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Population differentiation in *Spartina patens*: Water potential components and bulk modulus of elasticity

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Abstract

Pressure-volume technique was utilized to evaluate salinity response among three populations of *Spartina patens* (Ait.) Muhl. from Louisiana Gulf coast marshes. Plants were subjected to salinities of 85 and 425 mol m^{-3} for 77 d in a greenhouse. Ψ_w and Ψ_π decreased in all populations in response to increases in salinity. There were 32 % decrease in Ψ_{sat}, 42 % decrease in Ψ_{tlp} in response to salinity changes from 85 to 425 mol m^{-3} in the Ferblanc population. Similarly, there were 35 % and 41 % decrease in Ψ_{sat} in the Clovelly and Lake Tambour populations, respectively. All populations showed the ability to adapt to the increased salinity as was evidenced by osmotic adjustment. However, the Lake Tambour population appeared to have superior ability to adapt to high salinity through having a significantly lower osmotic potential at saturation (Ψ_{sat}), osmotic potential at turgor loss point (Ψ_{tlp}), and maximum turgor potential ($\psi_{P(max)}$) compared to other populations. Ferblanc and Clovelly populations revealed the ability to adapt to saline environments to a lesser extent as compared to the Lake Tambour population. Results indicate that there is a potential for selection of superior strains of *Spartina patens* for use in marsh restoration projects aiming at prevention of wetland loss in certain coastal areas.

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Abbreviation: Ψ_π, - osmotic potential; Ψ_p - pressure potential; E - bulk modulus of elasticity; Ψ_w - water potential; Ψ_{sat} - osmotic potential at saturation; Ψ_{tlp} - osmotic potential at turgor loss point; E_{max} - bulk modulus of elasticity at saturation; RWC_{sat} - relative water content at saturation; RWC_{tlp} - relative water content at turgor loss point; V_0 - symplasmic water volume; V_t - total water volume; V_e - expressed water volume; N_s - number of osmoles of solute; V_{tlp} - symplasmic water volume remaining in the cell at the turgor loss point; $\Psi_{p(max)}$ - maximum turgor potential; V_0/DM- symplasmic water volume per dry mass.

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Introduction

Evolution of differentiated populations which possess advantageous physiological characteristics at sub-species level under a given environmental stress condition have been reported for several species (Keeley 1979, Etherington and Thomas 1986). *Spartina patens* (Ait.) Muhl. is a dominant brackish marsh grass which grows in a wide range of flooding and salinity. Field observations along the Louisiana Gulf Coast suggest considerable variations in performance of populations of this species in response to flooding and salinity. These observations suggest a potential difference in the effects of increase in salinity on water potential components of these populations. However, to date no studies have been conducted to evaluate the water relations response of these populations to changes in salinity.

Increase in soil salinity affects plant water relations dramatically because of the necessity for osmotic adjustment allowing continuation of water uptake (Epstein 1980). As soil salinity increases, the plant must maintain a positive water balance through increasing solute concentrations (decreasing Ψ_π). The net effect is a positive Ψ_p despite decreasing Ψ_w (Epstein 1980, Kramer 1983). Osmotic adjustment, resulting from regulation of solute concentration, therefore, is an important means of plant adaptation to salinity in its root environment (Epstein 1980, Cheeseman 1988).

The present study was conducted to evaluate and compare the water potential components of three populations of *S. patens*. It was hypothesized that those populations which evolved under severe salinity regimes possess advantageous water relations characteristics. Hence, a water relations study using pressure-volume technique (*e.g.* Roberts *et al.* 1981, Tyree and Hammel 1972) may provide a screening method for evaluating the potential population differentiation. Pressure-volume technique allows calculation of plant water relation parameters such as Ψ_{sat}, E_{max}, Ψ_{tlp}, RWC_{tlp} and the fraction of apoplastic water (Tyree and Hammel 1972, Hellkvist *et al.* 1974, Tyree 1976).

