ECOLOGICAL ANATOMY INVESTIGATIONS RELATED TO SOME HALOPHYTE SPECIES FROM MOLDAVIA

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In this paper the vegetative organs of three halophyte species are investigated, evidencing some histo-anatomical features (aerenchyma, endodermis, bulliform cells, protective and secretory trichomes), which are discussed in relation to their ecological significance.

Key words: halophytes, ecological, endodermis, aerenchyma, trichomes.

INTRODUCTION

A plant is an opened biological system, which is in a continuous "negotiation" with ambiental factors, which have modellating and formative action on the vegetal organism. Related to an ambiental agent, having a normal value, but often modified (higher salinity of soil), the plants (the halophytes) react by some adaptive, histo-anatomical features. Next to this factor, soil salinity which induces a salt stress, others concur to the edification of polymorphic, convergent and multi-factorial stress; this certifies that all the environmental stressors have their importance and place in the ecological description of plants.

We present in this paper, related to the interest in halophyte's structure (Grigore and Toma, 2005), some histo-anatomical data, with ecological implications, regarding a three halophyte species.

MATERIAL AND METHODS

The next taxa were considered: *Juncus gerardi* Loisel. *(Juncaceae* family*)*, *Plantago schwarzenbergiana* Schur. (*Plantaginaceae* family) and *Spergularia media* (L.) C. Presl. (*Caryophyllaceae* family). The first two were collected from Valea lui David-Iași, the latter from Cotnari (Iași) in the summer of 2006. For the purpose of investigation, the material was fixed and preserved in 70° ethyl alcohol. The cross section of the vegetative organs was made using a manual microtome and a botanic razor. The resulted sections were subjected to the "classical" steps of histo-anatomical investigation: 20–30 minutes of sodium hypochlorite, washing with acetic water, then water, then, for one minute coloring with iodine green, wash in 90° ethyl alcohol, coloring with carmine red for 20 minutes, water wash

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and finally glycerol-gelatine fixing. Once the permanent materials were obtained, pictures were taken, using a Canon A95 camera and a Novex (Holland) light microscope.

RESULTS

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 ambiental 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, we could observe the typical secondary structure in the *Spergularia media* root and in the *Plantago schwarzenbergiana* rhizome and root, too. At this architecture, both lateral meristems, cambium and phellogen, play a role.

A common histo-anatomical adaptation for *Juncus gerardi, Spergularia media* and *Plantago schwarzenbergiana* consists of aeriferous cavities. We could evidence them in the cortical parenchyma belonging to the rhizome of *Juncus gerardi* (Figs. 1 and 2), in the primary cortex belonging to the root of *Spergularia media* (Fig. 3) and *Plantago schwarzenbergiana* (Fig. 4).

Another histo-anatomical adaptive feature of halophytes is the endodermis, often well developed. We could evidence the existence of a tertiary endodermis, in the *Juncus gerardi* rhizome (Figs. 1 and 2). Regarding the presence and the relative development of casparian strips, those appear not to be outside ecological signification, as we will see in discussion part.

We evidenced, in the central cylinder of *Juncus gerardi* aerial stem, a thick, sclerenchyma ring (Fig. 5), in which vascular bundles occur, and a sclerenchymatical pericycle, in the outer side (1–2 layers) of the superior level of the stem belonging of *Spergularia media* (Fig. 6), and in median level, the pericycle being almost totally sclerenchymatic and highly lignified. At the latter species, at the inferior level of the stem, the lignification of the sclerenchymatic pericycle and of the xylem is higher.

In the cortical parenchyma of the stem and in the mesophyll of *Spergularia media* we observed cells with calcium oxalate crystals (Fig. 8).

The stem of *Spergularia media*, especially in its superior level, presents multicellular, one-layered trichomes, of various size, having a unicellular, spheric gland, which were described by Chermezon (1910).

