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ELEMENTAL DISTRIBUTION IN SEEDS OF THE HALOPHYTES SALICORNIA PACIFICA VAR. UTAHENSIS AND ATRIPLEX CANESCENS[1]

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ABSTRACT

Little information is available on the distribution of ions in seeds of halophytes. Seeds of two halophytes, *Salicornia pacifica* var. *utahensis* (Tidestrom) Munz, a desert salt playa type, and *Atriplex canescens* (Pursh) Nutt., a desert shrub, were analyzed by energy dispersive X-ray micro-analysis. The relative ion concentration in three regions, the seed coat, endosperm, and embryo, were determined. The total relative concentration of elements was higher in seeds of *S. pacifica* var. *utahensis* as compared to *A. canescens*. The seed coats of *S. pacifica* var. *utahensis* contained the highest counts of sodium, chlorine, potassium, and calcium, whereas the embryo and endorsperm were both high in phosophorus. In *A. canescens*, sodium and chlorine were very low in all three tissues. The embryo contained the major amount of phosphorus, although potassium was high in both the seed coat and the embryo. These results support the concept of ion compartmentalization in the seeds of these two halophytes.

HALOPHYTES are plants that can grow and develop successfully in the presence of high salinity. In some plants, such as *Salicornia*, high salt concentration is a requirement for growth and development (Waisel, 1972). Other halophytes, such as *Atriplex canescens*, can survive low to moderate salinity, but they can complete their life cycle in the absence of salinity. Previous studies with *Salicornia pacifica* var. *utahensis* (Weber, Rasmussen and Hess, 1977) indicated that compartmentalization of elements occurs in the fleshy stem. The cortex cells had a higher ion concentration as compared to the photosynthetic palisade cells. Electron microscopy research using precipitation of chloride ions with silver nitrate indicated that compartmentalization of salt occurred even in the palisade cells (Hess, Hansen and Weber, 1975). It has been reported that ion concentrations in the fleshy material of *Salicornia* are very high (Hansen and Weber, 1975); therefore, it was assumed that the seeds would also have relatively high ionic concentrations.

Ionic content in shoots of *Salicornia* is about 15% of the total dry weight (Hansen and Weber, 1975; Poulin et al., 1978; Hocking, 1982; Ungar, 1984). This indicates that plants can control the amount of $Na+$ and $Cl-$ transported to seeds, since these elements are maintained at lower concentration in the seeds than in shoots. In this manner, halophytic plants avoid injury from ionic and osmotic stress during the period of embryonic development. There is no information available on the ion compartmentalization in seeds of halophytes. Although there are many artifacts that could occur when energy-dispersive X-ray microanalysis is used to analyze specimens (Fiori, Myklebust and Newbury, 1979), the use of scanning images of characteristic X-ray signals makes it possible to determine where elements are concentrated. This technology has been used to study a wide variety of biological specimens including both plant (Bennet and Parry, 1981; Saka, 1982; Strullu et al., 1981) and animal (Allen et al., 1981; Lechene, 1980) tissues.

It has been pointed out that for best results, tissues should be either freeze-dried or frozen-hydrated, and in all cases analysis should be performed using a cold stage with clean vacuum (Lechene, 1980). Data obtained with X-ray microanalysis should be evaluated with caution even when ideal conditions are used.

Two halophytes were selected to investigate the distribution of ions in halophyte seeds by use of scanning images of characteristic X-ray signals and computer tabulation of counts. *Salicornia pacifica* var. *utahensis* is an obligate halophyte that grows in salt playas and requires moist soils in order to survive in the saline environment. *Salicornia* tissue has high salt tolerance and is one of the last plants to survive under the highly saline conditions that occur in the salt playas (Hansen and Weber, 1975). *Atriplex canescens* is more drought resistant,

