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Vesiculated Hairs: A Mechanism for Salt Tolerance in *Atriplex halimus* L.

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ABSTRACT

The concentration of salts in the vesiculated hairs of *Atriplex halimus* L. was measured and was remarkably higher than that of the leaf sap and xylem exudate. In spite of their unusually high salt content, these hairs when immersed seemed unable to absorb water, in apparent contradiction to the previously held hypothesis that vesiculated hairs make it possible for such plants to absorb water from the atmosphere. Although growing the plants under saline conditions increased the salt content of the hairs from 2.3 M Na+K to 11.6 M, salt content of the expressed leaf sap from young leaves did not change significantly. This observation indicates that in *A. halimus* the vesiculated hairs play a significant role in removing salt from the remainder of the leaf and preventing the accumulation of toxic salts in the parenchyma and vascular tissues. Thus, a nearly constant salt content is maintained in leaf cells other than the hairs.

An important problem facing arid zone agriculture is the accumulation of excess soluble salts in many irrigated soils, which can greatly reduce productivity of crop plants (3). Plants vary in the degree of their salt tolerance and in the means by which they regulate salt content of their tissues. Mechanisms such as salt exclusion, salt accumulation, and salt secretion and export by means of salt glands are reported for different plants in coping with saline media (1, 16, 19).

Atriplex species are among the few salt-tolerant plants that have any agricultural value in extremely arid and saline areas because of their unusually high protein content and exceptional salt tolerance (6). These plants do not have salt glands but are characterized by the presence of several layers of balloon-like, vesiculated hairs (trichomes) on the leaf surface (4). Different functions have been assigned to these hairs, such as absorption of water from the atmosphere (4, 9, 20), water storage, and salt secretion (17, 22). Upon bursting, these cells deposit salt on the surface of the leaves. However, the salt content of these hairs has never been measured to see how effectively they function in salt removal (18). Nor has direct absorption of water by the hairs ever been studied. Wood (20) suspended *Atriplex* twigs in a humid atmosphere and observed an increase in weight. He concluded that water was absorbed through the hairs (4).

MATERIALS AND METHODS

Atriplex halimus L., an extremely salt-tolerant plant (8), was grown in the greenhouse for 10 weeks in Hoagland's solutions (10), 6 mM with respect to NaCl. Salinity treatments consisted of 16 levels of added NaCl and KCl in equal concentrations and each ranging from 0 to 100 mM. All salts were added at the beginning of the experiment. All treatments were replicated twice and each replicate consisted of three seedlings growing in a 7-liter container.

Plant twigs were suspended in large filter flasks and were washed for 48 hr with running distilled water to remove any superficial salt contamination caused by broken hairs.

Electrical Conductivity. With a stereoscopic microscope and a micro-needle, 5 hairs (90 μ in radius and 3×10^{-6} ml in volume, assuming a perfect sphere) were dissected from the third leaf below the apex and broken in 0.2 ml of glass-distilled water (dilution of 1.33×10^4 times). During this process, utmost care was exercised in handling the leaves and sampling the hairs in order to prevent rupture and contamination by adjacent hairs. Concentration of salts (EC)[1] in these diluted samples was measured by a microconductivity cell. In addition, approximately 100 μl of leaf sap were collected by forcing several unwashed leaves inside Tygon tubing and crushing with a metal rolling pin to express the leaf sap (11). A pressure chamber was used to collect approximately 50 μl of xylem exudate from shoots (10). The EC of these samples was also measured.

Absorption Spectrometry. Na^+ and K^+ concentrations were obtained by breaking 50 hairs (one at a time) in 7 ml of glass-distilled water and analyzing the solutions with a Perkin-Elmer atomic absorption spectrophotometer.

Microchemical Determination of Oxalate. Since oxalic acid constitutes up to 75% of the total acids in *Atriplex* species (13) and chloride salts are reported to accumulate in large quantities in *Atriplex* leaves (7, 20), the oxalic acid as well as the chloride content of the hairs was of interest with regard to the ionic balance inside the hairs. Oxalic acid content was measured by the following microtechnique derived from the method described by Palmer (14): 10 hairs were broken inside reaction vessels (300 μl volume) followed by the additon of 100 μl of glass-distilled water and 10 μl of concentrated H_2SO_4. After warming the reaction vessels to 65° and stirring with the aid of a magnetic bead, the solutions were titrated with 0.01 N $KMnO_4$ in a Linderstrom-Lang-Holter microburette (delivery accuracy of 0.02 μl).