Material and methods

Population samples of *Spartina patens* were collected from three populations along a salinity gradient extending from edge of freshwater to saltmarsh habitats in coastal Louisiana approximately 30 km apart. Ferblanc population grows near the higher salinity, brackish - saltmarsh zone (29° 15' N, 90° 5' W), Clovelly Population grows at the freshwater - brackish zone (29° 30' N, 90° 10' W) and Lake Tambour population was collected from a brackish - saltmarsh zone (29° 20' N, 90° 30' W). The predominant salinity ranges between 4 - 6 ppt in Clovelly site, 7 - 10 ppt in Ferblanc site and 8 - 12 ppt in Lake Tambour site. Sixty to 80 tillers per population were collected, transferred to a greenhouse and cloned. Newly grown culms and associated roots were planted in nursery pots filled with commercial potting soil (*Jiffy Mix Plus, Jiffy Products of America,* W. Chicago, IL). Study pots were watered to excess and fertilized once per week with a commercial water soluble plant food

(23 % N, 19 % P, 17 % K). Two weeks after the transplanting, salinity treatment was initiated.

Salt solutions were prepared using *Instant Ocean Synthetic Sea Salt* (*Aquarium Systems, Inc.*, Mentor, USA), with major ionic components of 47% Cl, 26% Na, 6 % SO_4, 3 % Mg, 1 % Ca and 1 % K (percentage of dry mass). Treatments began by flooding the pots with 17 mol m^{-3} (1 part per 1000) salt on the first day. Salinity level for low salinity (T_1) then increased to 51 mol m^{-3} on the 3rd day and 85 mol m^{-3} on the 7th day of the experiment. In the 2nd treatment, high salinity (T_2) plants were subjected to the same salinity levels as in T_1 except that salinity increased to 170 mol m^{-3} on day 10, 340 mol m^{-3} on day 12 and 425 mol m^{-3} on day #15. A *YSI model 33 meter* (*Yellow Springs Instrument Co.,* Yellow Springs, Ohio, USA) was used for measurements of salt levels in all pots throughout the experiment. In addition, throughout the study, pots were drained weekly and freshly made salt solution at respective concentration was added to the pots.

Measurements for construction of PV curves were made for 7 leaves per population per treatment collected at the end of the dark period on #18, 19, 21, 22, 23, 28, 31, 32, 36, 37, 71, 72, 73, 75, 76 and 77d following initiation of salinity treatment. The dark period was provided by transferring the pots to the laboratory and storing them in the dark at 22 °C overnight. At the beginning of each measurement, a leaf was dried with a tissue paper to remove any water adhering to the leaf, cut to 20 cm length and weighed to determine the saturated mass. The leaf sample was then inserted into a 2.5 cm wide membrane tubing (*Spectrapor* membrane, *Allied Fisher Scientific Co.*, Pittsburgh, PA, USA) of same length with end sealed to prevent air entry into the leaf tissue. The enclosed leaf was sealed into a pressure chamber (*PMS model 1001, PMS Instrument Co.,* Corvallis, Oregon, USA). The gas pressure of the chamber was increased until sapwater flowed out of the leaf; after a measured quantity has been expressed the pressure was fixed until sap water neither flowed in nor out (the balancing pressure). In the pressure chamber, the pressure was applied so that water was just detectable at the cut surface and recorded as initial balance pressure. Pressure was then increased in steps of 0.3 MPa over each previous balance pressure starting from 0.2 MPa and continuing up to about 3.2. The expressed water at each balance pressure was collected in preweighed polyethylene tubes filled with dry tissue paper and the absorbed polyethylene tubes were weighed at about 5-min intervals to determine when water efflux ceased. The tubes were exchanged at each pressure change to minimize the error from overwetting tissues. The fresh mass of the leaf was measured when it was removed from the chamber. The leaf was then dried at 80 °C for 48 h and its oven-dry mass was obtained. Pressure-volume curves were then constructed for 7 plants representing each population for each treatment. Several water relation parameters were determined from the PV curves (Fig. 1) and relative salinity resistance were partially evaluated from these parameters. Various informations from PV curves were obtained according to Tyree and Hammel (1972), Cheung *et al.* (1975), and Tyree *et al.* (1978).