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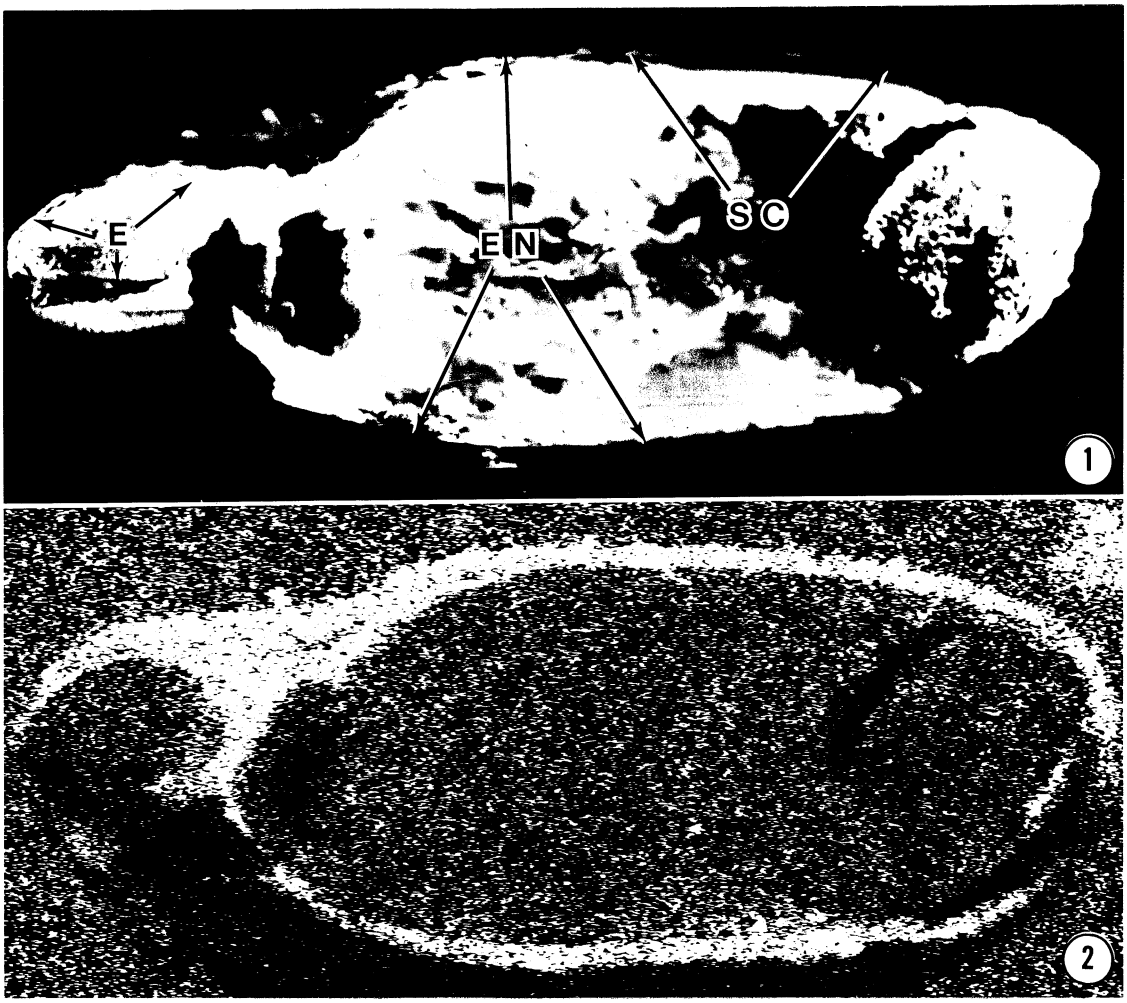

Fig. 1, 2. **1.** Scanning electron micrograph of a cross-section of an uncoated seed of *Salicornia pacifica* var. *utahensis* showing the embryo (E), endosperm (EN), and seed coat (SC). ×112. **2.** Scanning image of X-ray signals for chlorine of the same cross section of the seed of *Salicornia pacifica* var. *utahensis* shown in Fig. 1. × 112. The light area represents the location of the chlorine.

and, although it grows on saline soils, it cannot tolerate the high levels of salinity in salt playas. This investigation was undertaken to determine the ion distribution in seeds of these two plants from different habitats using energy-dispersive X-ray microanalysis.

MATERIALS AND METHODS—Seeds of *Salicornia pacifica* var. *utahensis* (Tidestrom) Munz were collected from natural habitats near Goshen, Utah. Seeds of *Atriplex canescens* (Pursh) Nutt. were obtained from Howard Stutz (Brigham Young University). These seeds were collected from field locations in central Utah.