Microchemical Determination of Chloride. The chloride content of the hairs was measured by the following microtechnique derived from the method described by Chapman and Pratt (5): 10 hairs were first crushed inside reaction vessels to which 50 μl of glass-distilled water were added. After adding 50 μl of the 1% aqueous solution (w/v) of potassium chromate, the solution was titrated by a microburette with 0.01 N $AgNO_3$ to a faint red color of the silver chromate precipitate.

[1] Abbreviation: EC: electrical conductivity.

The accuracy of both microtechniques was verified by placing known quantities of oxalic acid and sodium chloride in the vessels and following the above procedures.

RESULTS AND DISCUSSION

Leaves of *A. halimus* are covered with several layers of balloon-like hairs (Fig. 1). Concentration of Na^+ and K^+ inside the hairs was very high and increased significantly in plants growing on saline media (Table I). Cl^- content of the hairs also increased with salt treatment which balanced most of the Na^+ and K^+ in the hairs. Oxalate concentration did not change but contributed toward balancing Na^+ and K^+ (Table II).

In contrast to the dramatic increase in salt concentration of the hairs with salt treatment (Table I), the salt concentration of the expressed leaf sap did not change appreciably over the whole range of external salt concentrations (Fig. 2). The salt concentration of xylem exudate decreased at low salinities and increased slightly at higher ones, but it remained much below that of the leaf sap.

Vesiculated hairs are believed to increase transpiration and consequently salts are accumulated within the hairs (9). However, in our experiments, salt concentration in the hairs increased markedly with increase in salt concentration of the growing medium, while that of the expressed leaf sap did not (Table I).

A comparison of salt concentrations in the hairs and leaf sap reveals about a 60-fold difference, the EC of leaf sap being about 20 mmho/cm and the calculated EC of the hair sap about 1200 mmho/cm. This remarkable ability of the plant to remove salts from the leaf cells and accumulate them in the superficial hairs results in a comparatively low level of salts within the remainder of the leaf (Fig. 2). This phenomenon may be of physiological significance in maintaining low concentrations of toxic salts within parenchymatous and vascular tissues of plants growing under saline conditions.

It was not possible to dissect all vesiculated hairs from the leaf surface nor to measure the volume of hairs relative to the remainder of the leaf prior to extraction. Therefore, data obtained for electrolytes in the leaf sap may also include electrolytes present in the hairs. To minimize the contamination of expressed leaf sap by salts deposited on the leaf surface and by salts expressed from the hairs by rupture, we chose to use very young leaves (third leaf below the apex). We do not know how many hairs burst during extraction of leaf sap. If many hairs had burst, it may be argued that they did not contain a sufficient quantity of salts to significantly change the EC of leaf sap and, therefore, the hairs could not be of major importance in salt removal. However, workers have often observed that hairs form and burst continuously, deposit salt crystals on the surface of the leaf, build up a thick salt litter, and are replaced by new layers of hairs underneath (4, 12, 18). The surfaces of young leaves used in our experiments were covered with physiologically young vesicles, of which few, from microscopic observation, had burst. Therefore, salts accumulated on the surface of these young leaves were inconsequential in increasing the EC of leaf sap.

Furthermore, studies have shown that total salt content of the mature *A. halimus* leaves increases significantly with salinity treatments (12). Mature leaves of *A. halimus* were covered with a thick layer of broken vesiculated hairs, and the crystals of deposited salts on the surface could easily be seen under a stereoscopic microscope (12). Up to 50% of the total chloride present in *Atriplex* leaves may be on the surface (12). It is therefore apparent that these hairs, through constant formation and bursting, can remove salts from the leaves of *A. halimus*. The quantity of salts removed would depend on the age of leaves, rate of formation and bursting of hairs, and salinity of the growing medium.

