ECOLOGICAL IMPLICATIONS OF BULLIFORM CELLS ON HALOPHYTES, IN SALT AND WATER STRESS NATURAL CONDITIONS

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Abstract: Seven Romanian salt-tolerant species were histo-anatomical investigated. These species have been classified by the authors of the present study as “amphibious” halophytes, related to the field observations and anatomical considerations. All the analyzed taxa present bulliform cells at the foliar epidermis level. Despite the fact that there are different interpretations regarding the bulliform cells role and functional significance, we correlate these structures with the ecological factors, salinity and, respectively, drought conditions.

Key words: halophytes, bulliform cells, drought, salinity.

Introduction

There are so many definitions of halophytes, but often they are defined as those species that are able to survive and reproduce in environments where the salt concentration is around 200 mM NaCl or more [24]. As heterogeneous complex ecological class, an ecological interpretation of some anatomical features is far away to be easy to make. Besides many controversial discussions, it seems however like anatomical and morphological features typical of halophytes are usually considered to be adaptations to salinity [45].

In a nowadays, salt stress is the most intensely studied of all abiotic stress types, because the salinity is considered the main constraint for the agriculture worldwide [41; 42; 39; 6; 36; 50; 5]. As a rule, salinization and aridization are complementary phenomena, through some common pedologic processes, as well as by the fact that stress caused by salinity is both dehydration stress and ionic stress [51]. In fact, drought and salinity are two major environmental factors determining plant productivity and plant distribution. These two major stressful factors affect more than 10 percent of arable land; desertification and salinization are rapidly increasing on a global scale decreasing average yields for most major crops by more than 50 percent [8]. Plant responses to salt and water stress have much in common. Salinity reduces the ability of plants to take up water, and this quickly induces reduction in growth rate, along with a suite if metabolic changes identical to those caused by water stress.

Bulliform cells are structural features found especially on species included in Poaceae [19; 29; 30; 31; 60; 4; 44; 55; 34; 35; 25; ], Cyperaceae [18; 7; 38; 39] and Juncaceae [18]. Bulliform cells, sometimes named in various ways by different authors over time, are large, regularly with thin walled cell. Despite that they were recognized for many years, their ecological significance in plant adaptation to salinity remains unclear.

It is well known that many leaves are capable of rolling up in dry, unfavourable, conditions and reopening again under conditions when there is no water stress, have

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special, thin-walled water-containing cells enable them to make these movements [16]. These are the bulliform or motor cells, which under conditions of water deficit lose turgor and thus constrict in upon themselves, causing lamina to fold or roll inward edge to edge [17].

Although bulliform cells also occur on non-halophytic monocotyledons species – suggesting probably an origin in a common ancestor – their presence on species exposed both on drought and salinity conditions requires a detailed and prudent analysis in accordance with environmental factors.

The aim of this work is to find some correlations between bulliform cells occurring on halophytes and ecological conditions of saline habitats.

**Material and methods**

The sample material subjected to our analysis is represented by leaves of halophytes, collected from saline habitats, in plants anthesis phenophase. The investigated taxa that were anatomically investigated are: *Bolboschoenus maritimus* (L.) Palla ssp. *compactus* (Hoffm.) Dobrow, *Carex distans* L., *Carex vulpina* L. (*Cyperaceae*), *Juncus gerardi* Loisel. (*Juncaceae*), *Agrostis stolonifera* L., *Alopecurus arundinaceus* Poir. and *Puccinellia distans* (L.) Parl. ssp. *limosa* (Schur) Jáv (*Poaceae*). *J. gerardi* and *B. maritimus* were collected from saline areas on Valea lui David (Iași), and other taxa - from Valea Ilenei (Iași), during years of 2007-2008.

With reference to halophytic character of these species and considering also the categories in which these halophytes may be included, it goes without saying that here is no uniformity in this question. Anyway, these halophytes have been classified as *amphibious halophytes* [27], in a classification system which is based on halophytes anatomical features and their relation with ecological factors. The above mentioned taxa are regularly hygrophylous species, but in some circumstances the habitats where they live can be exposed to more or less prolonged drought short seasons.

Thus, *B. maritimus* is a neohalophyte species [12], preferential one [58], being included in the second category by Prodan [47]; *C. distans* is a preferential halophyte [58], mezohygrophyte, facultative halophyte [14]; *C. vulpina* was characterized as a neohalophyte [12]; *J. gerardi* was described as a euhalophyte [11], preferential halophyte [58], mezohygrohalophyte [14], included in the first category of Prodan [47]. *A. stolonifera* is a neohalophyte [12], supporting to salinity [58] and introduced in the first category by Prodan [47]; *A. arundinaceus* is a preferential halophyte [58], neohalophyte [12] and mezohygrophyte, facultative halophyte [14]. *P. distans* is considered a euhalophyte [11], preferential halophyte [58], first category including by Prodan [47]. It is obvious, considering the above exposed information that these taxa have been differently charaterized by various plant biology researchers. For further explanations and comments regarding the terminology used by these botanists, different classifications and equivalencies between them, see the Grigore’s work [26].