All seeds were cut with razor blades and mounted on carbon stubs with graphite blue. Seeds were not coated with carbon or gold before or after analysis. Although they are not as aesthetically pleasing to look at as shadowed specimens, morphological comparisons of uncoated seeds with scanning images of X-ray signals made it possible to compare ion concentration with seed morphology.

Energy-dispersive X-ray microanalysis was conducted with an EDAX 9100 interfaced with an AMRay 1000A scanning electron microscope (SEM). The voltage for analysis was 20 kv, and analysis times were 100 sec at about 3,000 counts per second (cps).

For each species, four different seeds were analyzed. On each seed, four similar regions were examined and analyzed to provide replications for statistical analysis using Duncan multiple-range analyses.

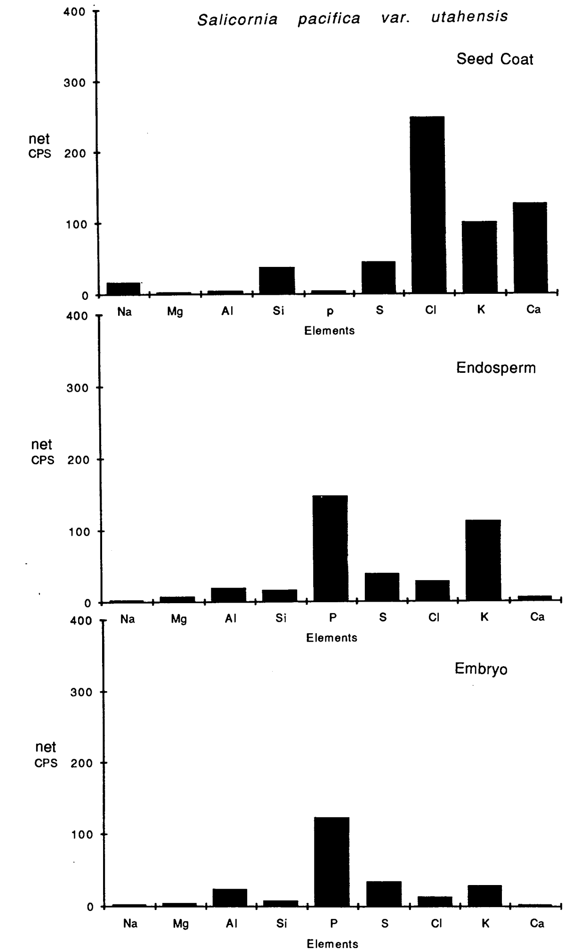

Fig. 3. Distribution of nine elements in seed coat, endosperm, and embryo of *Salicornia pacifica* var. *utahensis* as determined by energy dispersive X-ray microanalysis. Data is in net counts per second (cps).

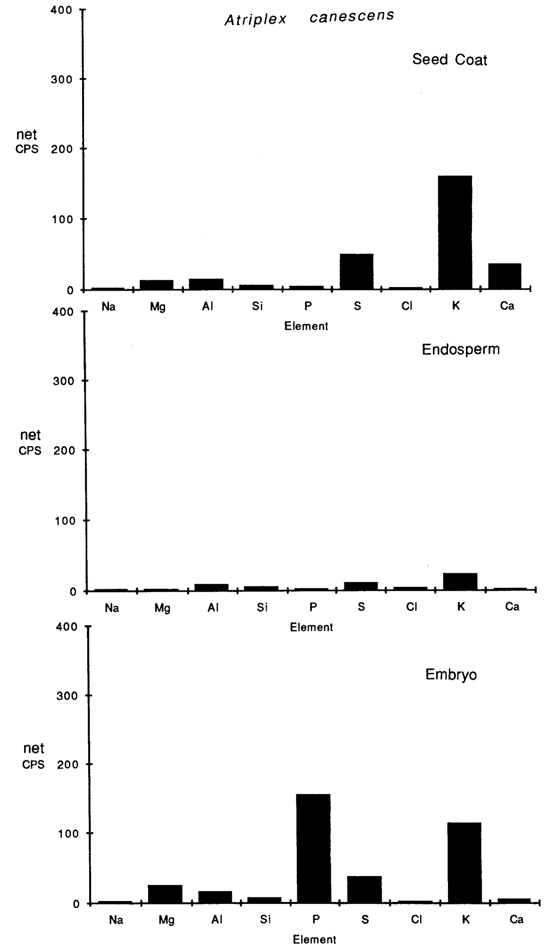

Fig. 4. Distribution of nine elements in seed coat, endosperm, and embryo of *Atriplex canescens* as determined by energy dispersive X-ray microanalysis. Data is in net counts per second (cps).