Regarding the leaf, as a lateral vegetative organ, we evidenced that the lower epidermis (front size view) of *Juncus gerardi* vagina (Fig. 7) has stomata of biperygen type. At its inferior level, vagina presents: lower epidermis with small,
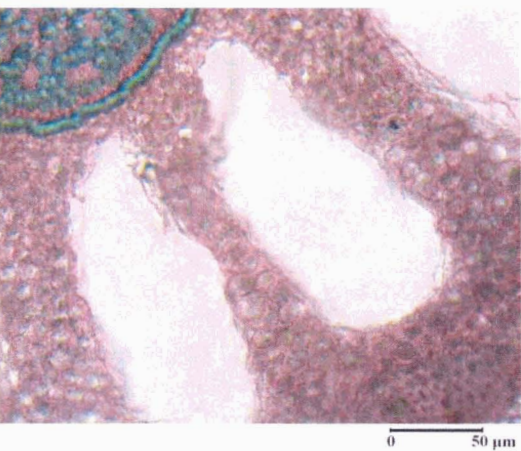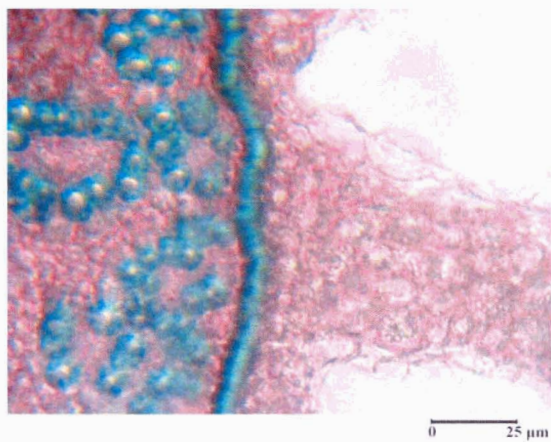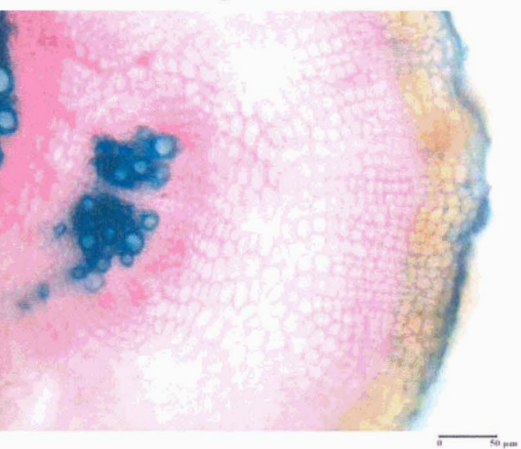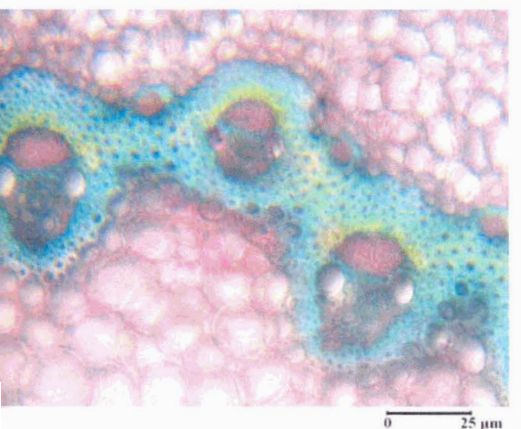
Fig. 1
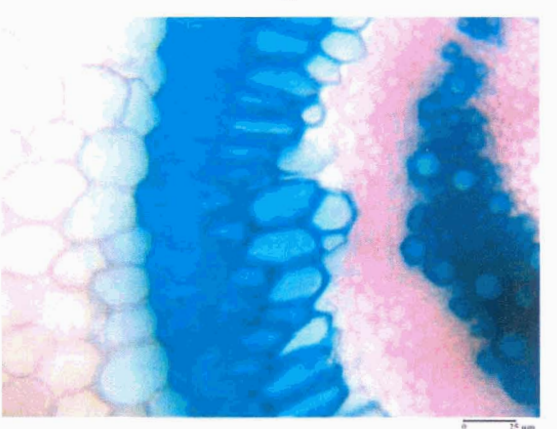

