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LEAF STRUCTURE AND DEVELOPMENT IN BUXUS SEMPERVIRENS L.

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SYNOPSIS

Immature and mature leaves (from first and second years) were observed in order to emphasize the histo-anatomical peculiarities related to their persistency. The foliar primordia have a semilunar shape determined by an intense activity of the abaxial meristem in the first stages of development. The marginal growth of the leaves is of median – submarginal type. The mesophyll is compact, dorsiventral, hypostomatic. At the young leaves, the palisade parenchyma consist in 2-3 layers of isodiametric cells which become elongated in mature leaves, especially in the second year. The vascular bundles present a girdle of sclerenchyma obviously more developed in the xylemic pole, contrary with the general case, when it is developed only or especially in the phloemic pole. The 2 years old leaf present isolate strands of sclerenchyma unconnected with the conducting tissue and two thick strands at the leaf edges. The diameter of the xylem vessels is very small. The stomata are localized at the same level with the epidermic cells, but the guard cells exhibit two extended crests which delimit a cavity.

INTRODUCTION

Buxus sempervirens is a shrub, but when left to grow naturally it will become a small tree. It belongs to the family *Buxacece*, a very small family of only six genera and about thirty species. Its twigs are densely leafy and the leaves are ovate, entire, smooth, thick, coriaceous and dark green. The leaves have been found to contain besides a small amount of tannin and unimportant constituents, a butyraceous volatile oil and three alkaloids: buxine, the important constituent, chiefly responsible for the bitter taste, parabuxine and parabuxonidine. The leaves have sudorific, alterative and cathartic properties being given in powder, in which form they are also an excellent vermifuge (Kohler and Bruckner, 1981, Grieve, 2005).

The anatomy of this species has not been greatly investigated. Balthazar and Endress (2002) have studied the structure of the gynoecium and androecium of representatives of all genera of *Buxaceae*. Hacker and Neuner (2007) investigated ice propagation in stems and leaves of various angiosperm deciduous and evergreen trees and shrubs and gymnosperms (including *Buxus sempervirens*).

MATERIAL AND METHODS

The vegetal material was provided from Botanical Garden Iasi.

For the anatomical analysis young leaves was fixed in FEA (formol, ethanol 70% and acetic acid 1:19:1), dehydrated with ascending alcohol series and embedded in paraffin wax. Transversal sections 12 µm thick were made with a rotary microtome. Tissues were stained with red-rutheniu and methyl-blue or with hematoxyline.

The mature leaves (from the first and the second years) were fixed and conserved in ethanol 70%. The sections made with free hand using a razor blade and colored with red-ruthen and methyl-blue. The photos were made after the obtained permanent slides using a Novex (Holland) microscope and a Canon digital photo camera.

RESULTS AND DISCUSSIONS

Xerophytes are desert plants that must carry out photosynthesis and conserve water at the same time. Xerophytes reduce water loss by having small, but thick, leaves, thus reducing the surface area for evaporative water loss. Light intensity is usually high in their environments, so sufficient light penetrates to all photosynthetic mesophyll cells, even in a thick leaf. Xerophytes have a thick cuticle and waxes on the leaf surface, further reducing water loss.

Leaf morphogenesis of *Buxus sempervirens* was investigated in order to emphasize the histo-anatomical peculiarities related to their persistency.

It is helpful to divide leaf development into several stages based on the time at which various features of the leaf become determined (Sylvester et al., 1996). During the first stage, the leaf primordium is initiated and acquires its identity as a leaf. During the second stage, the major parts of the leaf become determined and acquire their basic shape, and during the final phase, the histogenesis of the leaf is completed. Leaves are formed on the flanks of the shoot apical meristem. Leaf formation involves four overlapping stages: leaf initiation, morphogenesis, histogenesis, and expansion. The young foliar primordia have semilunar shape (photos 1-3). This is determined by an intense activity of the abaxial meristem in the first stages of development. Their structure is typical meristematic and consist in a homogenous tissue formed by isodiametical cells, rich in cytoplasmic content. At this stage of development, the distribution of growth is diffused, with the whole blade and petiole-midrib axis growing at an even rate.

Gradual, the foliar lamina breadth is growing; in their basal level the epidermis is differentiated first, then the meatic type parenchyma we can observe the differentiation of vascular bundles. In top of the foliar primordia three layers of isodiametrical cells represent the mesophil. They will take place of palisade parenchyma at the upper side and spongy parenchyma at the inner side. The marginal growth of the leaves is of median submarginal type. The mesophyll is compact, dorsiventral, hypostomatic (photos 4, 6). At the young leaves, the palisade parenchyma consist in 2-3 layers of isodiametric cells (photos 7, 8) which become elongated in mature leaves, especially in the second year (photo 12).

At the mature leaf, the lamina is slowly wavy; all the veins (but especially the middle vein) prevailed both of the upper and lower epidermis (photo 5).

The cuticle is very thick in the proximity of middle vein, and thinner between the veins. The epidermis in frontal view has polygonal cells (with straight lateral walls), stomata and tector hairs (but only in first developmental stages). Stomata are present only in lower epiderma (the foliar lamina is by hipostomatic type) (photo 13). The stomatal apparatus is from anomocytic type (photo 14).