RESULTS—Figures 1, 2 show a scanning electron micrograph and an elemental map of chlorine for an *S. pacifica* var. *utahensis* seed. The relative elemental concentrations were determined for the embryo, endosperm, and seed coat. Elements that were high in the seed coat were sodium, chlorine, potassium, calcium, and silicon. In contrast to the seed coat, sodium and chlorine were low in the endosperm and the embryo ($\alpha = 0.05$). The highest counts of potassium were in seed coats and endosperm. Phosphorus was present at low concentrations in the seed coats but was high in endosperm and embryo tissue ($\alpha = 0.05$). Magnesium and sulphur counts were low in all three regions of these seeds (Fig. 3). Aluminum was lower in the seed coat as compared to the endosperm and the embryo ($\alpha = 0.05$). The element with the highest concentration was chlorine in the seed coat of *S. pacifica* var. *utahensis* (Fig. 2).

The amount of detectable sodium was very low in the seed coat of *Atriplex canescens.* The bracts were removed from seeds that normally contain sodium. Chlorine was very low in all tissues including the seed coat (Fig. 4). These findings also suggest that little sodium and chlorine are moved into the seed or seed coat of *A. canescens.* In contrast, the concentration of potassium and calcium was highest in the seed coat. The amount of magnesium was significantly ($\alpha = 0.05$) higher in the embryo as compared to the seed coat and endorsperm. Low amounts of aluminum were present in all three tissues. Phosphorus was very high in the embryo and low in the endosperm and seed coat ($\alpha = 0.05$). The level of potassium were low and significantly different ($\alpha = 0.05$) from the concentration in the seed coat and embryo.

In comparing the elements in the seed coat of both species, the obvious difference is the high concentrations of sodium, calcium, and chlorine in *S. pacifica* var. *utahensis,* whereas in *A. canescens,* the elements with the highest concentrations are potassium, sulfur, and calcium. The amount of phosphorus in the endosperm is higher in *S. pacifica* var. *utahensis* than in *A. canescens.* Overall, the total concentration of the elements in the seed coat and endosperm of *S. pacifica* var. *utahensis* is higher than the embryo of *S. pacifica* var. *utahensis.* Concentrations of phosphorus and potassium are much higher in embryos than in endosperms of *A. canescens.*

DISCUSSION—Energy dispersive X-ray microanalysis of the seeds of these two halophytes supports the concept that compartmentalization occurs in seeds. Seeds usually have a very low concentration of salt as compared to leaf tissue (Ungar, 1984), which indicates that concentrations of ions vary in different plant parts.

Seed coats of *S. pacifica* var. *utahensis* had high levels of chlorine, potassium, calcium, and sodium, whereas embryos and endosperm had high levels of phosphorus and potassium. In contrast, seed coats of *A. canescens* contained little sodium and chlorine but larger amounts of potassium were present. Similar to *S. pacifica* var. *utahensis,* the embryo of *A. canescens* contained a high concentration of phosphorus. The phosphorus is assumed to be in organic compounds associated with the development of the embryo. The low concentration of sodium and chlorine in the embryo makes it possible for the embryo to avoid salt injury during germination (Ungar, 1984).

The two halophytes are from two different types of saline environments. The more moist and saline environment where *S. pacifica* var. *utahensis* grows appears to be correlated with ion concentration in the seeds. In contrast, the seeds of *A. canescens,* growing in a drier, less-saline environment, have a lower concentration of sodium and calcium. These findings suggest that ion concentration of seeds is related to environmental factors.

The seeds of *Atriplex* spp. are almost always formed between bracts, and the bracts contain high levels of Na and Cl (Osmond, Bjorkman and Anderson, 1980). Beadle (1952) demonstrated that the concentration of salt in moistened bracts of five species of *Atriplex* was sufficiently high to prevent germination. It may be that the bracts act as a salt accumulation area (Beadle, 1952), and that sodium and chlorine do not accumulate in the seed coat.

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