Fig. 2

Fig. 3

Fig. 3

Fig. 5

Fig. 6

Plate 1. Cross section: Figs. 1 and 2: rhizome (*Juncus gerardi)*; Fig. 3: root (*Spergularia media*); . 4: root (*Plantago schwarzenbergiana*); Fig. 5: aerial stem (*Juncus gerardi*); Fig. 6: stem (*Spergularia media*).
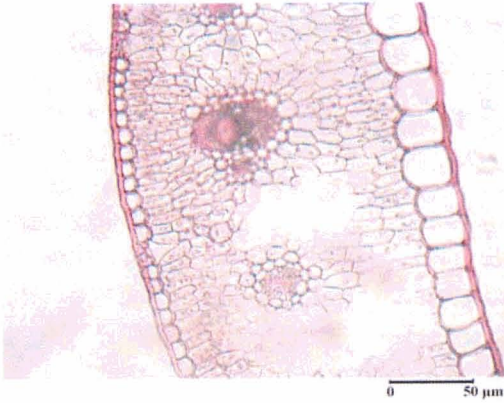
Fig. 7

Fig. 8
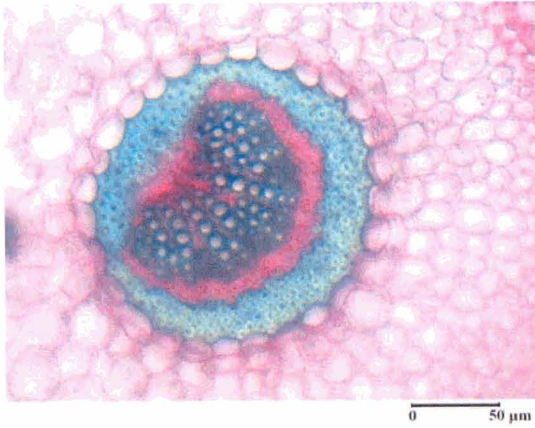

Fig. 9

Fig. 10

Fig. 11

Plate 2. Cross section: Fig. 7: vagina (*Juncus gerardi*); Fig. 8: lamina (*Spergularia media*); Fig. 9: petiole (*Plantago schwarzenbergiana*); Figs. 10 and 11: lamina (*Plantago schwarzenbergiana*).

tangentially elongated cells, having the external wall thicker than the other, being covered by a very thin cuticle; the upper epidermis consists of very large cells, with the external wall slightly thicker than the others. These cells remind us of the bulliform cells of *Poaceae* species.

At *Spergularia media,* both epidermis (in front size view) of the lamina present stomata of diacytic (caryophyllaceous) type; therefore, the lamina is of amphistomatic type (Zarrinkamar, 2001). The mesophyll consists in an almost totally palisade tissue at the superior level (Fig. 8), and is an almost totally lacunous tissue at the inferior one.

In the petiole of *Plantago schwarzenbergiana,* the biggest vascular bundles present at the periphery of the sclerenchyma one layer of parenchymatic cells which have Casparian strips in the radial walls, this layer representing a primary endodermis (Fig. 9), fact unspecified in the literature we consulted. The lamina epidermis (in front size view) presents stomata of anizocytic or anomocytic type and very long, multicellular, one layered protective trichomes (Fig. 11), with a narrowed terminal cell. In cross section, the lower epidermis presents a few secretory, short trichomes (Fig. 10), tri-celled, located in small depressions; some of these have one-celled gland, others bi- or tri-celled gland. The mesophyll consists of a 2–3 layered palisade tissue at the upper epidermis, whose cells are rich in chloroplasts; at the lower epidermis, the cells are shorter, sometimes even of palisade type, but they contain less chloroplasts.

