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Microstructure of *Salicornia bigelovii* Stems Under Photonic and Electron Microscopy

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Halophytes are plants that live in conditions of saline stress. Particularly, the genus *Salicornia* includes around 117 species distributed mainly in the northern hemisphere and in South Africa [1]. These halophyte plants can tolerate concentrations up to 1000 mM of NaCl. The more frequent is Salicornia europaea, but North America *Salicornia bigelovii* is found in California USA and cultivated and Baja California Mexico due to its uses as food, forage, oilseed for biofuel precursor, pharmaceutical and nutraceutical applications [2]. Although Salicornia plants are important for its widely uses and potential applications for remediation of saline soils, its microstructure has been scarcely studied, there are few works related to the microstructure of its stems, and these have only been conducted mainly with light microscopy. The knowledge of its microstructure can provide important information to know the mechanisms of adaptation to extreme saline conditions and the distribution of the zones where the absorbed salt is stored into the plant organelles. Therefore, the aim of this work was to study the stems microstructure of *S. bigelovii* by light microscopy, confocal microscopy and scanning electron microscopy. Cross-sections from fresh stems of *S. bigelovii* obtained from Ensenada Baja California, Mexico was prepared to its observation by microscopy techniques. For light microscopy (LM), the samples were cut in thin slices with a blade and with a cryostat (CM 1850, Leica, Germany) for the samples observed under polarized light. The cuts were stained with safranin and fast green to observe the lignin (red) and cellulose (blue-green) of the cell wall (Figure 1a). While polarized light was used to observe the distribution of NaCl into the cross-section of stems obtained with the cryostat and freezing tissue medium to inducing the stem shrinkage and formation of NaCl crystals (Figure 1b). These cuts were observed in an inverted optical microscope (Eclipse Ti-U, Nikon, Japan). For confocal laser scanning microscopy (CLSM) the samples were stain with calcofluor white M2R to observe the distribution of hemicellulose and cellulose into the tissues, while safranin was used for identify lignified structures and by autofluorescence the tissues with chlorophyll (Figure 1c), the samples were observed with a confocal microscope (LSM710 NLO, Carl Zeiss, Germany). For scanning electron microscopy (SEM) the samples were fixed with formaldehyde and glutaraldehyde, dried with alcohol solutions and at the critical point (K850, Quorum Technologies, England), and then coated with carbon coater (SPI supplies, USA). These samples were observed in a field emission scanning electron microscope (FE-SEM, JSM7800F, Jeol, Japan). Figure 1 is a gallery of images of different microscopy techniques, where can be seen the main structures of cross-sections of *S. bigelovii* stems. Figure 1a shows a LM image, where the epidermis (ep), palisade tissue (pt), parenchyma cells (pc) and vascular bundles are identified. The pt is constituted by 2-3 rows of elongated and densely arranged cells that contain a higher density of chloroplasts (ch), this is more evident in Figure 1c, where ch in red colour) are distributed along of pt. Close to pt can be seen the spongy parenchyma or storage parenchyma cells (pc). Below the endodermis (ed) there are about six or seven vascular bundles (vb) that are structured in a circular pattern. Polarized light allow to observe the distribution of NaCl crystals (cr) into the stem cross-section of S.

bigelovii, Thus, LM image in polarized light evidenced the abundance of NaCl crystals, localized mainly in the parenchyma cells and vb. Confocal laser scanning microscopy (CLSM) image (Figure 1c) allow identified the cellulosic compounds and safranin for lignified structures and it was evident the auto-fluorescence of palisade tissue (red colour) due to the chlorophyll present in chloroplasts (ch), pc rich in cellulose and hemicellulose (blue colour), and in green colour due to the presence of lignin compounds (green colour) ep, upper epidermal cells, lower epidermal cells and endodermis cells (ed) of palisade tissue as well as in the vascular bundles (vb). Finally, in the SEM image (Figure 1d) the microstructure of vascular bundles, xylem (xy) and phloem (ph) can be observed with more detail. In conclusion, the combined use of microscopy techniques was useful for identified properly the main structures of *S. bigelovii* stems.

