Elemental Distribution in Shoots of Salicornia pacifica var. Utahensis as Determined by Energy-Dispersive X-Ray Microanalysis Using a Cryochamber
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ELEMENTAL DISTRIBUTION IN SHOOTS OF SALICORNIA PACIFICA VAR. UTAHENSIS AS DETERMINED BY ENERGY-DISPERSIVE X-RAY MICROANALYSIS USING A CRYOCHAMBER

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Cells in different regions of the stem of *Salicornia pacifica* var. *utahensis* were analyzed by energy-dispersive X-ray microanalysis using a cryochamber that maintains the tissue at liquid nitrogen temperature during sample preparation and analysis. The elements detected were Na, Mg, Al, Si, S, Cl, K, and Ca. The highest concentration of elements was in the cortex. The Na concentration was lower in the palisade than in the cortex. The dominant element in all the regions was Cl. The cortex contained 50% more Cl than the palisade. The protoplasm of the cortex contained ca. 80% of the Cl and the cell wall, 20%. The results are consistent with the concept of element compartmentalization in halophytes.

Introduction

Osmotic regulation in plants can be achieved by changes in internal concentration of inorganic ions and low-molecular-weight organic solutes (FLOWERS et al. 1977; WYN JONES 1984). Halophytes absorb ions and accumulate them in their tissues. Accumulated salts increase the osmotic-absorbing capacity of halophytic plants, allowing them to compete for water in a saline environment: e.g., dried stem tissues of *Salicornia pacifica* var. *utahensis* contain ca. 10% Na (HANSEN and WEBER 1975).

Most enzymes investigated in halophytes were salt sensitive (FLOWERS 1975; FLOWERS et al. 1977). However, in *Salicornia* spp., a few enzymes, such as ATPase (KIM and WEBER 1980), malate dehydrogenase (YOPP 1974), and peroxisomal glycolate oxidase (AUSTENFELD 1976), were salt tolerant. The essential photosynthetic and metabolic processes in these halophytes require compartmentalization of salts. WEBER et al. (1977) analyzed the element concentration of *S. pacifica* var. *utahensis* using an electron-microprobe system and found that the central cortex region of the stem was high in Na, K, and Cl, whereas the outer photosynthetic region (palisade) of the stem contained small quantities of these ions.

Ion loss occurs in cells when normal electron-microscope (EM) fixation procedures are used to prepare thin sections because the preparation methods result in ion diffusion. Many specialized procedures have been used to minimize or eliminate ion movement prior to and during elemental analysis. The procedures that offer the most promise for reducing artifacts of preparation involve quick freezing and rapid drying (TERRACIO et al. 1981), quick freezing and freeze-hydrated sectioning (ROSS et al. 1981), or examination of freeze-hydrated specimens (ECHLIN 1979; FORREST and MARSHALL 1980; ECHLIN et al. 1981*a*, 1981*b*; TALMON 1982). BECKETT (1982) demonstrated that freeze-drying and critical-point drying cause tissue shrinkage that was not evident for freeze-hydrated tissue. Therefore, use of a cryochamber in combination with energy-dispersive X-ray microanalysis (EDS) should make it possible to examine samples of tissue and not disturb the ion distribution because the tissue remains at liquid nitrogen temperatures at all times.

The purpose of this study was to determine the ion distribution in tissues of different shoot regions of *S. pacifica* var. *utahensis* by using a cryochamber and EDS microanalysis.

Material and methods

Salicornia pacifica var. *utahensis* (Tidestrom) Munz was obtained from natural habitats near Goshen, Utah, in the fall 1984. The shoot pieces were mounted on special cup-shaped graphite-lined aluminum stubs, as suggested by PATRICK ECHLIN (Univ. of Cambridge, England, personal communication). The mounted shoot pieces were fractured under liquid nitrogen and then transferred to a cryochamber that maintains the tissue at liquid nitrogen temperatures. The tissue was then transferred, under vacuum, into the precooled chamber of a scanning electron microscope (SEM). Cells were analyzed with EDS in the freeze-hydrated tissues of the epidermis, cortex, palisade, and vascular stem. In addition, the cell walls of the cortex and palisade were also analyzed. Each tissue was analyzed four times in four different areas to provide replications for statistical analysis.