Osmotic adjustment, a parallel decrease in the osmotic potential of the leaf cells in response to a decrease in the osmotic potential of the growing medium, has been proposed as a mechanism

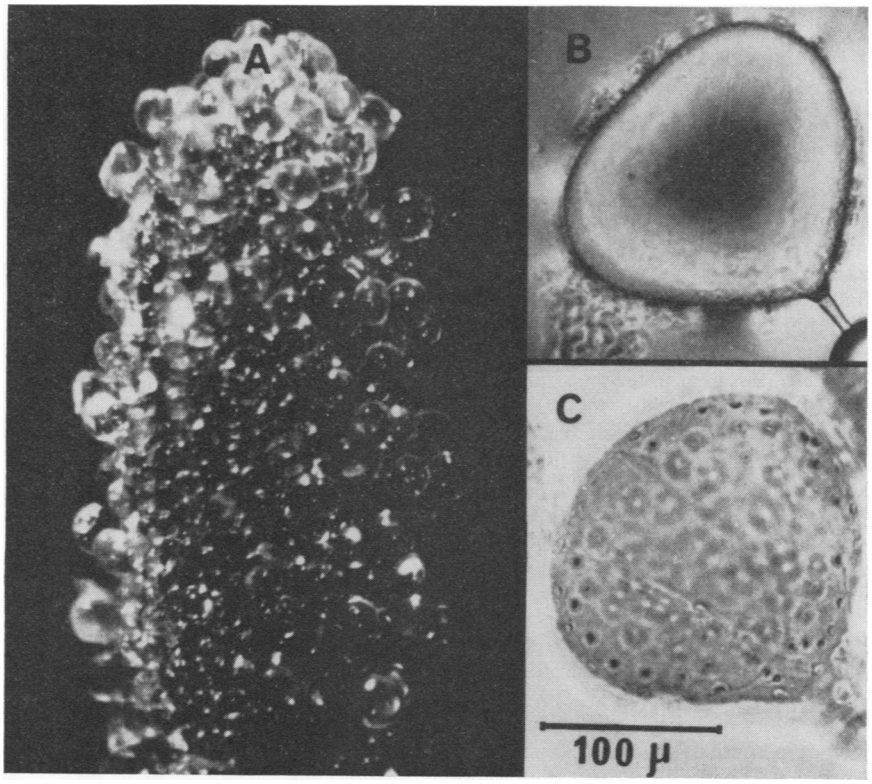

FIG. 1. A: Vesiculated hairs on the terminal bud of a 15-day-old *Atriplex halimus seedling*. B: Vesiculated hair attached to its stalk. Note the dark region at the point joining stalk and the balloon-like hair. C: Rugged surface of a vesiculated hair as seen under phase microscopy.

Table I. *Effect of Salt Treatment (100 mM NaCl + 100 mM KCl) on EC and Na^+ and K^+ Concentrations of Vesiculated Hairs of A. halimus*

Values are means of five replicates ± standard error.

Treatment	EC of Hair Contents Diluted 1.33 × 10^4	Na^+ in Hairs	K^+ in Hairs
	μmhos	M	M
Control	20.8 ± 4.3	1.33 ± 0.1	0.98 ± 0.1
Salt-treated	90.5 ± 6.8	5.48 ± 0.4	6.17 ± 0.6

Table II. *Effect of Salt Treatment (100 mM NaCl + 100 mM KCl) on Cl^- and Oxalate Concentrations in Vesiculated Hairs of A. halimus*

Values are means of three replicates ± standard error.

Treatment	Chloride	Oxalate
	M	M
Control	1.7 ± 0.27	1.3 ± 0.16
Salt-treated	9.2 ± 0.81	1.2 ± 0.11

by which plant cells maintain their turgidity under saline conditions (2).

In *A. halimus*, osmotic adjustment with respect to electrolytes in the leaf did not occur, as is evident from the dissimilar salt concentrations of the leaf sap and the growing medium (Fig. 2). The vesiculated hairs, in contrast, showed a striking increase in their salt concentration as a result of salinity (Table I). It seems that a powerful salt pump must be operative at one or more membranes located between the vesiculated hairs and the remainder of the leaf in order to produce and maintain such a high gradient of salt concentrations between the hairs and the adjoining leaf cells.