Artificial rehydration of leaf tissue prior to the construction of PV curves resulted in inconsistent values as was reported previously by Bolanos and Longstreth (1984). Therefore, water content at the end of the dark period was used as saturation reference (Cheung *et al.* 1975, Melkonian *et al.* 1982, Scholander *et al.* 1965, Bolanos and Longstreth 1984). The General Linear Models (GLM) procedure of the SAS System (*SAS Institute,* Cary, NC, USA) followed by Duncan's Multiple Range Test was used to test for differences in water relations parameters among populations within treatments. Other procedures such as the *t*-test were used where appropriate.

Results and discussion

Values of Ψ_w and Ψ_π decreased in all populations in response to increases in salinity. There was a 32 % decrease in Ψ_{sat}, 42 % decrease in Ψ_{tlp} in response to salinity changes from 5 to 25 ppt in Ferblanc population. Similarly, there were 35 % and 41 % decreases in Ψ_{sat} in Clovelly and Lake Tambour populations, respectively. These results are consistent with other reports of osmotic adjustment to salinity allowing maintenance of turgor in several species (Bernstein 1963, Gale *et al.* 1967, Bolanos and Longstreth 1984, Ben-hayyim 1987, Naidoo 1985).

The Ψ_{sat} indicates the amount of osmotic solutes that a leaf contains per unit volume of symplasmic water (Cheung *et al.* 1975). Maintaining a low value of Ψ_{sat}, if accompanied by positive turgor, could allow cell elongation under salinity conditions. Also, a decrease of Ψ_{tlp} would increase the potential salt tolerance capability through enabling the plant to develop a positive turgor pressure at low Ψ_w (Bowman 1988). In the T_1 treatment, values of Ψ_{sat} were not significantly different among populations (Table 1). However, Lake Tambour population in T_2 treatment had significantly lower Ψ_{sat} than other populations. In addition, Lake Tambour population had significantly lower Y_{tlp} compared to other populations in the T_1 treatment. This population exhibited a greater ability for osmotic adjustment in response to elevated salinity and appears to possess a higher salt tolerance adaptation capabilities compared to other populations.

There were some differences among populations in RWC_{tlp} in the T_1 treatment. However, in the T_2 treatment all populations showed almost the same degree of RWC_{tlp} (Table 1). This suggests that the decrease in Ψ_π resulted in approximately the same magnitude of decrease in RWC_{tlp}. A plant with higher RWC_{tlp} might conserve water more efficiently than a plant with lower RWC_{tlp} (Monson and Smith 1982). Accordingly these populations seem to have the same potential to conserve water in a saline environment.

In the T_1 treatment, there were no significant differences in $\Psi_{p(max)}$ and E_{max} among populations (Table 1). However, there were significant differences in $\Psi_{p(max)}$ among populations in T_2. A decrease of 0.1 MPa in Ψ_p would be expected to have a major effect on the growth rate (Neumann *et al.* 1988). Our results indicate that the Lake Tambour population had the highest salt tolerance ability preventing serious injury from salt stress at concentrations tested. Drake and Gallagher (1984) found similar results in their study of *Spartina alterniflora.*

Table 1. Comparison of water potential components among populations in *Spartina patens*. Values followed by the same letter within the same column do not differ significantly using Duncan's Multiple Range Test ($p \leq 0.05$).

Treatment	Population	Ψ_{sat} [MPa]	Ψ_{tlp} [MPa]	RWC$_{tlp}$ [%]	$\psi_{P(max)}$ [MPa]	E_{max} [MPa]	V_p/V_o [%]	V_o/DM [g(H_2O) g^{-1}(d.m.)]
(T_1)*	Ferblanc	- 9.87a	-14.27c	83.56a	8,46a	22.96a	67.23a	1.75a
	Clovelly	-10.74a	-16.33b	82.59ab	9.40a	22.14a	65.66a	1.88a
	Lake Tambour	-11.47a	-18.40a	78.54b	10.09a	21.46a	63.28a	2.11a
(T_2)	Ferblanc	-13.06b	-20.27c	77.15a	11.45c	18.35b	58.13a	1.80a
	Clovelly	-14.54b	-22.94b	77.18a	13.24b	26.77ab	62.80a	1.62a
	Lake Tambour	-16.54a	-27.66a	77.00a	15.13a	28.37a	60.28a	1.60a