The EDS was conducted with an EDAX 9100 interfaced with an AMRay 1000A SEM. The voltage for analysis was 10 kV, and analysis times were 100 s at ca. 3,000 cps. For SEM studies, tissues

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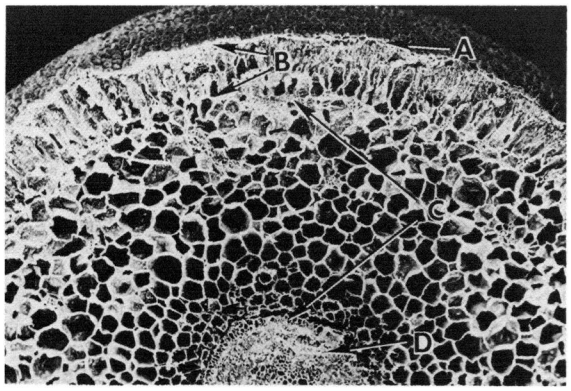

FIG. 1.—SEM micrograph of a cross section of a stem of *Salicornia pacifica* var. *utahensis* showing the four prominent tissue regions: epidermis (*A*), palisade (*B*), cortex (*C*), and vascular (*D*). ×36.

were fixed, stained with OsO_4, and dehydrated (HESS 1966). The samples were critical-point dried with liquid CO_2, mounted on stubs with double stick tape and gold-sputter coated before being photographed.

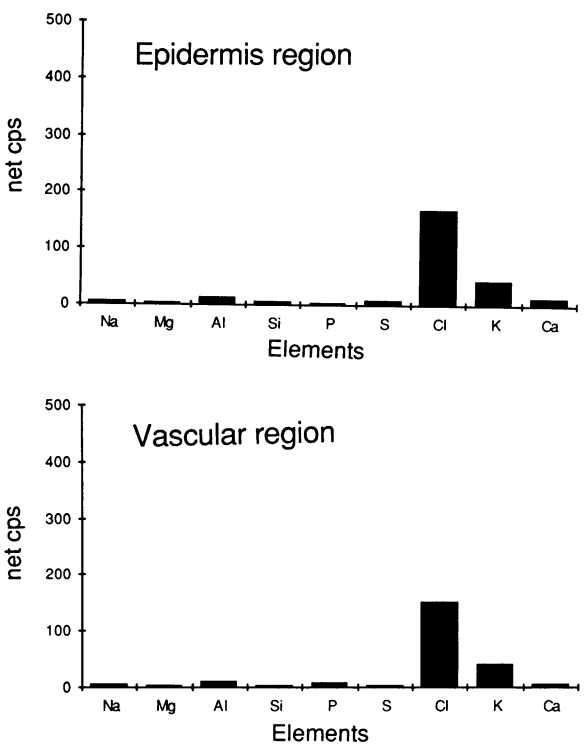

FIG. 2.—Ion distribution of epidermis and vascular regions of *Salicornia pacifica* var. *utahensis* as determined by EDS. The Na, K, and Cl content of the cortex region is significantly different (5% level) from the element content in the other regions. The Ca content of the palisade region is significantly different (5% level) from the element content of the other regions. The Al content of the cortex and palisade region is significantly different (5% level) from the Al content of the other two regions.

