (**a**) stem; (**b**) adaxial leaf epidermis with salt glands; (**c**) lamina; (**d**) lamina main vein

abaxially, with a layer of collenchyma on the abaxial side, and contains 4–6 vascular bundles, completely surrounded by sclerenchyma and a layer of parenchyma sheath cells containing starch grains.

A combination of halomorphic and xeromorphic anatomical adaptations has been recorded in this species. As crynohalophyte, it has developed salt glands for excretion of salt on both leaf surfaces, composed of 16 cells (four excretory cells arranged in a circle, having secreting pores, four internal cup-cells and two circles of four collector cells). The leaves have thick cuticle and a small number of stomata. Epidermal and mesophyll cells are rather large. Due to strong habitat insolation, it has well-developed palisade tissue, composed of elongated cells with the length/width ratio of 7.1. Branched, lignified sclereids occur in the mesophyll, together with well-developed mechanical tissue.

3.5 *Tripolium pannonicum* (Jacq.) Dobrocz.

Tripolium pannonicum is a widely distributed halophytic species (Karanovic et al. 2015). Its stem is characterized by rounded cross-section (Fig. 6). The epidermis consists of tiny cells with thickened walls, covered with a relatively thick, wrinkled cuticle, without trichomes. The cortex is composed of alternating collenchyma and chlorenchyma. Collenchyma is less developed and is located in the stem recesses, while chlorenchyma is loose and present in the ribs. Vascular bundles are arranged in one circle within the central cylinder. Secretory ducts have been observed in the cortex above the phloem. The central part of the stem is filled with the large pith

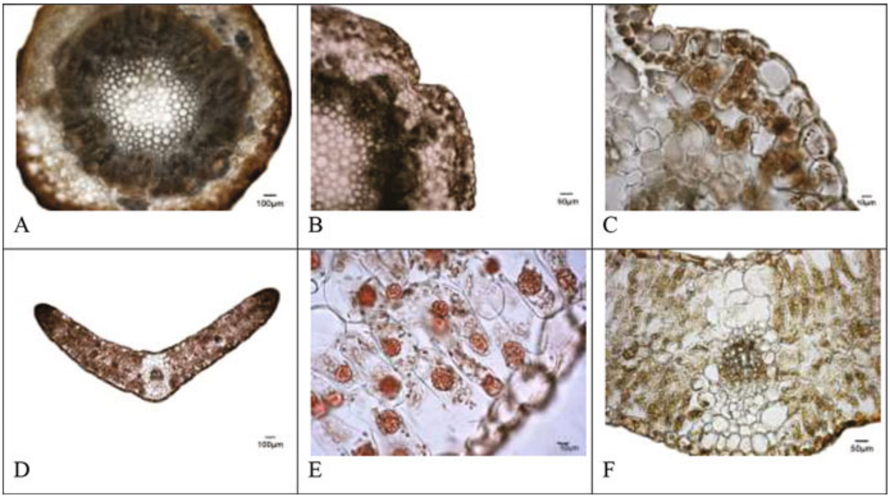

Fig. 6 Cross-sections of *Tripolium pannonicum.* (**a**–**c**) stem; (**d**) leaf; (**e**) oil bodies in palisade tissue cells; (**f**) main vein

parenchyma cells. Well-developed cortex is a halophytic characteristic that provides certain protection in the early stages of vascular tissue development, and the presence of aerenchyma is a characteristic of halophytic species, which explains the appearance of loose tissues in this species – chlorenchyma in the stem and palisade in the leaf blade.

The leaves have monolayered epidermis, with ribbed thickenings on the external periclinal walls. The leaf blade is glabrous and amphistomatous, with a small number of relatively large anomocytic stomata, leveled with the epidermis. It has an isolateral structure. Palisade tissue consists of large cells, rich in chloroplasts, arranged in 2–3 and 1–3 layers, on the adaxial and abaxial side, respectively. Large, individual oil bodies with a granular structure are clearly observed in the palisade tissue cells. Vascular bundles, surrounded by parenchymatous sheath, are arranged in a row. In the main vein, one vascular bundle is present, with strongly developed sheath extending to both epidermises. Schizogenous secretory ducts, with lumen lined with a layer of secretory epithelial cells, are present above the phloem of larger bundles. Anatomical features, such as epidermis without protective structures, and a smaller number of unprotected stomata leveled with the epidermis, are associated with mesomorphic structure, while large epidermal and palisade tissue cells indicate the halomorphic nature of this species.

3.6 *Lepidium cartilagineum* (J. Mayer) Thell.

