A PROPOSAL FOR A NEW HALOPHYTES CLASSIFICATION, BASED ON INTEGRATIVE ANATOMY OBSERVATIONS

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Abstract. This paper aims at proposing a new classification of halophytes, based on integrative anatomy observations. After the analysis of anatomical features and taking into account the ecological factors (salinity), we suggest the follow halophytes categories: extremehalophytes (irreversible and reversible) and mesohalophyes.

Keywords: halophytes, classification, integrative, proposal.

Rezumat. O nouă propunere de clasificare a halofitelor, bazată pe observaţii de anatomie integrativă. Această lucrare încearcă să propună o nouă clasificare a halofitelor, bazată pe observaţii de anatomie integrativă. În urma analizei adaptărilor anatomice şi luând în considerare unii factori ecologici (salinitatea), sugerăm următoarele categorii de halofite: extremohalofite (ireversibile şi reversibile) şi mezohalofitele.

Cuvinte cheie: halofite, clasificare, integrativ, propunere.

INTRODUCTION

There is still a lot of relativity and even arbitrary in what concerns the definition and classification of halophytes. Often, halophytes were defined as plants which grow and complete their life cycle in habitats with a high salt content (WAISEL, 1972). More recently, halophytes were described as plants that survive to reproduce in environments where the salt concentration is around 200 mM NaCl or more (FLOWERS & COLMER 2008). In addition, many of the existing classifications are not based on well-defined criteria; perhaps due to the fact that there is missing of a single definition of halophytes and saline environments.

However, especially in the last century, some general classifications of halophytes were proposed by CHERMEZON (1910), PRODAN (1922), STOCKER (1928), GUŞULEAC (1933), STEINER (1935), IVERSEN (1936), PRODAN (1939), VAN EIJK (1939), CHAPMAN (1942), ŢOPA (1939; 1954), BUCUR et al. (1957), PĂTRUŢ et al. (2005). The number of these classifications is greater and many attempts to classify halophytes were summarized and discussed in our previous works (GRIGORE, 2008a; GRIGORE, 2008b; GRIGORE & TOMA, 2010a). A part of these assays takes into account the physiological, ecological, and floristic criteria. The language used by many authors is often different, which complicate the way of approaching and understanding the halophytes classification. Moreover, some existing classifications represent modified and new adapted forms of the previous systems of classification, while others are based on ambiguous or more “intuitive” criteria. It is the case of the old Romanian classifications, where the deep intuition and large experience of some botanists allowed them to include halophytes in different classes. For an attempt to unify the equivalent major Romanian halophytes classifications, see GRIGORE’S paper (2008b).

The aim of our work is to propose a new system classification of halophytes based on integrative anatomy investigations. Integrative anatomy, as approach, represents the correlation of structure with plant function and ecological, functional, adaptive, and phylogenetic implications (GRIGORE, 2008a).