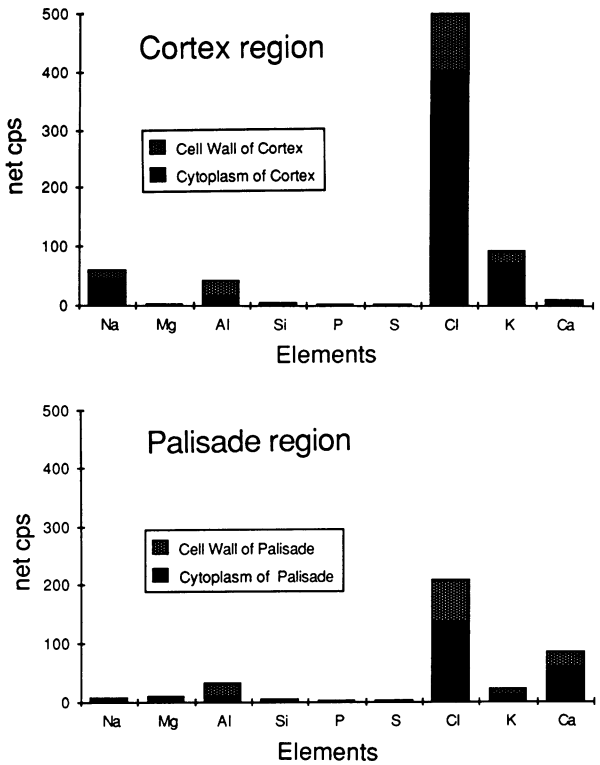

FIG. 3.—Ion distribution of cortex and palisade regions of *Salicornia pacifica* var. *utahensis* as determined by EDS.

Results

Salicornia shoots have four prominent tissue regions (fig. 1). The most abundant elements in the epidermis and vascular regions were Cl and K. The most dominant element in all four tissue regions was Cl. Elemental content in the epidermis and vascular region was similar (fig. 2). In the cortex, Cl and K were the most abundant; Na and Al were the next most abundant. In addition, Ca, K, Na, and Al were all present in the cell walls (fig. 3). In the palisade (fig. 3), all of the elements were present in low concentrations except Cl and Ca. Also present in cell walls were Cl, Ca, Al, and K. The amount of Cl in the protoplasm was more than twice as much as in cell walls, as were Na and K.

In the palisade cells, the concentration of Cl was only ca. 40% of the level in cortex cells (fig. 3); Cl, Ca, K, and Al were detectable in both cell walls and cytoplasm; and Cl and Ca were most abundant in the palisade.

Discussion

WEBER et al. (1977) analyzed the frozen shoots of *Salicornia pacifica* var. *utahensis* using an electron-probe X-ray analyzer. The electron probe had limited resolution and could not aid in localizing elements within specific cells and organelles. In addition, when frozen tissues were analyzed without the use of a cold stage and a clean vacuum,

there was a possibility of movement of ions from one cell to another and the formation of artifacts (FIORI et al. 1979; LECHENE 1980; MACINNES 1980).

The net CPS (counts per second) represents relative values of the different elements, and although they are not easily converted to absolute concentrations because of the pear-shaped area of analysis involved in bulk analysis with EDS, net CPS are useful in comparing different tissues and cells (LECHENE 1980).

The conclusion that the cortex contains the highest total ion concentration and concentrations of Na and Cl was consistent with the findings of WEBER et al. (1977) and with statistical analyses (5% level). Although salts accumulate in the cortex, the photosynthesizing palisades can continue to function. These results also indicate that ions accumulate in specific regions since elemental concentrations were different in different tissues. WEBER et al. (1977) showed that palisade cells in young shoots were low in salts and that the spongy cells near the center of the stem had relatively high concentrations. As shoots matured, the concentration of salts increased in the spongy cells, and the amount of salts in palisade cells also increased. ESHEL and WAISEL (1979) found that Na selectively accumulated in the outer layers of chlorenchyma cells in the halophyte *Suaeda monoica,* whereas Cl was concentrated mostly in the inner collenchyma cells.