Lepidium cartilagineum is considered Pannonian endemic species but is also distributed on salinated soils outside the Pannonian plain (Janjatovic et al. 1991). It exhibits xerohalomorphic and heliomorphic structure. Its stem epidermis is composed of thick-walled cells, covered with thick cuticle. Cortex is parenchymatic, with well-defined endodermis. Central cylinder is highly sclerenchymatous, with numerous vascular bundles. Sclerenchyma is present above vascular bundles, as well as in wide rays between the bundles, forming, together with xylem, wide mechanical ring. The pith is parenchymatic. Leaf epidermis is composed of large cells of nonuniform size that have water storage function. It is covered with cuticle, wax grains, and sporadic trichomes. Leaf has isolateral structure, with well-developed palisade tissue comprising 70% of the mesophyll, where sclereids are found.

3.7 *Plantago schwarzenbergiana* Schur.

Plantago schwarzenbergiana is a Pannonian endemic species which inhabits moderately salinated soils and has thus developed moderate halomorphic structure (Janjatovic et al. 1990). Its leaves are dorsiventral, with one layer of epidermal cells with thickened outer walls, covered with cuticle and small number of trichomes. Palisade tissue is composed of 2–3 cell layers, covering 4–5 layers of spongy cells. Vascular bundles are protected by sclerenchyma and endodermal sheath and contain narrow-lumen vessels. In peduncle, hypodermis composed of thick-walled cells can be found, and well-developed endodermis, surrounding central cylinder. Continuous ring of sclerenchyma surrounds vascular bundles. Root has strongly developed protective, multilayered exodermis. Cortex contains large radial intercellulars, as an adaptation to low soil aeration. Development of thick-walled tissues (protective, endodermal, and mechanical), together with narrow vessels, enables these plants to survive in moderately salinated soils.

3.8 *Artemisia maritima* L.

Artemisia maritima inhabits saline habitats in Pannonian plain but has wide ecological distribution (Janjatovic et al. 1989). On saline soils, its growth is compromised, and its leaves are smaller and thicker, while its stem mechanical tissue is better developed. Stem is ribbed and contains epidermal cells with thickened outer walls, covered with cuticle and trichomes. Stomata are present in sinuses between the ribs. Collenchyma is present in the ribs, while chlorenchyma is found between the ribs, subepidermally. Strong lignification in central cylinder is obvious. Sclerenchyma occurs above vascular bundles, and the ray cells between the bundles have thickened, lignified walls which, together with xylem composed of small-lumen vessels, form continuous mechanical ring. Leaves are isolateral, with epidermis covered with thick cuticle, T-shaped trichomes and glandular trichomes. Stomata are mostly hidden in leaf invaginations between the ribs. Palisade cells are distributed in 3–4 layers adaxially and 2–3 layers abaxially, with large water storage parenchyma cells located between them. Main vein parenchyma contains secretory ducts. Vascular tissue is well-developed, with small-lumen vessels. All these xerohalomorphic adaptations enable this species to survive dry conditions in saline environment.

3.9 *Atriplex littoralis* L.

Atriplex littoralis is obligatory halophytic species, distributed in Southwestern and Central Asia and continental and maritime halobiomes in Europe (Janjatovic et al. 1995). It is a characteristic species of meadow steppe vegetation of continental halobiomes in Pannonian plain. Its stem is rounded in shape, with four strong and four less expressed ribs (Fig. 7). Epidermis is monolayered, with thick cuticle. Cortex is relatively thin, composed of collenchyma tissue present in the ribs and 3–4 layers of small chlorenchyma cells. Starch sheath cells are large, tangentially elongated. In the central cylinder, phloem and xylem are arranged in a ring, with small groups of sclerenchyma above phloem. Centrally positioned parenchyma cells sometimes contain

