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Population differentiation in *Spartina patens*: Responses of photosynthesis and biomass partitioning to elevated salinity

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Abstract. Populations of *Spartina patens* (Ait) Muhl. were collected from three distinct habitats in Louisiana Gulf Coast marshes. The Lake Tambour and Ferblanc populations are associated with saltmarsh and saltmarsh-brackish marsh interface, respectively, where soil salinity is greater than at the brackish-freshwater transition zone where the Clovelly population occurs. The effects of salinity at 0, 5, and 15 ppt on gas exchange, growth and biomass partitioning were evaluated. All study populations performed well under the elevated salinities for 12 weeks (the duration of the study). However, differences in gas exchange, growth, biomass production, and patterns of biomass partitioning in response to the treatments were evident among the populations. For example, in the Lake Tambour population, there was no significant change in leaf conductance or net photosynthesis in response to the elevated salinities whereas, in others, significant reductions in net photosynthesis were found at the 15 ppt treatment. Generally, all populations had comparable gas exchange rates under control (0 ppt) treatment. However, the Clovelly and Lake Tambour populations exhibited significantly ($p < 0.05$) greater net photosynthesis compared to the Ferblanc population under the 5 ppt salinity treatment. In 5 and 15 ppt treatments, the Lake Tambour population maintained the greatest number of shoots, leaf area, and dry weight among the study populations. The population-by-treatment interaction was significant for all size-related traits except the gas exchange properties. Although certain measured parameters appeared to reflect the relative position of these populations in the natural range of *S. patens*, based on the present data salt-tolerance in this species can not generally be inferred from the field position. In addition, the within- and between-population variation in the characteristics studied suggest that collection of germplasm from a wide range of sites is needed to increase the likelihood of capturing the maximum salinity tolerance. Thus finding the greatest level of salt-tolerance among the population of this and other important coastal macrophyte species remains as a major task.

Keywords: Marsh restoration; Marsh vegetation; Photosynthesis; Population differentiation; Salinity stress.

Introduction

Population differentiation in marsh vegetation has been a topic of considerable interest to wetland scientists. For instance, the existence of height forms in *Spartina alterniflora* in saltmarshes of the US Atlantic and Gulf Coast (Nestler, 1977; DeLaune et al., 1983) and population differentiation in *S. patens* (Silander, 1985) prompted numerous research efforts centered around the question of whether these populations were genetically distinct (ecotypes) or homogeneous (ecophenes). Silander (1985) reported that genotypes of *S. patens* from adjacent saltmarsh, swale, and dune areas showed evidence of genetic differentiation. Nevertheless, the existence of ecotypes within a plant species have been recognized for sometime (Bradshaw, 1984; Kuiper, 1990). Such differentiation may provide one means of coping with environmental heterogeneity (Heslop-Harrison, 1964; Ehrlich and Raven, 1969). The genetic variations among populations of a given species are related to interactions among natural selection, mutation, drift, and genetic recombination. Under the selection pressure of a variable habitat, a homogeneous species can evolve into diverse specialized populations, which are distinct genetically (Eleuterius, 1989). Such differentiation has been reported for many marsh species (Boorman, 1967; Gray and Scott, 1980; Jefferies et al., 1981; Huiskes et al., 1985; Eleuterius, 1989; Warwick and Halloran, 1991).