MATERIAL AND METHODS

We have studied 30 halophyte species: *Petrosimonia oppositifolia* (PALL.) LITV., *Petrosimonia triandra* (PALL.) SIMONK., *Salicornia europaea* L., *Suaeda maritima* (L.) DUMORT., *Halimione verrucifera* (M. BIEB.) AELLEN, *Atriplex tatarica* L., *Atriplex littoralis* L., *Atriplex prostrata* (BOUCHER) ex DC, *Bassia hirsuta* (L.) ASCH., *Camphorosma annua* PALL., *Camphorosma monspeliaca* L. (Chenopodiaceae), *Artemisia santonicum* L., *Aster tripolium* L. subsp. *pannonicus* (JACQ.) SOO, *Lactuca saligna* L., *Scorzonera cana* (C. A. MEY.) O. HOFFM. (Asteraceae), *Lepidium cartilagineum* (J. C. MAYER) THELL. subsp. *crassifolium* (WALDST. & KIT.) THELL., *Lepidium latifolium* L., *Lepidium perfoliatum* L. (Brassicaceae), *Iris halophila* PALL. (Iridaceae), *Plantago schwarzenbergiana* SCHUR (Plantaginaceae), *Trifolium fragiferum* L. (Fabaceae), *Polygonum patulum* M. BIEB. (Polygonaceae), *Spergularia media* (L.) C. PRESL (Caryophyllaceae), *Scirpus maritimus* L. subsp. *maritima*, *Carex distans* L., *Carex vulpina* L. (Cyperaceae), *Juncus gerardi* LOISEL. (Juncaceae), *Puccinellia distans* (L.) PARL. ssp. *limosa* (SCHUR) JÁV., *Agrostis stolonifera* L., *Alopecurus arundinaceus* POIR. (Poaceae). We followed the nomenclature used by the digital version of *Flora Europaea* (http://rbg-web2.rbge.org.uk/FE/fe.html). These taxa form a very heterogeneous group, belonging to different families and having different ecological spectra. The species subjected to analysis were collected from different (wet, dry) saline habitats conditions. In the field, some short ecological notes were also made.

We followed the usual plant anatomy techniques, performing cross-sections through vegetative organs; the sections were stained with carmine red and green iodine, and finally fixed into glycerol-gelatin. The permanent preparations were examined with a light microscope and micrographs were taken using a Canon photo digital camera.

RESULTS AND DISCUSSION

After the histological analysis, we found out, as a general rule, that some halophytes have well expressed more complex anatomical features, as the soil salinity is higher. On an imaginary axis of salinity, with a minimum and maximum, the investigated species occupy different points, depending on the displayed adaptations. The well adapted halophytes show peculiar histo-anatomical features in a higher degree than less adapted species. Taking into account the anatomical adaptations, their relevance, ecological significance and general strategies of halophytes, we have divided the investigated taxa in the following categories:

1. Extremehalophytes (well adapted salinity species, extreme halophytes) are those species occurring exclusively (or almost exclusively) in saline environments. They have the strongest anatomical adaptations, in correlation with salinity factor. Two types of extreme halophytes were described:

Irreversible extremehalophytes: *Petrosimonia oppositifolia*, *P. triandra*, *Salicornia europaea*, *Suaeda maritima*, *Halimione verrucifera* (Chenopodiaceae). Some of these species display succulence (Figs. 1, 2, 3, 4), through a well developed water storage tissue in aerial organs; this feature is an important strategy in halophytes life, having a dilution effect on high salt content and also assuring the erect position of species with less developed mechanical system (GRIGORE, 2008a; GRIGORE & TOMA, 2010a). All taxa present successive cambia phenomenon in roots (Figs. 5, 6) and stems; we believe that this structure anomaly have an ecological and adaptive value. Moreover, the increased lignin content associated with this structure may confer cellular resistance to a high osmotic pressure. *S. europaea* has tracheoidioblasts (Fig. 7) in its fleshy tissues, whose function being perhaps involved in water storage or mechanical support. *H. verrucifera* has salt secreting hairs on leaves surfaces (Fig. 8); these are very important devices in salt removal from plant shoot. *P. oppositifolia* (Fig. 4) and *P. triandra* (Fig. 3) have foliar Kranz anatomy structure, an important configuration related to C_4 photosynthesis.

The attention must be drawn on the fact that until now, as far as we know, the term “extremophile” was reserved only for microorganisms (RAINEY & OREN, 2006).

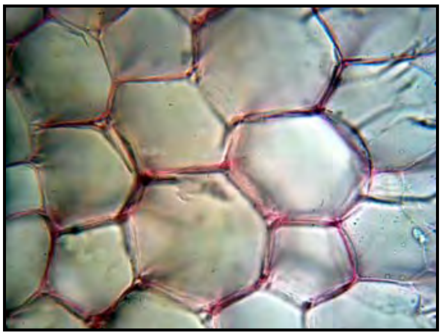

Figure 1. Micrograph of cross section through the fleshy tissue of *Salicornia europaea* (X400).
Figura 1. Secțiune transversală prin țesutul suculent de la *Salicornia europaea* (X400) (original).

