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Research article

Pressure-volume (P-V) curves in *Atriplex nummularia* Lindl. for evaluation of osmotic adjustment and water status under saline conditions

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ABSTRACT

The survival of *Atriplex nummularia* plants in saline environments is possible mainly due to the presence of salt-accumulating epidermal vesicles. Commonly, destructive methods, such as plant material maceration and subsequent reading in osmometers, are employed in studies on water relations and osmotic adjustment and are inconvenient due to their underestimation of the total water potential inside the cells, which can cause overestimation of an osmotic adjustment that is not present. As a result, methods that preserve leaf structure, such as pressure-volume (P-V) curves, which take into consideration only the salts that compose the symplastic solution, are more adequate. Thus, the main objectives of this study were to evaluate the effect of determination methods of osmotic potential (Ψ_o) in *Atriplex nummularia* through destructive and leaf structure-preserving techniques and to determine the water relations of the species under increasing NaCl concentrations. Plants were subjected to daily irrigations, maintaining soil moisture at 80% of field capacity, with solutions of increasing NaCl concentration (0, 0.05, 0.1, 0.2, 0.25 and 0.3 M) for 84 days. Water potential, osmotic potential and osmotic adjustment were determined. In addition, P-V curves were constructed using pressure chambers. Water and osmotic potentials decreased linearly with increasing NaCl concentration in the irrigation solution. The main discrepancies observed were related to the osmotic adjustments determined through maceration and P-V curves. Based on the present research, it was possible to conclude that in studies with species that have salt-accumulating vesicles in the epidermis, such as the plants in the genus *Atriplex*, constructing P-V curves is more adequate than destructive methods.

1. Introduction

In saline environments, halophyte plants have mechanisms that promote the reduction of their total water potential - in greater extent by compartmentalizing ions present in the soil solution in the vacuole (especially Na^+ and Cl^-) and in smaller extent by accumulating compatible organic solutes in the cytosol - allowing water absorption under water and salt stress conditions (Flowers and Colmer, 2015; Silveira et al., 2009). Under these conditions, preserving turgor potential is crucial to maintaining cell expansion and, consequently, to continuing growth (Munns, 2002; Feng et al., 2016). In addition to the osmotic adjustment and protection of cell structures, stomatal regulation controlled by the abscisic acid (ABA) also seems to be an efficient strategy to reduce the water deficit caused by both salinity and drought (Ren et al., 2007).

Knowledge of the water relations of plant cells, tissues and organs is fundamental to understanding the ecophysiological importance of water in plants. In the quantification of these relations, the variables commonly used are the water volume and water potential, because these variables explain the ability of the plant to maintain leaf water content at optimum levels even under conditions of limited water availability in stressful environments (Álvarez et al., 2012; Negrão et al., 2017).

The components of the total water potential in the plant can be quantified using pressure chambers or osmometers or through the construction of pressure-volume curves (Hassine and Lutts, 2010; Kirkham, 2014).

In the last 50 years, pressure chambers have been widely employed

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in studies on plant physiology and ecology to characterize various aspects of water relations (Roderick and Canny, 2005). These devices allow researchers to infer, based on a single leaf or branch, values ranging from water potential to cell elasticity modulus (Bartlett et al., 2012). Thus, water parameters derived from pressure-volume curves have complemented studies on morphology, physiology and anatomy in different plant species, allowing characterization of the strategies adopted in response to environmental stress (Benzarti et al., 2014).

In studies with halophyte species, especially those belonging to the Chenopodiaceae, such as *Atriplex nummularia*, the maintenance of the integrity of plant tissues is very important. Plants belonging to this species generally have salt accumulating vesicles in the leaf epidermis (Souza et al., 2012). Consequently, in evaluations of osmotic potential performed through the freezing and maceration of leaf tissue, these vesicles are ruptured and leak the inorganic contents stored. The solutes that were previously stored begin to be counted as components of the cell osmotic potential and can interfere with the data in calculations of osmotic adjustment (Callister et al., 2006).

Thus, the main objectives of this study were to evaluate the effect of determination methods of osmotic potential (Ψ_o) in *Atriplex nummularia* through destructive and leaf structure-preserving techniques and to determine the water relations of the species under increasing NaCl concentrations.

2. Material and methods

2.1. Plant material and stress conditions

The cultivated plants of *Atriplex nummularia* were asexually propagated through cutting, using one single plant as a matrix to minimize genetic variability. Cuttings with height of approximately 12 cm were collected in January 2014 and placed to root in polyethylene tubes containing washed sand in a protected environment. When the cuttings were completely rooted (60 days) and acclimated, they were transplanted to pots in March 2014. During the entire experimental period, plants were cultivated in soil with moisture at 80% of pot capacity and a matric potential of 0.08 Bar. Irrigation was applied always in the late afternoon to replace the water lost through evapotranspiration. The applied treatments were irrigation with water containing six different NaCl concentrations (0, 0.05, 0.1, 0.2, 0.25 and 0.3 M).