Spartina patens (Ait) Muhl. is a dominant brackish macrophyte species in US Gulf Coast marshes which grows over a wide range of salinities ranging from the edge of freshwater habitats to saline marshes where it occurs with *S. alterniflora*. Along the US Gulf Coast, *S. patens* has been used extensively in marsh planting efforts in different coastal areas along with other species (Woodhouse et al., 1974, 1976). In its natural range, *S. patens* shows a relatively high level of tolerance to salinity. Although it is considered a salt-tolerant species, growth of this species is adversely affected by excess salinity (Parrondo et al., 1978; Gosselink, 1984). Previous studies have indicated considerable variation in the performance among certain populations of this species in response to salinity regimes (Pezeshki and DeLaune, 1991; Pezeshki, 1991; Pezeshki et al., 1993). Finding the high-est level of salt tolerance among existing US Gulf Coast populations of this and other important marsh species remains as a major objective. If successful, such stocks may be used in marsh restoration projects with a much greater chance of success. Identification of populations tolerant to elevated salinities is useful in developing strategies for the stabilization of deteriorating marshes. Selecting the best physiologically adapted species or populations for wetland creation and restoration involves the matching of the planting stock with the site conditions. Such conditions include timing, depth, and duration of flooding, salinity, and soil substrate.

Previous studies on populations of this species were conducted on limited populations collected from relatively narrow ecological zones. The present study was conducted to evaluate individuals from a wide range, including high, medium, low salinity zones in US Gulf Coast, and to test whether there are differences in responses of selected populations from different habitats to elevated salinities. The specific objectives were (1) to evaluate populations of *S. patens* from three distinct habitats (high, medium, and low salinities) in the US Gulf Coast marshes under various flooding-salinity combinations and (2) to examine the differences in physiological and growth responses to elevated salinities.

Materials and Methods

To evaluate the responses of ecotypes of *S. patens* to salinity, phenotypes from its natural range were tested together under uniform conditions (Goodman, 1973; Silander, 1985). Population samples were collected from three locations along a salinity gradient extending from the edge of freshwater to saltmarsh habitats in coastal Louisiana, approximately 30 km apart. The Ferblanc population grows near the higher salinity, brackish-saltmarsh zone (29°15' N, 90°5' W); the Clovelly population grows at the freshwater-brackish zone (29°30' N, 90°10' W); and the Lake Tambour population was collected from a brackish-saltmarsh zone (29°20' N, 90°30' W). The predominant salinity ranges are 4–6 ppt in the Clovelly site, 7–10 ppt in the Ferblanc site, and 8–12 ppt the in Lake Tambour site. Sixty to 100 tillers per population were collected, transplanted in a greenhouse, and cloned. Newly sprouted culms and associated roots were planted in nursery pots filled with commercial potting soil in a ventilated greenhouse. The environmental conditions in the greenhouse were as follows: mean maximum and minimum temperatures, 33.8°C and 23.5°C, respectively; midday photosynthetic photon flux was 1400 μmol m^{-2} s^{-1} during sunny days and ranged from 150 to 700 μmol m^{-2} s^{-1} during cloudy days. The greenhouse used did not have supplemental light systems. Study pots (15 cm diameter, 20 cm deep) were watered to excess and fertilized with a commercial water soluble fertilizer (23-19-17, N, P, K, respective percentages, 0.05 g per pot) once per week. Four weeks after transplanting, salinity treatments were initiated.

Salt solutions were prepared using Instant Ocean Synthetic Sea Salt (Aquarium Systems, Inc., Mentor, Ohio, USA), with major ionic components of 47% Cl, 26% Na, 6% SO_4, 3% Mg, 1% Ca, and 1% K (percentage of dry weight). The first treatment (control) was flooded with water (no salt), the second treatment (T2) began by flooding plants with saltwater containing 1 part per 1000 (17 mol m^{-3}) salt on the first day (Day # -15 of the experiment), 3 ppt on the third day (Day # -12), the salinity then was increased to 4 ppt on the seventh day (Day # -8) and to 5 ppt on the fifteenth day (Day # -1). The third treatment (T3) consisted of salinities which were added at the same rate as in T2 except that salinity was increased to 10 ppt on the eighth (Day # -7), 12 ppt on the tenth day (Day # -5) and 15 ppt on the fifteenth day (Day # -1). A YSI model 33 meter (Yellow Springs Instrument Co., Yellow Springs, Ohio, USA) was used to measure salt concentrations in all pots throughout the experiment. The experiment began immediately following the day of completion of all salintiy concentrations in designated treatments (Day #1 of the experiment). Throughout the study, which lasted 84 days following the completion of the treatment initiation, pots were drained on a biweekly schedule and freshly made salt solution (at respective concentrations) and fertilizer were reapplied. The experimental design was a completely randomized design with 3 populations, 3 treatments (control plus 2 salinity levels) and 64 replications (pots) per population/salinity combination. Each pot had one plantlet at the beginning which subsequently reproduced new culms.