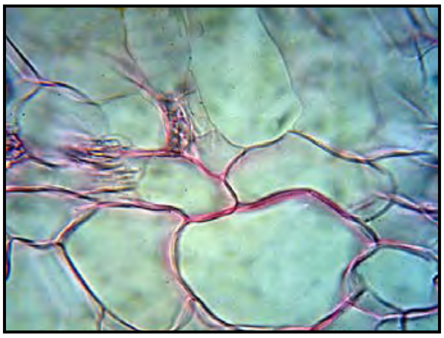

Figure 2. Micrograph of cross section through the lamina of *Suaeda maritima* (X400).
Figura 2. Secțiune transversală prin limbul foliar de la *Suaeda maritima* (X400) (original).

Figure 3. Micrograph of cross section through the lamina of *Petrosimonia triandra* (X400).
Figura 3. Secțiune transversală prin limbul foliar de la *Petrosimonia triandra* (X400) (original).

Figure 4. Micrograph of cross section through the lamina of *Petrosimonia oppositifolia* (X200).
Figura 4. Secțiune transversală prin limbul foliar de la *Petrosimonia oppositifolia* (X200) (original).

Reversible extreme halophytes: *Atriplex tatarica*, *Atriplex littoralis*, *Atriplex prostrata*, *Bassia hirsuta*, *Camphorosma annua*, *C. monspeliaca*. The adaptations displayed by these species are a little bit less developed than those of the previous group, despite the fact that some important anatomical features may also occur: salt hairs (Fig. 9), successive cambia phenomenon, Kranz anatomy (Fig. 10), and succulence. But taxa may also vegetate in other salinized habitats, such as salty sands or anthropized environments; that are the reason for calling these halophytes "reversible", because they can pass from much salinized areas through less salinized soils.

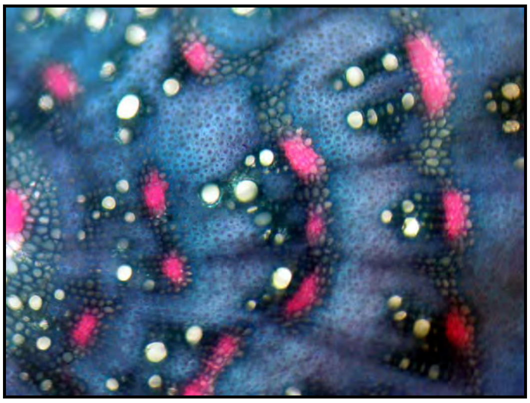

Figure 5. Micrograph of cross section through the root of *Atriplex littoralis* (X200). / Figura 5. Secţiune transversală prin rădăcina de la *Atriplex littoralis* (X200) (original).

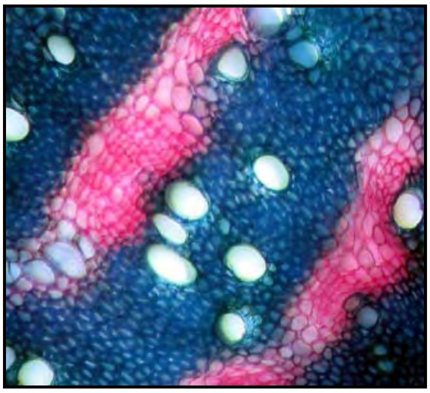

Figure 6. Micrograph of cross section through the root of *Atriplex prostrata* (X400). / Figura 6. Secţiune transversală prin rădăcina de la *Atriplex prostrata* (X400) (original).

Figure 7. Micrograph of cross section through the fleshy tissue of *Salicornia europaea* (X400).
Figura 7. Secţiune transversală prin ţesutul suculent de la *Salicornia europaea* (X400) (original).