*T_1 = low salinity treatment; T_2 = high salinity treatment

Many salt-tolerant plants adjust osmotically, when exposed to salinity, and thereby maintain favorable turgor (Cheesman 1988). In species capable of osmotic adjustment, Ψ_p increases with sublethal salinity increases (Gale *et al.* 1967, Hoffman *et al.* 1980). However, Ψ_p is primarily dependent on the water content and small changes in water content result in substantial decrease in Ψ_p. The bulk modulus of elasticity (E) governs the relationship between Ψ_p and tissue water content (Roberts *et al.* 1981). E is the rate of the change in Ψ_p in response to changes in tissue water content (Melkonian *et al.* 1982, Roberts *et al.* 1981). As such, E is closely correlated with physical properties of cells in tissues (Roberts *et al.* 1981). In tissues with rigid cell walls E is greater than in tissues consisting of cells with elastic cell walls (Cheung *et al.* 1975, Dainty 1972). Bolanos and Longstreth (1984) found that E increased with increasing salinity concentration.

Cells with low elasticity could increase the water adsorption yet reduce positive turgor in response to salt stress (Cowan 1960). This feature, if accompanied by osmotic adjustment, could aid the plant to adapt to a saline environment (Neumann *et al.* 1988). Our study indicates that in the T_2 treatment, the Lake Tambour population had significantly higher E_{max} (lower elasticity) and lower Ψ_{sat} than other populations, which suggests active osmotic adjustment in response to salt stress. In the present study, the Lake Tambour population displayed a greater ability for osmotic adjustment than other populations under the saline environment through increasing cell solute concentrations. The ratio of RWC at the turgor loss point per symplasmic water volume (V_{tlp}/V_0) indicates the effect of tissue elasticity on the relative water content at which turgor loss occurred. If accompanied by low values of osmotic potential, high values of V_{tlp}/V_0 enable plant to sustain water uptake through generating a large drop in Ψ_w and thereby a steep gradient in water potential, which is required for maintaining relatively high values of relative water content as total water potential declines (Cheung *et al.* 1975, Parker *et al.* 1982). A high value of V_p/V_0 (or RWC$_{tlp}$) indicate that the turgor loss point is reached after a small loss of water, a consequence of rigid cell walls (high E). Thus for a small drop in RWC, the plant can generate a large drop in Ψ_w and hence a substantial gradient in water potential for maintaining water uptake capability. However, if cell walls are more elastic, then much more water can be lost before wilting point is reached and consequently the Ψ_π can fall further, due to the concentrating effects of water loss. Hence, for the same initial osmotic potential, the more elastic cells will reach a more negative Ψ_w before they lose turgor, a potential advantage for maintaining growth in face of increasing salinity. There was a rapid decrease in V_{tlp}/V_o in the Ferblanc population from T_1 to T_2. However, in Clovelly and Lake Tambour populations V_{tlp}/V_0 did not decrease much in T_2 (Table 1). This, coupled with the lower Ψ_{sat}, suggests that Lake Tambour population may have better ability to adapt to higher salinity environment than other populations. Although there were no significant differences in symplasmic water volume per dry mass (V_0/DM) among populations in both T_1 and T_2 treatments, Lake Tambour population generally had higher V_0/DM values in T_1 and lower values in T_2 than that of other populations. Moreover, in T_2 treatment the V_0/DM of Ferblanc and Clovelly populations remained relatively constant as compared with T_1. However, V_0/DM of the Lake Tambour population decreased from T_1 to T_2 (Table 1). Decrease in V_0/DM may be attributed to the increase of membrane permeability (Beevers 1976, Parker *et al.* 1982) or the increase of dry matter content. Therefore, the decrease of V_0/DM in Lake Tambour population imply a greater capability of this population to adapt to high salinity environments compared with the other populations.

