BRIEF COMMUNICATION

Presence of internal photosynthetic cylinder surrounding the stele in stems of the tribe *Salicornieae* (Chenopodiaceae) from SW Iberian Peninsula

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Abstract

Fluorescence microscopy and physiological examination revealed the presence of an inner cylinder of active photosynthetic cells located below the endodermis-like layer in stems of four of the six taxa of the tribe *Salicornieae* (Chenopodiaceae).

Additional key words: anatomy; *Arthrocnemum macrostachyum*; O_2 evolution; *Salicornia ramosissima*; *Sarcocornia fruticosa*; *Sarcocornia perennis* ssp. *alpini*; *Sarcocornia perennis* ssp. *perennis*; *Sarcocornia perennis*×*fruticosa*.

The family of Chenopodiaceae contains a number of halophytic genera which have stems composed of succulent assimilating internodes, giving the plants an articulated appearance (Castroviejo 1990). Each internode has a pair of oppositely arranged, but very reduced, scale leaves and the main photosynthetic organ is the stem. Phase contrast optics and fluorescence microscopy were used in order to describe the distribution of chloroplasts in the stems of six taxa of the tribe *Salicornieae* (Chenopodiaceae) from salt marshes of the SW Iberian Peninsula.

Clumps of *Arthrocnemum macrostachyum* Moric., *Salicornia ramosissima* J., *Sarcocornia perennis* ssp. *perennis* Miller, and the hybrid *Sarcocornia perennis*× *fruticosa* (Figueroa *et al.* 2003) were collected from the marshes surrounding the River Odiel (37º15´N, 6º58´W), *Sarcocornia fruticosa* L. clumps were collected from the marshes of the River Piedras (37º13´N, 7º9´W) and *Sarcocornia perennis* ssp. *alpini* Lag. from the Guadalquivir River marshes (37º7´N, 6º10´W). The classification of these six taxa is according to the Iberian Flora (Castroviejo 1990) and supported by evidence from RAPD markers (Luque *et al.* 1995).

Samples were taken from side stem branches rather than the principal stem of the plant. Stem fragments (succulent assimilating internodes) were fixed in 4 % glutaraldehyde diluted in a 0.1 M sodium cacodylate buffer solution, pH = 7.2, at 4 ºC for 2 h. After fixation, all the samples were frozen for cutting; 10 μm sections perpendicular to the stem axis were obtained using a cryomicrotome (*Kryomat 1703, Leitz*). The sections mixed with a glycerol-PBS solution were deposited onto slides and observed using phase contrast optics and fluorescence microscopy (*Aristoplan, Leitz*). Chloroplasts appeared red (662–728 nm) when exposed to green radiation (520–560 nm) due to their natural fluorescence. The images were captured by means of a digital camera *Leica DC-100*.

Photosynthesis was measured as O_2 evolution in order to confirm that the inner cylinder of chloroplast-containing cells was photosynthetically active. The inner core of the cylindrical stems was separated mechanically from the outer one. O_2 evolution was measured in an *LD2 Hansatech* leaf chamber with a gas phase O_2 electrode (Delieu and Walker 1983) at 25 ºC ($n = 3$). A buffer of 1 M carbonate/bicarbonate (pH 9.0) was used to provide a CO_2 saturated atmosphere. Irradiation (330 μmol m^{-2} s^{-1})

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Abbreviations: PPFD, photosynthetic photon flux density; RAPD, random amplified polymorphic DNA.
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was provided by a *Hansatech* source *LS2*. Photosynthetic photon flux density (PPFD) was modified with neutral density filters (*Balzers*) and measured with *Li-Cor* integrating quantum/radiometer/photometer using a *Li-190 SB* quantum sensor cell.

Phase contrast optical microscopy of transverse sections showed that all six taxa have the anatomic features described by SaadEddin and Doddema (1986) for *Sarcocornia fruticosa*; an outermost epidermis layer, a layer of palisade parenchyma, many layers of parenchyma with thin walls and few intercellular spaces, a layer of cells forming an endodermis-like layer surrounding the stele and within the stele, and six to eight collateral vascular bundles with parenchyma in between. In addition, a band (in fact, a cylinder) composed of several layers of small round cells situated just below the endodermis-like layer was evident in *A. macrostachyum*, *S. fruticosa*, *S. perennis*×*fruticosa*, and *S. perennis* ssp. *alpini* but not in *S. perennis* ssp. *perennis* and *S. ramosissima* (Fig. 1*A*).