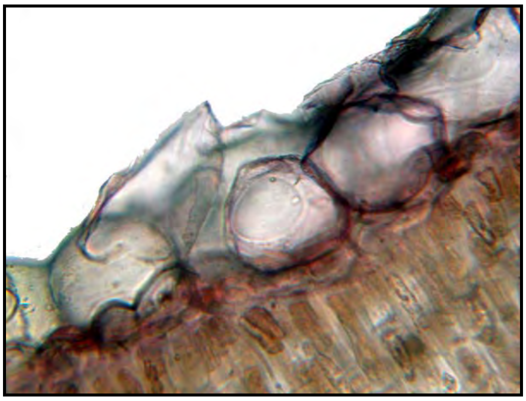

Figure 8. Micrograph of cross section through the lamina of *Halimione verrucifera* (X400).
Figura 8. Secţiune transversală prin limbul foliar de la *Halimione verrucifera* (X400) (original).

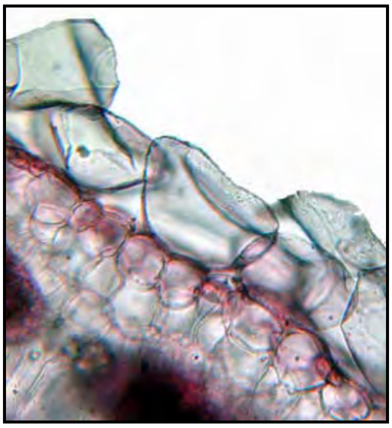

Figure 9. Micrograph of cross section through the lamina of *Atriplex tararica* (X400).
Figura 9. Secţiune transversală prin limbul foliar de la *Atriplex tatarica* (X400) (original).

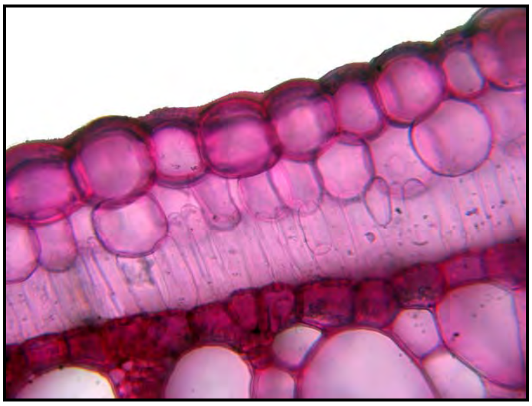

Figure 10. Micrograph of cross section through the lamina of *Camphorosma annua* (X400).
Figura 10. Secţiune transversală prin limbul foliar de la *Camphorosma annua* (X400) (original).

2. Mesohalophytes are species with intermediary anatomical adaptations between extremehalophytes and glycophytes: *Aster tripolium* subsp. *pannonicus*, *Lactuca saligna*, *Scorzonera cana*, *Lepidium cartilagineum* subsp. *crassifolium*, *Lepidium latifolium*, *Lepidium perfoliatum*, *Iris halophila*, *Plantago schwarzenbergiana*, *Trifolium fragiferum*, *Spergularia media.* They can vegetate in many environments, not only salinized ones, although some of these may have relevant features in accordance with salinity. Thus, a well developed endodermis, aerenchyma, xerophytic features (sunken stomata, water storage tissue) occur, many of these adaptations being also related with other ecological factors: flooding, dryness, humidity. In this group we also included a separated class – *amphibious halophytes* – having bulliform cells (Figs. 11, 12), which we regarded as an adaptation to temporary dry conditions of the habitats, despite the fact that these species are hygrophylous: *Scirpus maritimus*, *Carex distans*, *Juncus gerardi*, *Puccinellia distans* subsp. *limosa*, *Carex vulpina*, *Alopecurus arundinaceus*.