DISCUSSION

The aeriferous cavities in *Juncus gerardi, Spergularia media* and *Plantago schwarzenbergiana* vegetative organs are common features for more halophyte species such as *Spartina* (Waisel, 1972; Anderson, 1974). A similar situation is suggested in the case of mangroves; in this case, the stem cortex of the old plants turns in being used as aeriferous tissue, and these tissues become more developed in the lower level of the stem (Mullan, 1932 quoted by Waisel, 1972). It might be a convergence phenomenon, because plants which have in common salinity and moisture as combined ecological agents may develop similar adaptive strategies. We believe that the existence and the role of these aeriferous lacunae are in agreement with the local environment agents, integrate in a certain ecosystem, where the soil salinity, ant the possibility, at least temporally, of flooding, even waterlogging at the soil level (which increases considerably its moisture) are present. It is known that a saline soil has defectuous ventilation and water circulation – when it is moist, it becomes marshy, and when it is dry it clops (Davidescu, Calancea, Davidescu, Lixandru, Țârdea, 1981).

Therefore, the porosity and permeability for air and water decreased, as a result of some changes in the dispersion of soil's colloids (Milică, 1982). Whether

it is dry or moist, a saline soil offers basically similar conditions for the plants in terms of effects on them. In both cases, the plants suffer from a "physiological drought" (Poljakoff-Mayber, 1975), because Na^+ hydrates more than other ions, which explains why the water retaining is higher even if the saline soil is moist.

Actually, it consists in water deficit ("drought stress") arising from the low (more negative) water potential of the rooting medium (Marschner, 2002). That is why, always, at least partly, a salt stress is a drought stress and vice versa. Moreover, sodium and chlorine are biologically "aggressive osmolytes" inducing, due to their features, a triple stress: a salt, ionic and a dehydrative one (Schulze, Beck, Müller-Hohenstein, 2005). But, the clear presence of the aerenchyma, at least at *Juncus gerardi*, can be explained through the fact that it is a halophyte of wet saline; it is a hygrophilous species, more or less halophilous, intermediate from hygrohalophilous to hygrophilous; it develops in clayed and salinized humic gley soil, with permanently moist soil (Bucur, 1960).

It has a wide ecological exigency, constitutes phytocenosis around the swamps, lakes and marshy areas more or less salinized (Şerbănescu, 1965). In such conditions, when the plant is subjected to waterlogging, for example, to the salt stress, named by us "primary", adds another one, called "secondary", named hypoxic, induced by physico-chemical peculiarities of soil in flooding conditions, often discontinuous ones.

We emphasized, however, that this hierarchy of stress type is rather didactically arbitrary. Actually, it is very difficult to quantize precisely the ponderosity and importance of a certain factor in the delineation of some particular type of stress. Beyond the multiple effects of hypoxic and even anoxic soils on plants (Adam, 1990; Ungar, 1991; Kozlowski, 1997; Marschner, 2002; Schulze, Beck, Müller-Hohenstein, 2005), on which we do not insist (this is not the subject of the present paper), we reveal that the aerenchyma is a histo-anatomical feature of plants in the habitats subjected to flooding conditions (Waisel, 1972; Lawton, Todd and Naidoo, 1981; Justin and Armstrong, 1987; Keiffer, McCarthy, Ungar, 1994; Feller and Sitnik, 1996; Kozlowski, 1997; Evans, 2003). Plants affected by waterlogging are subjected to hypoxia or even anoxia, and store oxygen in aerenchyma of roots or stems (Kozlowski, 1997; Schulze, Beck, Müller-Hohenstein, 2005). Hypoxia and anoxia produce negative redox potentials of the soil and may, finally, shift the pH of the soil into the alkaline range (Schulze, Beck, Müller-Hohenstein, 2005).

Along all these processes, the ethylene intervenes too, as a phytohormone involved in the plant's reaction to stress. This induces, *via* a so-called "programmed cell death" (PCD) (Evans, 2003; Schulze, Beck, Müller-Hohenstein, 2005), the formation of aerenchyma – histologically expressed by the cells separation and disintegration –, followed by the enlargement of the spaces among the collapsed cells (Esau, 1965).

The whole subtle and complex mechanism is not, of course, completely understood yet. That is an additional proof to the fact that the environmental agents influence is conjugated, multi-factorial and quite hard to view in its entire complexity.