In vesiculated hairs of *A. halimus* the osmotic potential due to sodium and potassium chloride, estimated by van't Hoff's law (15), would range from −110.4 ± 9.9 atm to −559.4 ± 44.0 atm in the control and the salt-treated plants, respectively. Although van't Hoff's law is valid only for dilute solutions, its application to solutions of higher concentrations only underestimates the absolute values of the osmotic potentials. The estimated values for hairs from *A. halimus* treated with salt are considerably lower than the minimum of −150 atm previously reported for plant cells (21).

Observations via phase microscopy of cytoplasmic streaming inside these hairs proved that even at such a high salt content the hairs were alive. Since the internal salt concentrations are so high, salt crystals might be present inside the hairs, but none could be detected. The hair contents crystallized in a few seconds after being broken on the surface of glass slides, but when the hairs were covered with a layer of mineral oil before being broken, the salt crystals did not form. This indicates that the wall pressure did not contribute to keeping the salts in solution inside the hairs.

Considering the concentration of salts within the hairs (Table I), one would expect them to be able to adsorb water, but when we suspended them in glass-distilled water and watched them carefully under the microscope, they did not increase in size, even after 48 hr. Study of the hairs under a phase microscope revealed their rugged surface (Fig. 1, B and C), which appears to be of a waxy material that may render the hairs impermeable to water. In fact, after application of a mild surfactant (0.01% Tween 20) to the water, the volume of the hairs began to increase in 2 to 3 hr. This suggests that the lack of increase in size of the hairs in the absence of surfactant was not due to a rigid cell wall that could prevent the change in volume, but rather to the hydrophobic covering of the hair, which prevented the absorption of water.

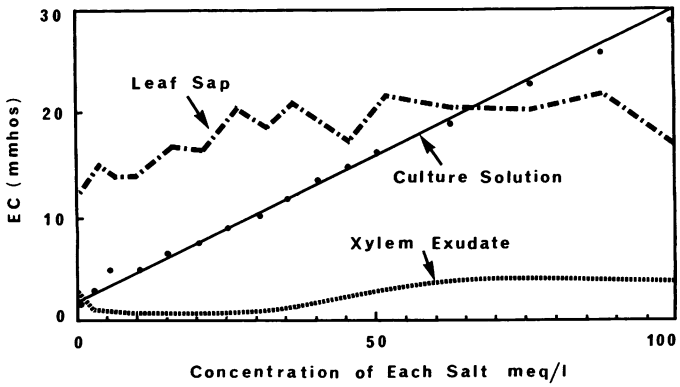

FIG. 2. Effect of increasing concentrations of NaCl and KCl on the salt concentration (expressed as EC) of leaf sap and xylem exudate of *A. halimus*. The EC of the culture solution is shown for comparison.

Furthermore, our preliminary experiments showed that prolonged washing of the leaves prior to sampling did not decrease the salt concentration in the hairs, which indicates that salt did not leach from the hairs.

It may be argued that, in intact hairs, absorption of water by the hairs may not necessarily be accompanied by an increase in their size since the absorbed water may move from the hairs to other parts of the leaf. Therefore, our observations of the lack of increase in size of dissected hairs does not necessarily mean the absence of water absorption by the hairs. It seems unlikely that total water potential inside the hairs would ever be higher than that of the other parts of the leaf unless pressure potential approaches the absolute value of osmotic potential.

In order to verify our results with still another method, we used vapor pressure osmometry to determine the osmotic potential of the hairs. Fifty vesiculated hairs of the salt-treated plants were diluted in 50 μl of glass-distilled water, and their osmotic potential was measured. The osmotic potential obtained by this method was lower than that calculated from salt measurements, indicating a possibly considerable contribution of nonelectrolytes to total osmotic potential. However, some of the discrepancy between the osmometric and calculated values may be due to errors in estimating hair volumes and to deviations from van't Hoff law estimates of osmotic potential as previously noted.

From this experimental evidence, it can be concluded that vesiculated hairs play an important role in the removal of salts from *A. halimus* leaves and therefore have a definite significance in the over-all salt tolerance of the plant. Atmospheric absorption of water by these hairs, in spite of their high salt content, cannot be confirmed at this time.

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