The 2 years old leaf present isolate strands of sclerenchyma unconnected with the conducting tissue and two thick strands at the leaf edges (photo 9). The diameter of the xylem vessels is very small. The vascular bundles present a girdle of sclerenchyma obviously more developed in the xylemic pole, contrary with the general case, when it is developed only or especially in the phloemic pole (photo 11).

The cuticle is very thick especially over the upper epidermis cells (2 yaears old leaves) (photo. 10) The significantly thicker cuticle may be a mechanism to conserve water. By forming a hydrophobic barrier, the cuticle restricts water vapours loss (Pallardy, 1981). Thickening of the cuticle is the simplest method by which to conserve water (and cuticle biosynthesis is energetically inexpensive (Shulze, 1982).

The stomata are localized at the same level with the epidermic cells, but the guard cells exhibit two extended crests which delimit a cavity. In the mesophyll, some large cavities could be observed (photos 11, 12). In mature leaves of *Buxus sempervirens* L. large ice lenses formed in the central mesophyll lacunas (Hacker and Neuner, 2007).

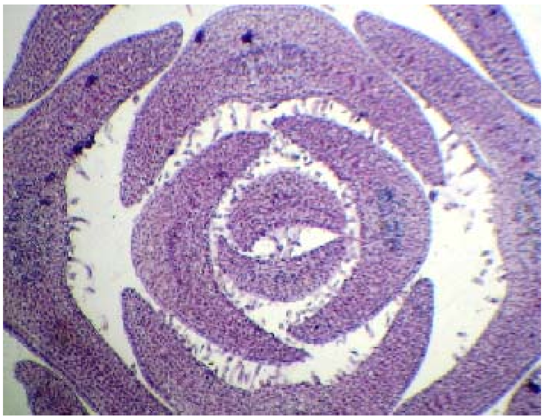

Photo 1 - Crossection through foliar primordia (x40)

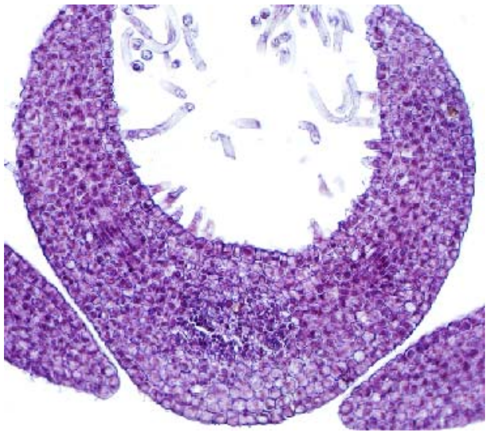

Photo 2 - Crossection thruogh a foliar primordia (x200)

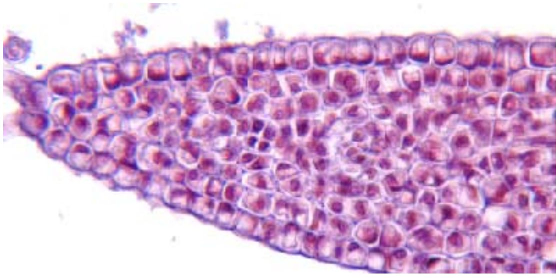

Photo 3 - Crossection thruogh a foliar primordia (x400)

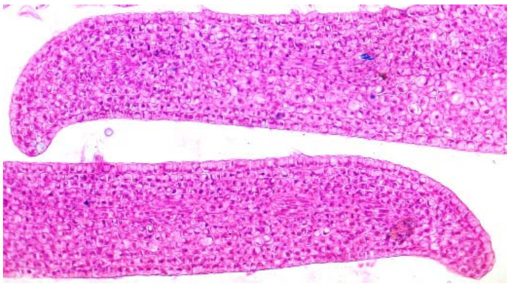

Photo 4 - Crossection thruogh very young leaves (x200)

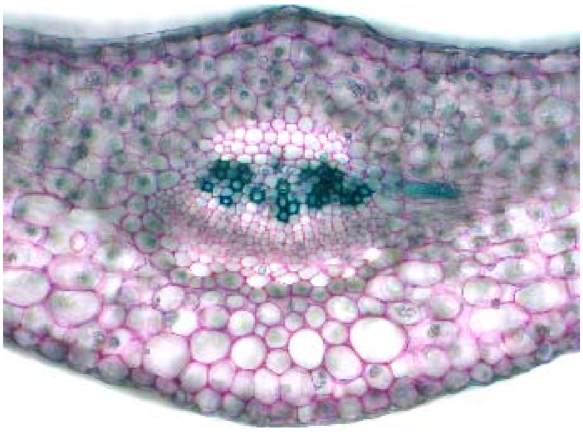

Photo 5 - Crossection thruogh a mature leaf (first year) (detail from midvein) (x200)