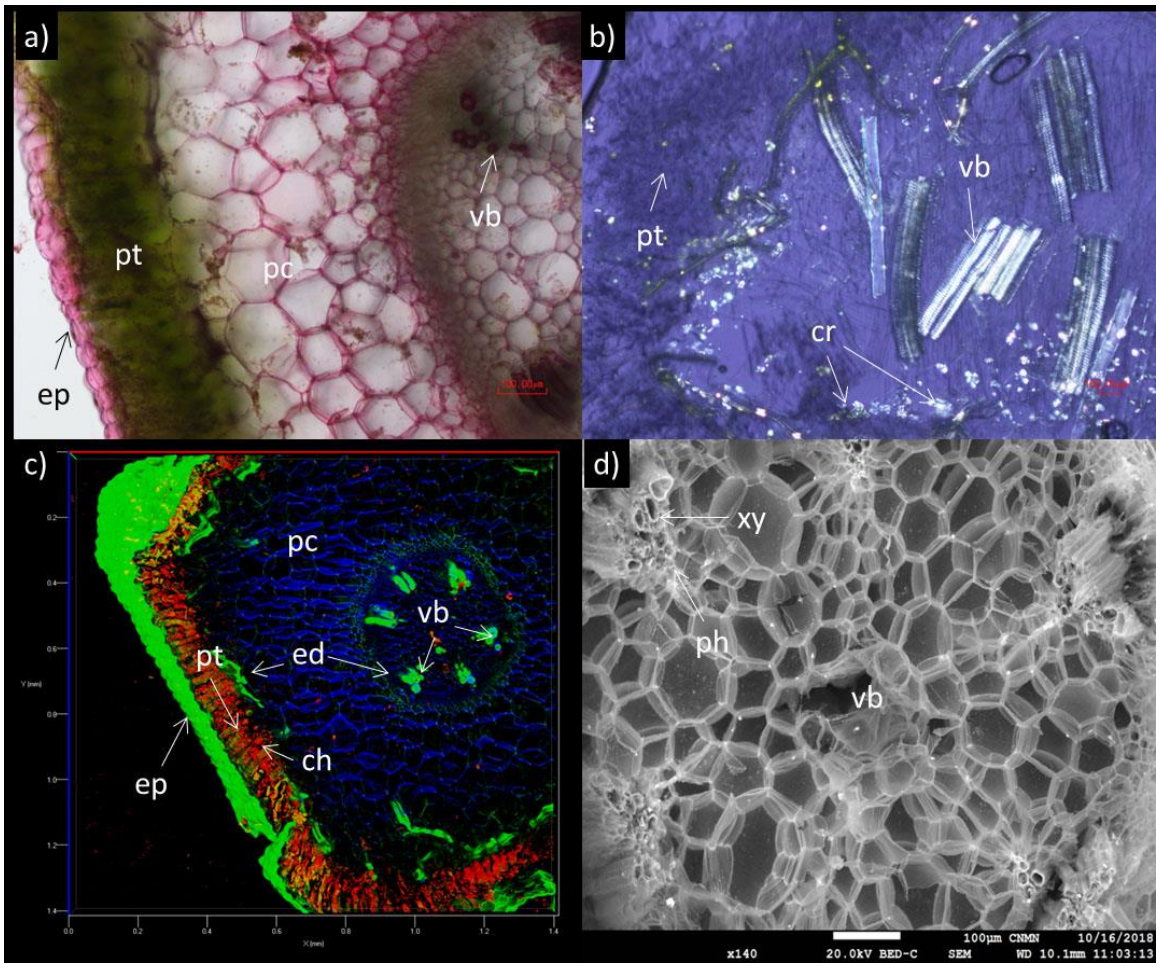

Figure 1. Microscopy images of stem cross sections of Salicornia bigelovii a) light microscopy (LM) image, b) LM image in polarized light, c) 3D confocal laser scanning microscopy (CLSM) image, d) field emission scanning electron microscopy (FE-SEM) image. ep: epidermis, pt: palisade tissue, vb: vascular bundle, pc: parenchyma cells, ed: endodermis, ph: phloem, xy: xylem, cr: NaCl crystals, In CLSM image, ch: chloroplasts and pt in red colour, pc in blue colour, stained in green colour due to lignin compounds ep, upper and lower ed and vb.

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