For subsequent histo-anatomical investigations, the material was fixed and preserved in ethanol (70°). Sectioning of the leaf samples was made using a botanical razor and a microtome. The cross sections thus obtained have been subsequently subjected to the “classical” stages of a common histo-anatomical procedure: immersion in sodium hypochlorite for 20-30 min,
washing with acetic water and tap water, then staining: first with iodine green (for 1 minute) and washing in ethylic alcohol (90°) bath then second with red carmine (for 20 min.), washing with water and finally fixation in glycerol-gelatine.

After obtaining permanent slides, micrographs have been taken using a NOVEX (Holland) photonic microscope, with a Canon photo digital camera.

Results

After histo-anatomical analysis we found that all investigated taxa have bulliform cells, at epidermis level of the blade - if we are referring on Cyperaceae and Poaceae taxa – and the leaf sheath level – referring on Juncaceae species. These cells are regularly confined on upper epidermis, distributed in isolated groups or in long rows, forming bands of bulliform cells.

In Bolboschoenus maritimus, epidermis has isodiametric cells, the external cell wall being thicker than others and covered by a thin cuticle; here and there, some stomata could be also observed. In the midrib region, the epidermis presents larger cells, which remind the bulliform-like cell form. As we gradually are moving away from the midrib, bulliform cells become less prominent (Fig. 1).

In Carex distans, the contour line of lamina has “V” letter general form, with largely opened two lateral parts, sometimes disposed even at the same level. The midvein is well protruding on lower surface of the lamina; at this level, the medial and the inferior epidermal cells have papillae like hair aspect, with thick wall cells. The upper epidermal cells are very big, forming a bulliform cells arch corresponding to the midvein (Fig. 2).

Carex vulpina has bulliform cells clustering in the midvein level, as in the case of previously mentioned species. They are forming a more restricted arch, regarding the number of cells (Fig. 3).

In Juncus gerardi, the upper epidermis of leaf sheath displays very large cells (Fig. 4), with external wall cell a bit thicker than others. This kind of cells was not evidenced in the case of two other Juncus species: J. acutus L. and J. maritimus Lam. [13]; the first mentioned species has been collected from less wet sandy salinized soils, while the second, from more wet and salinized depressions, localized between maritime dunes of Sărăturile - Dobrogea. Contrariwise, the bulliform cells were observed on J. trifidus [57], a species collected from subalpine region of Ceahlău Mountains.

In Agrostis stolonifera, the bulliform cells (Fig. 5) are located in a small number at the bottom of some grooves (Fig. 6), having a variable depth; these grooves form, regularly, between the big vascular bundles. Moreover, these grooves are protected, in the top, by short protecting trichomes.

Alopecurus arundinaceus has huge bulliform cells, disposed in clusters consisting of 3-5 cells (Fig. 7), at the bottom of small grooves (Fig. 8), localized between the mesophyll areas comprising vascular bundles.

Puccinellia distans shows enlarged bulliform cells (Fig. 9), confined on upper epidermis, in the line of some grooves having various depth (Fig. 10).
Discussions

We have underlined in the introductory part of this work that the function and ecological significance of these bulliform cells are still controversial and intensely discussed.

Haberlandt [28] included the *Poaceae* bulliform cells among motor, hygroscopic system of plants; the author stated that the leaves of many xerophilous grasses become folded, or curl up, when they are insufficiently supplied with water, in order to avoid excessive transpiration. Other botanists have also assigned a similar function of these cells, closely related with xerophytic environment value. Beal [7] called also these cells “blister” cells; according to him, when dry, these cells contract and aid in closing the leaf in two or three ways. When moist the leaf expands again. Sometimes, their role in leaves rolling in drought conditions is so intensely asserted, so authors such Mazel [38] refers to “cellules de plissement”, “appareil de plissement” or even “tissue d’articulation”, related to their role among some *Carex* species.

Brongniart [10] is among the first botanist which observed movements of leaves on a *Poaceae* species. Nothing is mentioned about bulliform cells, but it is interesting that the author was able to distinguish between this kind of movement and typical, motor movement of dicotyledons species.

Duval-Jouve [19] has gone more deeply concerning the presence and the role of bulliform cells on *Poaceae*; moreover, he even proposed a classification system of grasses, according to the number and disposition of bulliform cells. This French botanist discerned exactly that the rolling movement of grasses leaves in drought conditions is different from that expressed on dicotyledons. The movement induced by bulliform cells is very slow and is involved in diminution of leaves transpiration surface.