Observations of the transverse sections under green radiation using fluorescence microscopy revealed that both the cells in this internal band (Fig. 1*B*) and those of the palisade parenchyma layer contained a high density of chloroplasts. Measurements of O_2 evolution showed that this inner band was photosynthetically active; taxa with the band showed high positive rates of O_2 evolution, while S. *ramosissima* and *S. perennis* ssp. *perennis* demonstrated, respectively, a very low and negative rate. Net photosynthetic rate varied between 6 and 12 µmol m^{-2} s^{-1} for the four taxa with the band but this difference was not found to be significant (ANOVA, $F = 1.53$; $p>0.05$) (Table 1).

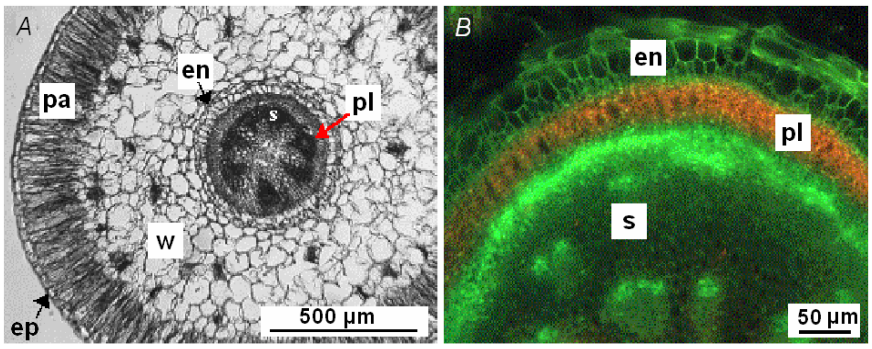

Fig. 1. *A*: Transverse section of succulent assimilating internodes of *Sarcocornia fruticosa* observed using phase contrast optics, showing epidermis (ep), palisade parenchyma (pa), water-storing parenchyma cells (w), endodermis (en), inner photosynthetic layer of cells (pl), and stele (s). Bar = 500 µm. *B*: Image of part of the centre of *Sarcocornia perennis* ssp. *alpini* under green radiation, using fluorescence microscopy. Chloroplasts appear as red. Bar = 50 µm.

Table 1. O_2 evolution [µmol m^{-2} s^{-1}] measured in a leaf chamber with a gas phase O_2 electrode at 25 ºC and PPFD of 330 µmol m^{-2} s^{-1}. Means ± standard error.

Taxa	O_2 evolution
Arthrocnemum macrostachyum	6.28 ± 0.36
Sarcocornia perennis ssp. *alpini*	12.28 ± 2.07
Sarcocornia perennis ssp. *perennis*	-2.11 ± 0.24
Sarcocornia perennis×*fruticosa*	10.48 ± 1.85
Sarcocornia fruticosa	9.04 ± 2.02
Salicornia ramosissima	0.35 ± 0.02

The presence of such an inner cylinder has not been described previously for the Chenopodiaceae family. Internal photosynthetic structures, such as the assimilatory cells found in the ground tissue of the centre of stems in *Vanilla* (Orchidaceae) (Stern and Judd 1999) and in the pericarp of grains of *Hordeum* (Cochrane and Duffus 1979), have been described in the literature. However, these are groupings of cells rather than a continuous layer next to the stele. C_4 species of the *Suaeda* genus of Chenopodiaceae have a specialised leaf anatomy where two distinct cell layers of chlorenchyma are located between the epidermis and central water-storage tissue but the C_3 species in this genus do not have these distinct layers (Fisher *et al.* 1997). None of the six taxa in this study have been shown to display C_4 properties (Nieva *et al.* 1999, Davy *et al.* 2001, Redondo Gómez 2004). If it is the case that the inner cylinder of chloroplasts is photosynthetically active under field conditions as well as in the laboratory, the source of CO_2 is uncertain. The inner chloroplasts are separated from the stomata by many layers of cells, including a double-layered endodermis-like structure and there are few intercellular spaces. However, research on tobacco, a typical C_3 plant, has shown that chloroplasts in cells of stems and petioles that surround the xylem and phloem are supplied with carbon for photosynthesis from the vascular system rather than the stomata (Hibberd and Quick 2002).

The distribution of chloroplasts in two distinct and separate layers raises interesting questions about both the origins and function of the inner cylinder. Further studies are necessary to see if the band has a role to play in the adaptation of the plant to the various environmental stresses experienced throughout the elevation gradient of the marshes.

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