As for the endodermis, Fahn (1964) and Ginzburg (1964) (quoted by Waisel, 1972), studying especially this aspect at the desertic halophytes, found in their roots the casparian strips wide and thick. It was thus suggested that in habitats in which an easy penetration of salts into the plant body may endanger their normal existence, the endodermic barrier appears in a highly developed form.

We should say that some authors (Van Andel, 1953; Steward and Sutcliffe, 1959; Weigel and Lüttge, 1962, quoted by Waisel, 1972) suppose that the active transport mechanism resides either in the endodermis or in the xylem parenchyma. As a matter of fact, when it was tried to compare the anatomical features of plants which occur in saline or drought environments with those of glycophytes, the most important and studied aspect was the thickness of casparian strips (Poljakoff-Mayber, Gale, 1975).

Casparian bands contain both aliphatic and aromatic suberins (Schreiber *et al.*, 1999, quoted by Peng *et al*, 2004), which suggest that the Casparian bands are fairly impermeable to ions and to high molecular weight polar solutes, but may allow some passage of water and small solutes.

On certain species, Casparian strips cover almost all the radiall walls of the endodermic cells, comparing to less than ¼ covered in dicotyledonous glycophytes case (Waisel, 1972). These modifications of the endodermis are related to the ions, to their absorption.

It is known that K^+ has a major role in the salt tolerance. The "ideal" scheme for salt tolerance of halophytes would consist in maintaining a high level of K^+ and a low level of Na^+ in the cells, but these two ions compete one against another. Above many molecular biology aspects, we just mention that solutions such as Na^+, K^+, Ca^+, Mg^+, and ABA (abscisic acid) move freely in the apoplast of root cells and must pass through Casparian strips to reach the apoplast of xylemic vessels.

The relative lignification of central cylinder, pointed on our materials (sclerenchyma ring of *Juncus gerardi* and sclerenchymatic and high lignified pericycle of *Spergularia media*), can be related to an excessive salinity in soil (Bickenbach, 1932, quoted by Waisel, 1972). Kozlowski (1997) underlines that the salinity increases the fiber production. The lignin may be a cellular resistance element against the high osmotic pressure inside the plant body.

The presence of cells with calcium oxalate crystals in some halophytes species may be significant, because there is an apparent connection between calcium and salt tolerance. It is known that salinity increases the production of calcium oxalate (Eckstein *et al.*, 1976, quoted by Kozlowski, 1997).

The presence of calcium is related to ion absorption, ionic antagonism and facilitates selective absorption of some ions. Calcium plays also an important role in maintaining the integrity of plant cell membrane; it is a physiological barrier to free diffusion of potentially toxic ions prevalent in a saline environment. The relation between Ca^{2+} and ion transport is resolved to an even finer degree when plants are exposed to a high salt environment. The increasing of the salts level in the surroundings of the plants roots results in greater demands on the salt-regulating processes of plants. The presence of potentially toxic ions will increase the possibility of membrane damage. The role of calcium therefore becomes even more important as the system is increasingly saline (Rains, 1972).

La Haye and Epstein (1969) demonstrated a definite role of calcium in increasing the tolerance of beans to high salt.

We can assume, therefore, that calcium ions are involved in increasing the salt tolerance, on different ways, such as by regulating ion transfer into cells exposed to saline conditions (Rains, 1972); calcium also influences physiological reactions, membrane structure, and redistribution of ions in the plants exposed to salinity.

The presence of bulliform cells which occur in the vagina's upper epidermis of *Juncus gerardi* was not mentioned, for example, for other two *Juncus* species: *J. acutus* L. and *J. maritimus* Lam. (Burduja C., Toniuc Angela, 1984), the first one being collected from sands, quite salinized, less moist, and the second one from the saline, moist depressions between the dunes of Sărăturile, but it was mentioned for *J. trifidus* (Toma *et al.*, 1989).