Our data indicate (1% level) that there is a higher concentration of elements in the cytoplasm than in the cell wall. Histochemical studies at the light-microscope level with *S. pacifica* var. *utahensis* indicated that the Cl concentration was low in the chloroplasts and high in the cytoplasm (HESS et al. 1975). STOREY et al. (1983) studied freeze-hydrated cells of young and mature tissues of *Atriplex spongiosa* with X-ray microanalysis and reported low K, Na, and Cl and high K selectivity in the bundle sheath cytoplasm. VAN STEVENINCK et al. (1976) studied the mangrove *Aegiceras* and found Cl both in cytoplasm and vacuoles of leaf cells by silver-chloride precipitation. HARVEY et al. (1978) used silver-chloride precipitation and X-ray microanalysis techniques and found Cl in chloroplasts and in vacuoles of *Suaeda maritima* leaf cells. The content of Na and K in the cytoplasm of these cells was low.

It is surprising that high levels of Cl are present in all four of the tissue regions of *S. pacifica* var. *utahensis*. Also, the total amount of Cl within a particular tissue was not balanced by an equal amount of cation elements. This raises the question of whether there are organic compounds with positive charges present. One other factor may be that the detection of sodium is at the lower level of sensitivity of the Si(Li) detecting crystal. Correction factors are included in the computer program, but these factors require analysis of a perfectly flat surface. Although surface geometry is better with freeze-hydrated tissue than with freeze-dried or critical-point-dried tissue, it is still not possible to have a perfectly flat surface for analysis, and detection efficiency is hampered by the presence of ice (TALMON 1982). MARSHALL (1982) and JAFFEE and GLAESER (1984) discussed how some of the disadvantages of hydration can be overcome. On the other hand, the use of a cryochamber to study freeze-hydrated tissues in conjunction with EDS provides a method to study elemental composition in tissues without exposing ions to leaching solutions, but there are still many questions that need to be answered concerning ion localization and distribution.

LECHENE (1980) pointed out that, for best results, tissues should be either freeze-dried or freeze-hydrated and thin sections or bulk samples should be analyzed. All analyses should be performed using a cold stage with a clean vacuum.

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LITERATURE CITED

AUSTENFELD, F. A. 1976. The effect of various alkaline salts on the glycolate oxidase of *Salicornia europaea* and *Pisum sativum* in vitro. Physiol. Plantarum **36**:82–87.

BECKETT, A. 1982. Low-temperature scanning electron microscopy of the bean rust fungus *Uromyces viciae-fabae*. Philips Electron Optics Bull. no. 117, pp. 6–8.

ECHLIN, P. 1979. Low temperature scanning electron microscopy and X-ray microanalysis of biological material. Microskopie **35**:45–49.

ECHLIN, P., C. E. LAI, and T. L. HAYES. 1981*a*. The distribution and relative concentration of potassium in the root-tips of *Lemna minor* L. Scanning Electron Microsc. **11**:383–394.

———. 1981*b*. The distribution and relative concentration of potassium in the root-tips of *Lemna minor* L. analyzed by low temperature X-ray microanalysis. Scanning Electron Microsc. **11**:489–498.

ESHEL, A., and Y. WAISEL. 1979. Distribution of sodium and chloride in leaves of *Suaeda monoica*. Physiol. Plantarum **46**:151–154.

FIORI, C. E., R. L. MYKELBUST, and D. E. NEWBURY. 1979. A catalogue of artifacts observed in energy-dispersive X-ray spectrometry and their influence on analysis. Pages 225–263 *in* C. P. LECHENE and R. R. WARNER, eds. Microbeam analysis in biology. Academic Press, New York.

FLOWERS, T. J. 1975. Halophytes. Pages 309–340 *in* D. A. BAKER and J. L. HALL, eds. Ion transport in plant cells and tissues. North Holland, Amsterdam.

FLOWERS, T. J., P. F. TROKE, and A. R. YEO. 1977. The mechanism of salt tolerance in halophytes. Annu. Rev. Plant Physiol. **28**:89–121.