3. Glycophytes are plants that normally cannot grow in saline environments. These species are conventionally called glycophytes, and their category is just listed here for having an imaginary view of species disposition on salinity axe (Fig. 13).

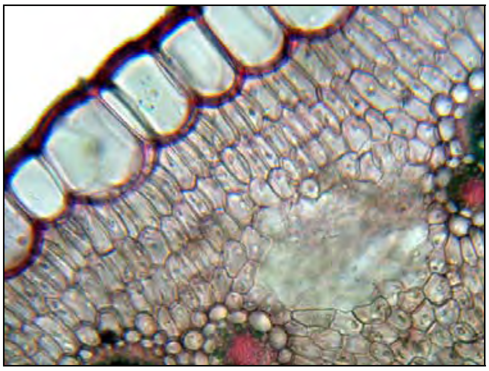

Figure 11. Micrograph of cross section through the leaf sheat of *Juncus gerardi* (X400). / Figura 11. Secțiune transversală prin teaca limbului de la *Juncus gerardi* (X400) (original).

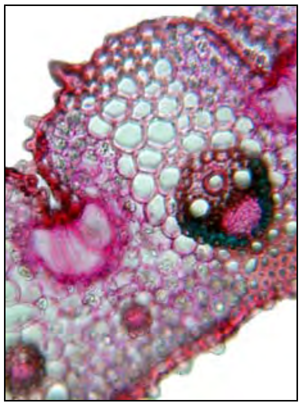

Figure 12. Micrograph of cross section through the lamina of *Puccinellia distans* (X400). Figura 12. Secțiune transversală prin limbul foliar de la *Puccinellia distans* (X400) (original).