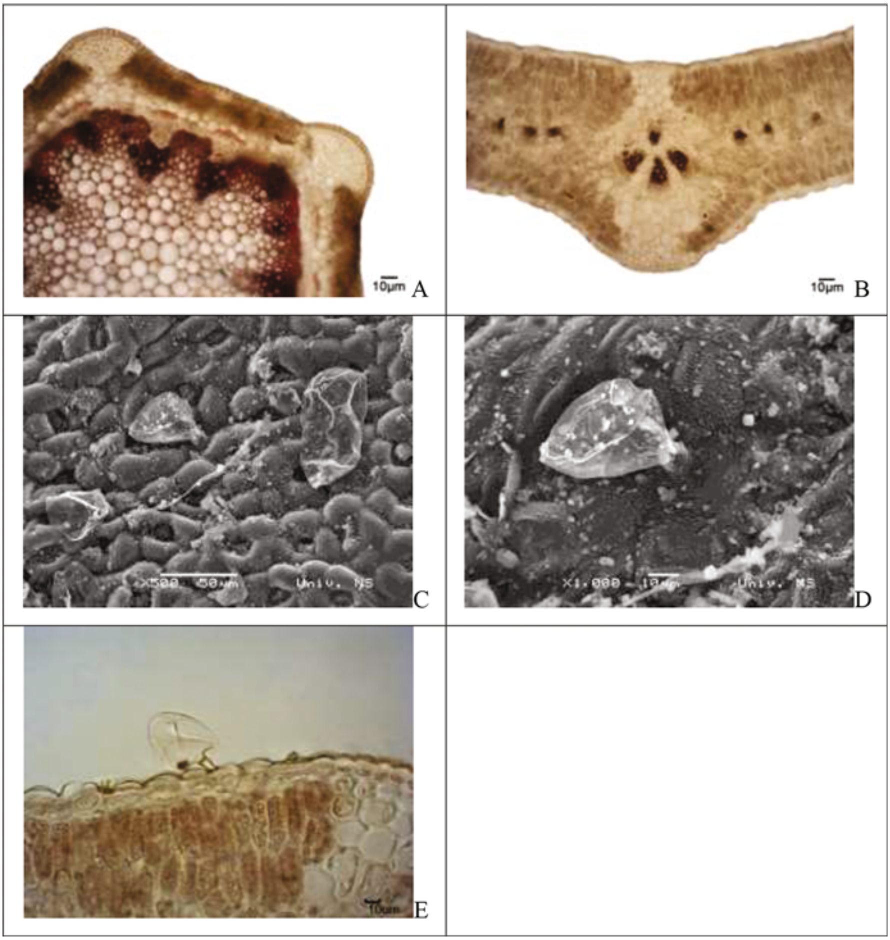

Fig. 7 Cross-section of *Atriplex littoralis*. (**a**) stem; (**b**) lamina main vein; (**c**–**d**) SEM of leaf adaxial epidermis with salt bladders; (**e**) salt bladder

crystal druses. Leaves are thick, isolateral, with epidermis composed of relatively large cells, covered with thick cuticle, and slightly sunken stomata. Salt bladders, composed of a short stalk cell and a large bladder cell, aid in salt elimination through abruption. The bladders are mostly present on young leaves, and abrupt in older. Mesophyll is composed of large cells – three layers of palisade cells on both leaf sides with two layers of spongy cells between them. A certain number of mesophyll cells contain crystal druses or prismatic calcium oxalate crystals. Small vascular bundles are surrounded by large parenchyma sheath cells. Main vein contains 1–4 vascular bundles, surrounded by parenchyma sheath, which extends to both epidermises. Sheath extensions are composed of large cells which have water storage function. This is C_4 atriplicoid type of leaf structure, an important ecological adaptation of *Atriplex* species, where chlorenchyma forms continuous sheath around the vascular bundles.

Very important halomorphic adaptation of this species is the presence of salt bladders, as the main tool for salt elimination from vegetative organs. Moreover, anatomical adaptations of leaves, such as their increased thickness, isolateral structure, thick cuticle, well-developed palisade tissue, and presence of water storage tissue, help plants to survive in dry, saline conditions.

3.10 *Puccinellia limosa* (Schur) Holmb.

Puccinellia limosa is widespread in Central and Southeastern Europe, facultatively present on saline habitats, while in Serbia, it is obligatorily distributed on saline soils of Pannonian region (Knezevic et al. 1998). Stem cross-section is round to oval, with central cavity. Subepidermally, a continuous ring of sclerenchyma is present, with assimilatory tissue embedded in it. Outer vascular bundles are smaller, embedded in the sclerenchyma ring, whereas inner bundles are larger, usually distributed in two circles in parenchyma tissue.

Puccinellia limosa has typical festucoid leaf, with more or less pronounced ribs on adaxial side, while bulliform cells are located at the bases of the furrows. Sunken stomata and numerous papillae are present on both surfaces, whereas short microhairs appear only adaxially. Abaxial surface is more exposed to environmental influences, with better developed chlorenchyma consisting of 2–3 layers of palisade-like cells, which perform most of the photosynthesis processes. The reminder of the mesophyll is filled with rounded parenchyma cells, containing fewer chloroplasts along with numerous oil droplets. Vascular bundles are placed closer to abaxial side, surrounded by a double sheath, with the inner one serving mechanical and the outer one parenchymatous function. Larger vascular bundles have sclerenchyma strands, forming columns of thick-walled cells. Among those anatomical characteristics, leaf scleromorphic structure is the strongest adaptation of this species to dry, saline soils.

3.11 *Rorippa kerneri* Menyh.