Kearney [33] identified bulliform cells on some grasses, observing that the margins of leaves become more or less involute, when the supply of water is small, becoming flat when moisture is plentiful.

Britton [9] opined that the presence of bulliform cells, considered by him “water-cells”, on grasses and sedges represent one of the most interesting and striking examples of special adaptations to xerophytic conditions. These cells are found at the bottom of the grooves of the upper surface of the leaf. The stomata are situated along the slopes of the groove and when the bulliform cells give up their water the grooves close up, thus preventing in a large extent the further escape of water through the stomata.

Warming [62] considered that leaves’ rolling of Poaceae and Cyperaceae species represents a manner in which the transpiring surface is reduced. In these movements a part is played by bulliform cells (called by Warming “hinge-cells”) lying in furrows on the upper face of the leaves. These cells are deeper than the other epidermal cells and their cellulose walls are easily folded as the leaf curls.

Fahn and Cutler stated that bulliform cells of grasses are a xeromorphic adaptation. Moreover, the bulliform cells were found to be more developed in desert ecotypes than in mesophytic ecotypes of some plants investigated [61], clearly suggesting that these cells are involved in plant adaptive response to water and salt stress.

For other authors, these cells were considered as water storage [46; 20; 59] and can participate in the young leaf expansion. Their implication in leaf rolling and/or folding of mature leaves was discussed by some researchers [52; 32]. According to Esau [22],
during excessive water loss, the bulliform cells, together with or without colorless cells, became flaccid and enabled to leaf either to fold or to roll. Clayton and Renvoize (quoted in [2]), opined that bulliform cells favoured the light entrance in the mesophyll cells. In some species, bulliform cells were not actively or specifically related to unfolding and hygroscopic leaf movement, since they accumulated large amounts of silicon and their outermost walls might thicken and cutinize, becoming stiff [21].

It was shown that in water stress conditions, the activity of these cells become more intensely. For instance, Loudetiopsis chrysothrix and Tristachya leiostachya showed leaf rolling of mature and young leaves during water stress [2]. According to Moulia (1994) (quoted in [2]), the leaf rolling is a xeromorphic characteristic and has adaptive value, reducing light interception, transpiration and protecting the leaf from dehydration and overheating. This it would be a mechanism to minimize light exposition and water transpiration, thus keeping the stomata in a microclimate with higher humidity, preventing drought conditions [15; 54].

Other species exposed to water stress show, among different adaptations, bulliform cells, such as Carex ligerica [56], Zea mays [48], common bean [53] and tomato [49].

Nawazish et al. [43] showed that on a species collected from xeric and saline habitat, Cenchrus ciliaris, the bulliform cells were well developed in severe drought; it was assumed that these cells are very crucial under moisture limited environments as these are responsible for leaf curling and ultimately checking water loss through leaf surface [1; 3].

But Ellis [21] suggested caution in assigning bulliform cells a role in leaf movement; Shields [52] described that the subepidermal sclerenchyma and other elements of mesophyll rather than bulliform cells contributed to involution in some xeric grasses.

Our ecological short notes in the field sustain the above mentioned observations. Our investigated taxa are mainly hygrophilous, some of them being salt marshes species. The temporary characters of soil moisture and atmosphere humidity induce anyway the necessity of some xeromorphic adaptations, as a response to both water stress and salt stress. It was already stated that salt stress has a high dehydration component.

Conclusions

The results of our investigations, the ecological observations in the field and the interpretations made by many authors sustain the idea that bulliform cells are involved in the way that halophytes response to salt and water stress. These investigated taxa were nominated by us “amphibious halophytes”, because they regularly vegetate in wet habitats, but sometimes these environments could be exposed to drought conditions. It is an integrative way to see these bulliform cells as a logic adaptation to environmental factors.

Anyway, an opened vision must be followed; we consider that some additional studies-mainly in experimental conditions - are required in order to obtain more exactly data regarding the role bulliform cells play in halophytes strategies.
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Explanation of plates
Micrographs of cross section through:

Plate I: Fig. 1. Lamina of Bolboschoenus maritimus (X 200); Fig. 2. Lamina of Carex distans (X 200); Fig. 3. Lamina of Carex vulpina (X 200); Fig. 4. Leaf sheath of Juncus gerardi (X 400); Fig. 5. Lamina of Agrostis stolonifera (X 400); Fig. 6. Lamina of Agrostis stolonifera (X 200).
Plate II: Fig. 7. Lamina of Alopecurus arundinaceus (X 400); Fig. 8. Lamina of Alopecurus arundinaceus (X 200); Fig. 9. Lamina of Puccinellia distans (X 400); Fig. 10. Lamina of Puccinellia distans (X 200).