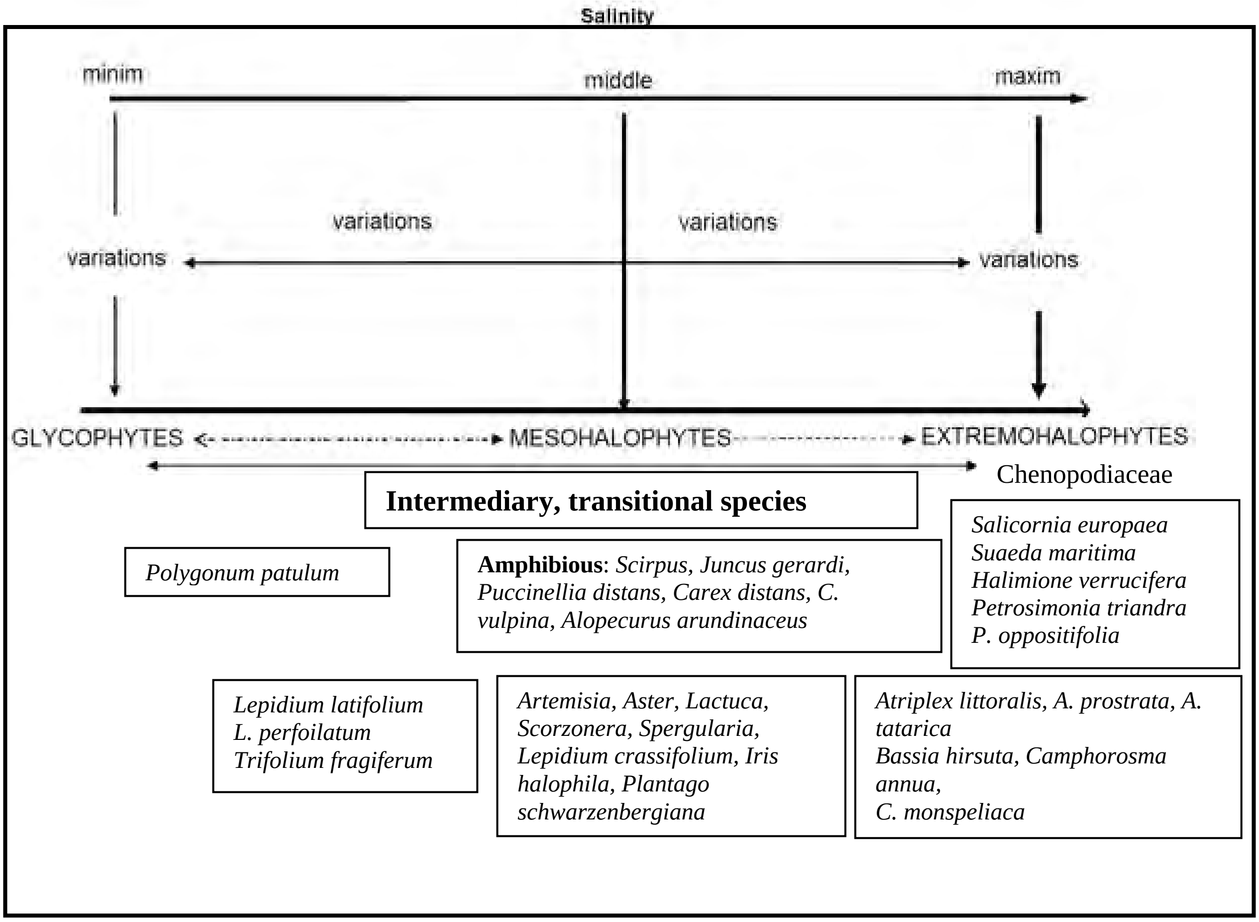

Figure 13. A proposal of new halophytes classification, based on integrative anatomy observations (after GRIGORE & TOMA, 2010a). Figura 13. O nouă propunere de clasificare a halofitelor, bazată pe observații de anatomie integrativă (după GRIGORE & TOMA, 2010a).

CONCLUSIONS

We must assert that not all the halophytes display the same relevance concerning the expression of anatomical adaptations. Here is no doubt that evolution has selected, during the time, some “branches” (botanical families, as Chenopodiaceae) with more spectacular anatomical features, in connection with salinity factor (see GRIGORE & TOMA, 2010b for some evolutionary comments). It is also true that many species are not so strictly related to hypersaline environments, if we consider them as anatomical approach.

In general, as a result of our investigations, we can say that there are some obvious anatomical adaptations of halophytes, well defined, enabling them to survive in hypersaline environments. These features characterize the “obligatory” halophytes - species adapted to high salinity conditions, which are strictly restricted to salty areas, and are not found on other soil types than the saline ones. These observations allowed us to formulate a hypothesis regarding the “extreme” adaptations of halophytes to salinity. The most evident and “spectacular” characters, linked to halophilous profile were observed on halophyte species adapted to higher salinity conditions (and usually on the species that occur only in high salinized areas).

Following our investigations and given interpretations, we have proposed a new system of halophytes classification, based on the relevance of anatomical adaptations correlated with the intensity of environmental factors (most often, soil salinity). Thus, the Chenopodiaceae succulent species best adapted to high salinity conditions (*Salicornia*, *Suaeda*, *Halimione*, *Petrosimonia oppositifolia* and *P. triandra*) were nominated as extreme halophytes; in addition, because they grow only in very salinized environments, we applied to them the term irreversible halophytes.

Atriplex littoralis, A. prostrata, A. tatarica, Bassia hirsuta, Camphorosma annua, C. monspeliaca species, which have a large ecological spectra and are not so strictly related to increased salinity, were called by us as reversible halophytes. These species may also pass in less salinized environments.

The supporting, accidental or preferential halophytes which do not have histo-anatomical characters very clearly correlated to salinity factor were called mesohalophytes. They are more subjected to multiple influences of environmental factors, including soil salinity, but this is not the major factor inducing spectacular adaptations within this group of halophyte species. In the mesohalophytes group, the above mentioned amphibious halophytes could be included.

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