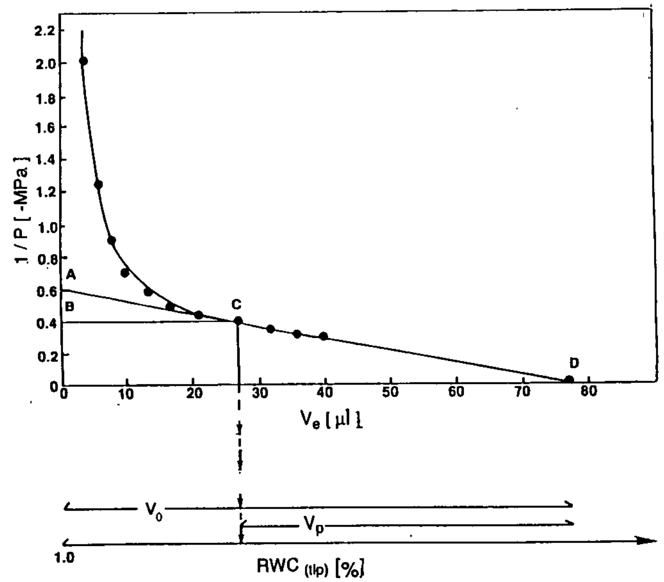

Fig. 1. A typical pressure-volume curve for the Lake Tambour population of *Spartina patens* under high salinity regime. V_e is the expressed water volume at turgor loss point; A is the inverse of the original bulk osmotic pressure, Ψ_{sat}; B is the inverse of the osmotic pressure at turgor loss point, Ψ_{tlp}; C is the turgor loss point; D is the original symplasmic water volume; D-C is the symplasmic water remaining in the cell at turgor loss point, V_{tlp}.

If cellular osmotic potentials are to be maintained at levels lower than those in the root environment, the intracellular concentrations of dissolved solutes in the cell sap must increase with increasing salinity. The major site of such solute accumulation is the vacuole (Ball 1988) which governs the symplasmic water volume. As cells mature, dry mass increases, while relative V_0 decreases (Doi *et al.* 1986). There were significant differences in V_0/DM among populations which implies the high growth potential of these populations in saline environments. A decrease in V_0/DM also indicates the concentrating effect of removing water from the vacuoles due to the stress. However, maintenance of this ratio in face of increasing external salinity may indicate an adaptation characteristic to stress conditions. Lake Tambour population had a slightly lower V_0/DM value than other populations which suggest that the Lake Tambour population may have a greater ability to adapt to a high salinity environment compared to other populations.

Population differentiation in marsh grasses has been reported in *S. patens* (Silander 1985) and many other marsh grasses (Gray and Scott, 1980; Jefferies *et al.* 1981; Boorman 1967; Huiskes *et al.* 1985; Eleuterius 1989). Silander (1985) reported that *S. patens* populations from adjacent saltmarsh, swale and dune areas showed evidence of genetic differentiation. Through the evolution process under a selection pressure of a distinct habitat, a homogeneous species can gradually evolve into diverse specialized populations. As a result, these populations become distinct genetically (Eleuterius 1989). In coastal marshes, the primary environmental factor for population differentiation is the sediment water salinity which creates a substantial selection pressure for plants. In the present study, there is no evidence of restricted gene flow among the populations of *S. patens* studied. Thus it is difficult to distinguish between evolutionary divergence of "isolated" populations and site-specific differential survival of offspring from a common gene pool.

In conclusion, all study populations showed the ability to adapt to the increased salinity through osmotic adjustment. However, Lake Tambour population appears to have superior ability to adapt to high salinity through maintaining the lowest Ψ_{sat}, Ψ_{tlp}, and the highest value of $\psi_{P(max)}$ as compared to other populations. The results suggest that there may be a potential for selection of superior strains of *Spartina patens* for use in marsh restoration projects aimed at preventing wetland loss in certain coastal areas where saltwater intrusion occurs. The effort requires selection of numerous populations of this species from various coastal areas and tests of their salinity tolerance under field conditions.

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