FORREST, Q. G., and A. T. MARSHALL. 1980. Electron-probe X-ray microanalysis of fully-frozen hydrated bulk-specimens of axons from connective, Micron **11**:399–400.

HANSEN, D. J., and D. J. WEBER. 1975. Environmental factors in relation to the salt content of *Salicornia pacifica* var. *utahensis*. Great Basin Nat. **35**:86–96.

HARVEY, D. M. R., T. J. FLOWERS, and J. L. HALL. 1978. Intracellular localization of ions in the halophyte *Suaeda maritima*. Proc. 1st Meet. Eur. Soc. Plant Physiol. Pp. 248–249.

HESS, W. M. 1966. Fixation and staining of fungus hyphae and host plant root tissues for electron microscopy. Stain Technol. **41**:27–35.

HESS, W. M., D. J. HANSEN, and D. J. WEBER. 1975. Light and electron microscopy localization of chloride ions in cells of *Salicornia pacifica* var. *utahensis*. Can. J. Bot. **53**:1176–1187.

JAFFEE, J. S., and R. M. GLAESER. 1984. Preparation of frozen-hydrated specimens for high resolution electron microscopy. Ultramicroscopy **13**:373–378.

KIM, C. K., and D. J. WEBER. 1980. Isolation and characterization of adenosine triphosphatase from *Salicornia pacifica* var. *utahensis*. Plant Cell Physiol. **21**:755–763.

LECHENE, C. 1980. Electron probe microanalysis of biological soft tissue: principle and technique. Federation Proc. **39**:2871–2880.

MACINNES, D. G. 1980. A contamination free method for the transfer of specimens from liquid nitrogen onto a S.E.M. cold stage. Cryo-Letters **1**:343–348.

MARSHALL, A. T. 1982. Application of $\phi(\rho z)$ curves and a windowless detector to the quantitative X-ray microanalysis of frozen-hydrated bulk biological specimens. Scanning Electron Microsc. **1**:243–260.

ROSS, A., A. T. SUMNER, and A. R. ROSS. 1981. Preparation and assessment of frozen-hydrated sections of mammalian tissue for electron microscopy and X-ray microprobe analysis. J. Microsc. **121**:261–272.

STOREY, R., M. G. PITMAN, R. STELZER, and C. CARTER. 1983. X-ray microanalysis of cells and cell components of *Atriplex spongiosa*. J. Exp. Bot. **34**:778–794.

TALMON, Y. 1982. Thermal and radiation damage to frozen hydrated specimens. J. Microsc. **125**:227–237.

TERRACIO, L., P. W. BANKSTON, and J. A. MCATEER. 1981. Ultrastructural observations on tissues processed by a quick-freezing, rapid-drying method: comparison with conventional specimen preparation. Cryobiology **18**:55–71.

VAN STEVENINCK, R. F. M., W. D. ARMSTRONG, P. D. PETERS, and T. A. HALL. 1976. Ultrastructural localization of ions. III. Distribution of chloride in mesophyll cells of mangrove (*Aegiceras corniculatum blanco*). Aust. J. Plant Physiol. **3**:359–365.

WEBER, D. J., H. P. RASMUSSEN, and W. M. HESS. 1977. Electron microprobe analyses of salt distribution in the halophyte *Salicornia pacifica* var. *utahensis*. Can. J. Bot. **55**:1516–1523.

WYN JONES, R. G. 1984. Phytochemical aspects of osmotic adaptation. Pages 55–78 *in* B. N. TIMMERMAN, C. STEELINK, and F. A. LEOWUS, eds. Phytochemical adaptation to stress. Plenum, New York.

YOPP, J. A. 1974. Effect of low water potential on the activity of mitochondrial, chloroplast and supernatant malic dehydrogenase from the halophyte *Salicornia pacifica*. Trans. Ill. State Acad. Sci. **67**:20–27.