Diurnal measurements of air temperature, relative humidity, photosynthetic photon flux density (PPFD), leaf temperature, leaf conductance (g), and net carbon assimilation (A) were made on 5 sample leaves per treatment per population every 3 h from 0900 to 1500 hours each sample day. There were 6 sample days during the experiment (Days #8, 14, 24, 38, 48, and 57). Leaf conductance was measured using a steady-state porometer (LI-1600, LiCor, Inc., Lincoln, Nebraska, USA). After recording g, the same leaf was used for measurement of A, which was performed rapidly using a portable gas-exchange system (Model A120, ADC, Field Analytical System, Analytical Development Co., England). The system was composed of: an ADC Parkinson Leaf Chamber; an infrared CO_2 analyzer, ADC Model LCA-2; and an air-supply unit controlling humidity and flow of incoming air. The leaf was enclosed in the chamber, and PPFD and differential CO_2 levels were recorded. Mature, developed leaves were used for all measurements. Net carbon assimilation rates were calculated from the flow rate of air through the chamber and from the CO_2 partial pressure differences between incoming and outgoing air, as outlined by Caemmerer and Farquhar (1981). Both g and A were calculated per unit leaf area (single surface), determined with a surface-area meter (Model SI701, SKYE Instrument, Inc., Buckingham, Pennsylvania, USA). The General Linear Models procedure of the SAS System (SAS Institute, Inc., Cary, North Carolina, USA) was used to test for differences in g and A among treatment means, by applying a repeated-measures design which included the day and hour of measurement (Moser et al., 1990).

Changes in biomass (difference between final biomass values and initial values) were evaluated from replicated destructive samples taken at the conclusion of the study (Day # 84, the last day of the experiment). The initial values were obtained using the means from destructively harvested subsamples of 12 plants per population at the initiation of the treatment for the respective parameters. The initial total biomass dry weights were not significant among the study populations, averaging 76, 69, and 81 mg per plant for Ferblanc, Clovelly, and Lake Tambour, respectively. The final harvest consisted of destructively sampling 8 pots per population per treatment and washing and dividing samples into above-ground and below-ground components. Leaf surface area (LA) was determined using a Leaf Area Analysis System (SI-701, SKYE Instrument, Inc., Buckingham, PA). Biomass samples were then oven dried at 70°C to a constant weight, and dry weights were recorded. Plant growth (relative growth rate, RGR) in response to various treatments was analyzed using classical growth analysis techniques (Radford, 1967; Ledig et al., 1970). The General Linear Models (GLM) and Duncan's Multiple Range Test (DRT) procedures of SAS were used to compare means for each population among the salinity treatments.

Table 1. F- ratios and significance (*p<0.05, **p<0.01, ***p<0.001) of population, treatment, and the interaction resulting from analysis of variance for net carbon assimilation (A, μmol $m^{-2}s^{-1}$), stomatal conductance (g, μmol $m^{-2}s^{-1}$) and several growth parameters of *Spartina patens*.

Source of variation	d.f.	A	g
Population	2	5.32**	3.84
Treatment	2	4.36*	1.18
Time	5	17.96***	24.94***
Population × Treat	4	0.86	0.54
Treatment × Time	10	2.18*	0.45

Table 2. F- ratios and significance (*p<0.05, **p<0.01, ***p <0.001) of population, treatment, and the interaction resulting from analysis of variance for growth trains of number of shoots per pot (LA), root dryweight per pot (RDW), total dry weight per pot (TDW), relative growth rate (RGR), and root shoot ratio (RSR), for Spartina patens.