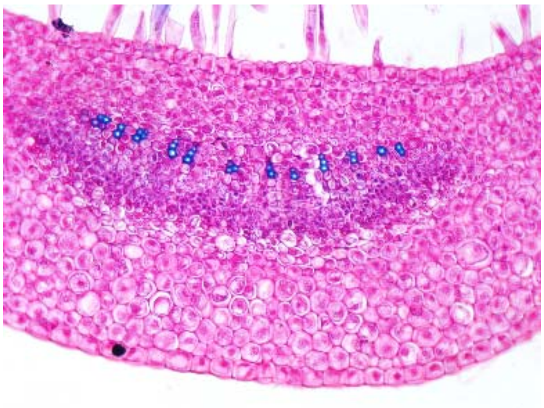

Photo 6 - Crossection thruogh a young leaf (first year) (detail from midvein) (x200)

Photo 7 - Crossection thruogh a young leaf (x200)

Photo 8 - Crossection thruogh a young leaf (x200)

Photo 10 - Crossection thruogh mature leaf (x400)

Photo 9 - Crossection thruogh mature leaf (second year)(x200)

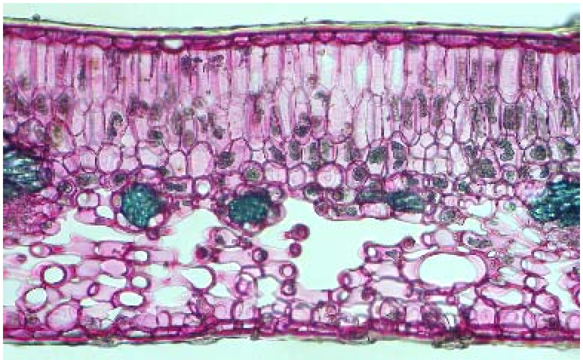

Photo 12 - Crossection through a mature leaf (second year) (x200)

Photo 11 - Crossection thruogh a mature leaf (second year) (detail from midvein) (x200)

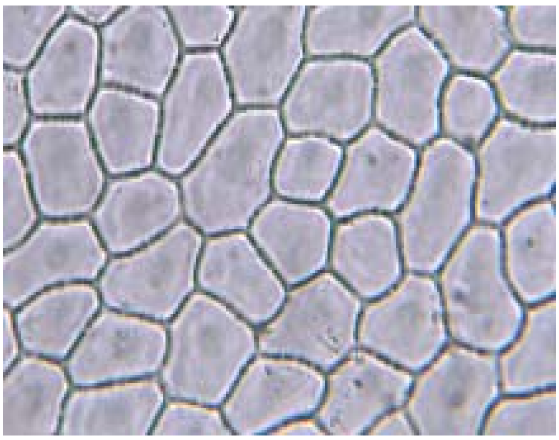

Photo 13 – Upper epidermis in front view (x400)

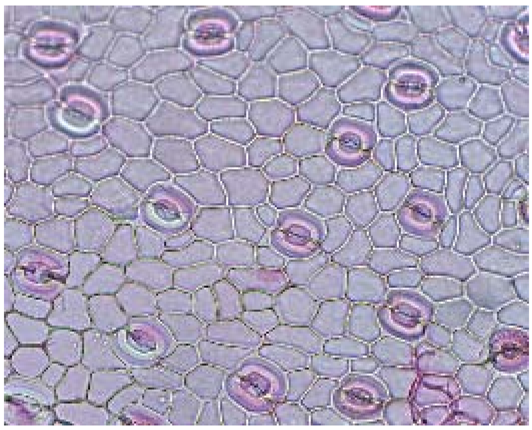

Photo 14 – Lower epidermis in front view (x200)

CONCLUSIONS

The investigation of young and mature leaves anatomy reveals ecological peculiarities. They have xeromorphic features (thick cuticle, especially in 2 years old leaves, compact and thick mesophyll), despite the relative high humidity from their environment.

REFERENCES

VON BALTHAZAR, M., ENDRESS, P. K. (2002) Reproductive structures and systematics of Buxaceae, *Botanical Journal of the Linnean Society*, 140 (3),193 - 202

GRIEVE, M. (2005) Botanical. Com, http://www.botanical.com/botanical/mgmh/b/box---67.html

HACKER, J., NEUNER, G. (2007) Ice propagation in plants visualized at the tissue level by infrared differential thermal analysis (IDTA), *Three Physio*logy, 27:1661–1670.

KOHLER, E., BRUCKNER, P. (1981): Pharmacognostic evaluation of Buxus microphylla var. sinica and Buxus microphylla var. japonica, Farmaceuticky Obzor, 50, 595-604, Bratislava

SHULZE, E D. (1982). Plant life forms and their carbon, water and nutrient relations. In: Lange, O. L., Nobel, P. S., Osmond, C. B. & Ziegler, H. (Eds.) Water relations and carbon assimilation: Physiological plant ecology II. Encyclopedia of plant physiology, vol 12B, *Springer, Berlin*, pp: 615–676.

SYLVESTER, A.W., SMITH, L., FREELING, M. (1996). Acquisition of identity in the developing leaf. *Annual review of cell and developmental biology*. 12, 257-304.

PALLARDY S.G. (1981) Closely related woody plants. In: Kozlowski T.T. (Ed.) Water deficits and plant growth, Vol. 4, *Academic Press, New York*.