Rorippa kerneri is spring ephemeroid, facultative halophyte with somewhat succulent leaves, and xeromorphous stem (Knezevic et al. 1996). Stem is rounded to oval in cross-section, sometimes with six weakly expressed ribs (Fig. 8). Large epidermal

Fig. 8 Cross-sections of *Rorippa kerneri.* (**a**) stem; (**b**) leaf

cells are covered with thick cuticle. Cortex is thin, with peripherally positioned chlorenchyma. Collateral open vascular bundles, arranged in a circle, have groups of sclerenchyma adjacent to the phloem. Parenchyma cells between the bundles have strongly lignified walls. Together with xylem elements, they form continuous, thick mechanical ring. Parenchyma pith cells are centrally positioned.

Lobed lamina is composed of relatively large cells. Single epidermal cells at lamina margins, as well as the groups of cells at the midrib, are larger than the other epidermal cells and probably conserve water. Mesophyll is composed of three layers of large palisade and 3–4 layers of similarly large spongy tissue cells. Closer to the abaxial side, small vascular bundles, surrounded with sheath and accompanied by small groups of sclerenchyma, are present.

Voluminous leaves, large cells, and weakly developed mechanical and vascular tissues are characteristics of succulent halomorphic adaptations. Stem xeromorphic features, such as small diameter, well-developed cuticle, mechanical and vascular tissue, as well as small cortex and cylinder cells, help plants to survive in dry conditions on saline habitats.

3.12 *Hordeum hystrix* Roth.

Hordeum hystrix is facultative halophytic species, but in Serbia present only on salines, with soils characterized by low salinity. Stem epidermis is monolayered, with sclerenchyma ring present subepidermally, composed of 2–4 layers of cells, and containing groups of imbedded chlorenchyma cells and small vascular bundles (Nedeva and Vutov 1981). Large vascular bundles are distributed in a circle, embedded in parenchyma tissue surrounding large central cavity. The leaf is of festucoid type, with prominent bulliform cells. Numerous short and several long unicellular microhairs are present on both epidermises. The leaf has 15–21 ribs, each containing a vascular bundle, surrounded by double – mechanical and parenchymatic – sheath. Sclerenchyma is presented adjacent to the bundles, extending to both epidermises. The rest of the mesophyll is occupied by chlorenchyma. Anatomical adaptations of this species, tending toward drought tolerance (such as scleromorphic leaf structure and strong stem sclerification), enable these plants to survive on low salinated soils.

3.13 *Camphorosma annua* Pall.

Camphorosma annua is a species well adapted to sunny, thermophilic, continental halobiomes, which occurs in Central and Eastern Europe, but is in Serbia only found within moderately salinated parts of Pannonian region (Janjatovic et al. 1992). Stem has one layer of epidermal cells with thickened outer walls, covered with thick cuticle and long nonglandular trichomes. Narrow zone of collenchyma, parenchyma, and endodermis comprise thin cortex. Collateral open vascular bundles, with groups of sclerenchyma above the phloem, form a ring in the central cylinder. The leaf has succulent structure. Relatively large epidermal cells are covered with thick cuticle and have long nonglandular and glandular trichomes. Stomata of paracytic type are sunken. Hypodermal layer has water storage function. The leaf has C_4 anatomy of *Camphorosma* type, with one continuous layer of palisade cells and one inner continuous Kranz layer. Each of the inner cells corresponds to 2–3 palisade cells. Numerous small vascular bundles are located beneath chlorenchyma, and large, main vascular bundle is situated in the middle part of the leaf. The reminder of the mesophyll is filled with large water storage cells.

4 Conclusion

Halophytes inhabiting continental halobiomes of Pannonian plain in Northern Serbia have developed a wide range of anatomical adaptations to salt stress, as well as to physiological drought on saline habitats. Their physiological and biochemical adaptive strategies are closely linked to and dependent on their morpho-anatomical characteristics. Salt ion transport interruption commences at the root level, due to specific anatomical barriers along the water transport pathway, such as thick-walled cortex cells and endodermal cells with Casparian strips. Dilution of absorbed ions, as well as salt deposition is achieved through the development of succulent structure, water storage tissue and voluminous cells, which enhances plant's osmotic potential. Elimination of surplus salt outside of the plant body is facilitated by specific secretory structures or by shedding off plant parts. Halophytes of Pannonian plain exhibit a considerable diversity of anatomical mechanisms, which equip them for successful survival on dry, saline habitats. They have developed succulent structure, Kranz leaf anatomy, and various types of salt secretory tissues, but also some xerohalomorphous anatomical adaptations, including strong lignification and modifications of epidermal and vascular tissue.

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