The mesophyll is quite a homogeneous and entire palisade: 2–3 layers of higher cells at the upper epidermis and 3 layers of short cells at the lower epidermis. *Juncus gerardi* is related to the mesophyte group, if we consider that it vegetates on less saline and moist solonchaks and solonetz. By morphology and anatomy, it is related to the meadow mesophytes, near which it lives. For this reason, it was considered as a halomesophyte (Şennikov, 1950). In extension, Chermezon (1910) considered that the histo-anatomical structure of *Juncus* species does not denote especially the halophilous features (Chermezon, 1910).

CONCLUSIONS

Our investigation emphasizes the following considerations:

The presence of aerenchyma in *Juncus gerardi* and *Plantago schwarzenbergiana* rhizome and *Spergularia media* root, which represent an adaptive feature, characteristic of other halophyte species, too (Waisel, 1972), which may be correlated with poor ventilation of saline soils.

The presence of a tertiary endodermis in *Juncus gerardi* rhizome, and of an endodermoid in the *Plantago schwarzenbergiana* and *Juncus gerardi* aerial stem,

can restrict the penetration of salts in the plant (Fahn and Ginzburg, 1964, quoted by Waisel, 1972 and Poljakoff-Mayber, 1975).

The presence of a sclerenchymatious pericycle in *Plantago schwarzenbergiana* and *Spergularia media* aerial stem, a feature which is probably induced by a higher salinity (Strogonov, 1962 and Waisel, 1972, quoted by Poljakoff-Mayber, 1975).

The observation of multicellular protective trichomes in the epidermis of the aerial stem and of the secretory trichomes in the epidermis of the lamina of *Plantago schwarzenbergiana*; the former plays a role in hydric implications (decreases the transpiration); the latter, probably, in salt exclusion.

The presence of the cells with calcium oxalate crystals in the cortical parenchyma of the aerial stem and the lamina of *Spergularia media*, which increase the salt tolerance of plants (Rains, 1972).

The existence of some bulliform cells in the upper epidermis of *Juncus gerardi* vagina.

The analysis of all these observations may conduce to some preliminary ecological conclusions: *Plantago schwarzenbergiana* is a xero-mesophilous halophyte, *Spergularia media* a mesophilous to hygrophilous, and *Juncus gerardi* is relatively hygrophilous, intermediary between hygrohalophytes to hygrophytes, fact confirmed on field work.

REFERENCES

1. Adam P., 1990, *Saltmarsh ecology*, Cambridge University Press.
2. Bucur N., Dobrescu C., Turcu GH., Lixandru GH., Teşu C., 1960, Contribuţii la studiul halofiliei plantelor din păşuni şi fâneţe de sărătură din Depresiunea Jijia-Bahlui (partea a II-a). *Stud. şi Cerc. (Biol. şi Şt. Agricole)*, Acad. R.P.R., filiala Iaşi, an. XI, fasc., **2**, pp. 333–347.
3. Burduja C., Toniuc A., 1984, Données céno-écologiques et histo-anatomiques sur les espèces *Juncus acutus* L. et *J. maritimus* Lam. *Acta Botanica Horti Bucurestiensis*, Vol. omagial, pp. 93–96.
4. Chermezon H., 1910, Recherches anatomiques sur les plantes littorales, *Ann. Sci. Nat.*, sér. 9, Bot., tome XII, pps. 117–129, 270–274, 299–307.
5. Cutler D.F., 1969, *Anatomy of the Monocotyledons* **4**, *Juncales*, Ed. Clarendon Press, Oxford, pp. 17–77.
6. Davidescu D., Calancea L., Davidescu V., Lixandru Gh., Ţârdea C., 1981, *Agrochimie*. Edit. Did. şi Ped., Bucureşti.
7. Esau K., 1965, *Plant anatomy* (second edition). Eds. John Wiley and Sons, New York, London, Sydney.
8. Evans D.E., 2003, Aerenchyma formation, *New Phytol.*, **161**, pp. 35–49.
9. Feller I.C., Sitnik M., 1996, *Mangrove ecology: a manual for a field course*. Smithsonian Institution, Washington DC.
10. Grigore M., Toma C., 2005, Contributions to the knowledge of anatomical structure of some halophytes. I. *Stud. Cerc. Şt. Biol.*, Univ. Bacău, **10**, pp. 125–128.
11. Justin S.H.F.W., Armstrong W., 1987, The anatomical characteristics of roots and plant response to soil flooding, *New Phytol.*, **106**, pp. 465–495.