Irrigation with saline water was gradually applied, to avoid osmotic shock to the plants. Thus, saline treatments were applied through the addition of a 0.05 M NaCl solution until the concentration of the respective treatment was reached.

Polyethylene pots with a capacity of 5 L were used in the experiment. The pots were filled with soil classified as Fluvic Neosol (Fluvisol). The soil collection site is located at the Nossa Senhora do Rosário Farm, at the coordinates 8°34′11″S and 37°48′54″ W, at an altitude of 630 m above sea level. The soil was collected from the 0–30 cm layer, air-dried, pounded to break up clods, sieved through a 4-mm mesh to preserve the microaggregates and subsequently used to fill the pots. For its chemical characterization (Table 1), ten individual samples of the total soil volume were collected and sieved through a 2-mm mesh to obtain the air-dried fine earth (ADFE). The value of the initial characterization refers to the mean of ten samples. According to Köppen's classification, the climate of the region is BSh (extremely hot and semi-arid), with total mean annual rainfall of 730 mm and mean annual reference evapotranspiration of 1.683 mm.

2.2. Water relations

2.2.1. Water potential (Ψw) and osmotic potential (Ψo)

Leaf water potential was determined according to the procedure of Scholander, using a Scholander pressure chamber (model 1515D Pressure Chamber Instrument- PMS Instrument Company).

The total osmolality of the leaf tissue was evaluated using the leaves from the branch used in the determination of leaf water potential. The plant material was macerated with liquid nitrogen in a mortar and pestle. The macerated leaf tissue was filtered and centrifuged at 10000 g for 15 min at 4 °C. A 10-μL aliquot of the supernatant was used to determine the osmolality of the tissue, using a vapor pressure osmometer (VAPRO WESCOR model 5600). The values obtained with the osmometer were converted to osmotic potential through the Van't Hoff equation.

Table 1
Mean values (n = 10) of the chemical characteristics of the saturation extract, exchange complex and the soil physical characteristics used for the cultivation of *Atriplex nummularia* under different levels of salinity.

Characteristics	Values
Saturation extract[a]	
PHes	7.77
Electrical Conductivity (dS m^{-1})	2.17
Na^+ ($mmol_c$ L^{-1})	13.26
K^+ ($mmol_c$ L^{-1})	1.83
Ca^{+2} ($mmol_c$ L^{-1})	3.15
Mg^{+2} ($mmol_c$ L^{-1})	1.36
Cl^- ($mmol_c$ L^{-1})	14.37
Sodium adsorption ratio	8.50
Exchange complex[a]	
pH (1:2,5)	6.85
Na^+ ($cmol_c$ kg^{-1})	1.645
K^+ ($cmol_c$ kg^{-1})	3.70
Ca^{2+} ($cmol_c$ kg^{-1})	7.78
Mg^{2+} ($cmol_c$ kg^{-1})	1.73
Sum of bases ($cmol_c$ kg^{-1})	14.85
Exchangeable sodium percentage (%)	11.07
Physical characteristics[b]	
Sand Fine (g kg^{-1})	329.79
Sand coarse (g kg^{-1})	143.15
Total sand (g kg^{-1})	433.09
Silt (g kg^{-1})	466.04
Clay (g kg^{-1})	100.87
Clay dispersed in wate (g cm^{-3})	50.40
Soil bulk density (g cm^{-3})	1.51
Soil particle density (g cm^{-3})	2.63
Degree of flocculation (%)	50.00
Degree of clay dispersion (%)	50.00
Total porosity (%)	42.00

[a] USSLS (1954).
[b] EMBRAPA (1997).

2.2.2. Osmotic adjustment (OA)

The osmotic adjustment was estimated based on the collection of 5 leaves per plant from branches close to those collected for water potential determination. The collected leaves were stored in aluminum foil envelopes, placed in polystyrene boxes containing ice and transported to the laboratory, where they were immediately saturated in Petri dishes for 24 h at 4 °C in the dark.

After achieving complete turgor, the leaves were dried with paper towels and macerated in a mortar and pestle. The extracted sap was filtered with mousseline fabric, placed in Eppendorf® tubes and centrifuged at 10000 g for 15 min at 4 °C.

Osmolality readings were taken with an osmometer using the supernatant from the centrifugation. A 10-μL aliquot was used in the readings. The readings were obtained in mmol kg^{-1} and converted to Bar using the Van't Hoff equation. The total osmotic adjustment was calculated based on the difference between the osmotic potentials of the plants in the control treatment (irrigated with distilled water) and stressed plants: $OA_{tot} = \Psi_{o_c}^{100} - \Psi_{o_s}^{100}$ (Blum, 1989), in which OA_{tot} is the total osmotic adjustment, $\Psi_{o_c}^{100}$ is the osmotic potential of plants in the control treatment at full turgor and $\Psi_{o_s}^{100}$ corresponds to the osmotic potential of stressed plants at full turgor.