12. Keiffer C.H., McCarthy B.C., Ungar I.A., 1994, Effect of salinity and waterlogging on growth
    and survival of *Salicornia europaea* L., an inland halophyte, *Ohio J. Sci.*, **94** (3), pp. 70–73.
13. Kozlowski T.T., 1997, Response of woody plants to flooding and salinity, *The Physiology
    Monograph*, **1**, pp. 1–29.
14. La Haye P., Epstein E., 1969, Salt toleration by plants: enhancement with Ca^+, *Science*, **166**,
    pp. 395–396.
15. Lawton J.R., Todd Ann, Naidoo D.K., 1981, Preliminary investigations into the structure of the
    roots of the mangroves *Avicennia marina* and *Bruguiera gymnorhiza*, in relation to ion
    uptake. *New Phytol.*, **88**, pp. 713–722.
16. Marschner H., 2002, *Mineral nutrition of higher plants*. Fifth printing, Academic Press.
17. Metcalfe C.R., Chalk L., 1972, *Anatomy of the Dicotyledons*, 1–2, Clarendon Press, Oxford,
    pp. 147–153, 1053–1059.
18. Milică C., 1982, *Fiziologie Vegetală*. Ed. Did. şi Ped., Bucureşti.
19. Napp-Zinn Kl., 1984, *Anatomie des Blattes. II. Blattananatomie der Angiospermen. B.
    Experimentelle und ökologische Anatomie der Anngiospermenblattes*, **8**, 2B, Gebrüder
    Bontrager, Berlin, Stuttgart.
20. Peng Yan-Hui, Zhu Ya-Fang, Mao Yong-Qiang, Wang Suo-Min, Su Wei-Ai, Tang Zhang-
    Cheng, 2004, Alkali grass resists salt stress through high $[K^+]$ and an endodermis barrier to
    Na^+, *Journ. Exp. Bot.*, **55** (398), pp. 939–949.
21. Poljakoff-Mayber A., Gale J., 1975, *Plants in saline environments*. Springer Verlag, Berlin,
    Heidelberg, New York.
22. Rains D.W., 1972, Salt transport by plants in relation to salinity, *Ann. Rev. Plant. Physiol.*, **23**,
    pp. 367–388.
23. Roland Albert, 1975, Salt regulation in halophytes, *Oecologia* (Berlin), **21**, pp. 57–71.
24. Schulze E.-D., Beck E., Müller-Hohenstein K., 2005, *Plant Ecology*. Springer Verlag, Berlin,
    Heidelberg, New York.
25. Sennikov A.P., 1950, *Ecologhia rastenij*, Izdat. Sovetskaia Nauka, Moskva, pp. 233–250.
26. Şerbănescu I., 1965, Asociaţiile halofite din Câmpia Română, *Com. Geol., Instit. Geol. Şt. Tehn.
    şi Econ., seria C, Pedologie*, **15**, Bucureşti, pp. 1–148.
27. Toma C., Moţiu T., Niţă M., Filipescu G., 1989, Cercetări anatomo-ecologice asupra unor plante
    ierboase din etajul subalpin al munţilor Călimani. *An. Şt. Univ. "Al. I. Cuza" Iaşi, ser.II a*
    (Biol.) **35** (supl.), pp. 5–26.
28. Ungar I.A., 1991, Ecophysiology of vascular halophytes, CRC Press, Boca Raton, FL.
29. Waisel Yoav, 1972, *Biology of halophytes*, Academic Press, New York and London.
30. Walsh G.E., 1974, *Mangroves*, In: REIMOLD R.J., QUEEN W.M., *Ecology of halophytes*.
    Academic Press Inc., New York, London, pp. 51–174.
31. Zarrinkamar F., 2001, Foliar anatomy of the *Caryophyllaceae* family in Arasbaran, NW. Iran,
    Iran. Journ. Bot., **9** (1), pp. 93–102.