Growth trait	Population	Treatment	Pop x Treat
NOS	8.29***	4.15*	3.82*
LDW	11.22***	0.37	3.44*
LA	11.38***	0.46	3.58*
RDW	10.41***	1.48	3.05*
TDW	11.24***	0.45	3.41*
RGR	9.16**	1.02	3.89*
RSR	31.10***	0.32	1.25

Results

Population and treatment had significant effects on A (Table 1). However, the effects of population and treatment on g were not significant. Also, there was no significant population × treatment interaction effects on g or A. Statistical analyses showed that population had significant effects for the biomass parameters (Table 2). Treatment had significant effects on number of shoots but no significant effect on other measured biomass parameters. The population-treatment interaction was significant for all biomass parameters and size-related traits, including RGR, except RSR (Table 2). It was not significant for gas exchange properties (Table 1). Whereas in 0 ppt treatment all populations produced a comparable number of new shoots (vegetative culms), in the 5 and 15 ppt treatments, the Lake Tambour populations produced more new shoots than the Clovelly population, indicating a potentially advantageous characteristic for this population at higher salinities. For example, in the 15 ppt treatment, the average number of new vegetative culms were 36, 26, and 54 in the Ferblanc, Clovelly, and Lake Tambour populations, respectively (Figure 2). The difference was statistically significant ($p < 0.01$) between the

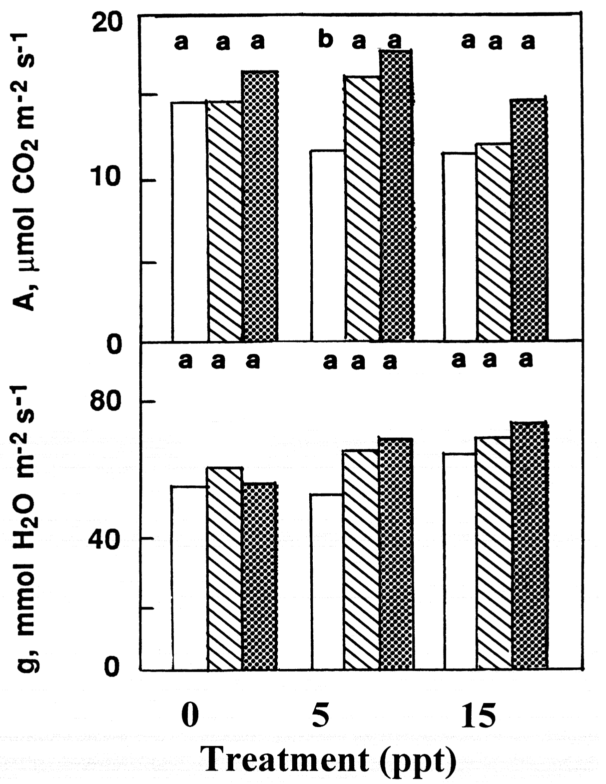

Figure 1. Mean net carbon assimilation (A) and stomatal conductance (g) responses over the experimental period for three populations of *Spartina patens*, Ferblanc (open bars), Clovelly (hatched bars) and Lake Tambour (dotted bars) under salinity treatment of 0 ppt, 5 ppt, and 15 ppt. Bars within each treatment not marked with the same letter are significantly different (p<0.05) according to the Duncan's Multiple Range Test.

Clovelly and Lake Tambour populations. With progression of the study, in 5 ppt treatment, the Lake Tambour population maintained significantly greater leaf area and total biomass per pot compared to the Ferblanc and Clovelly populations. In the 15 ppt treatment, the Lake Tambour population had significantly greater leaf area and dry weight than the Ferblanc population.

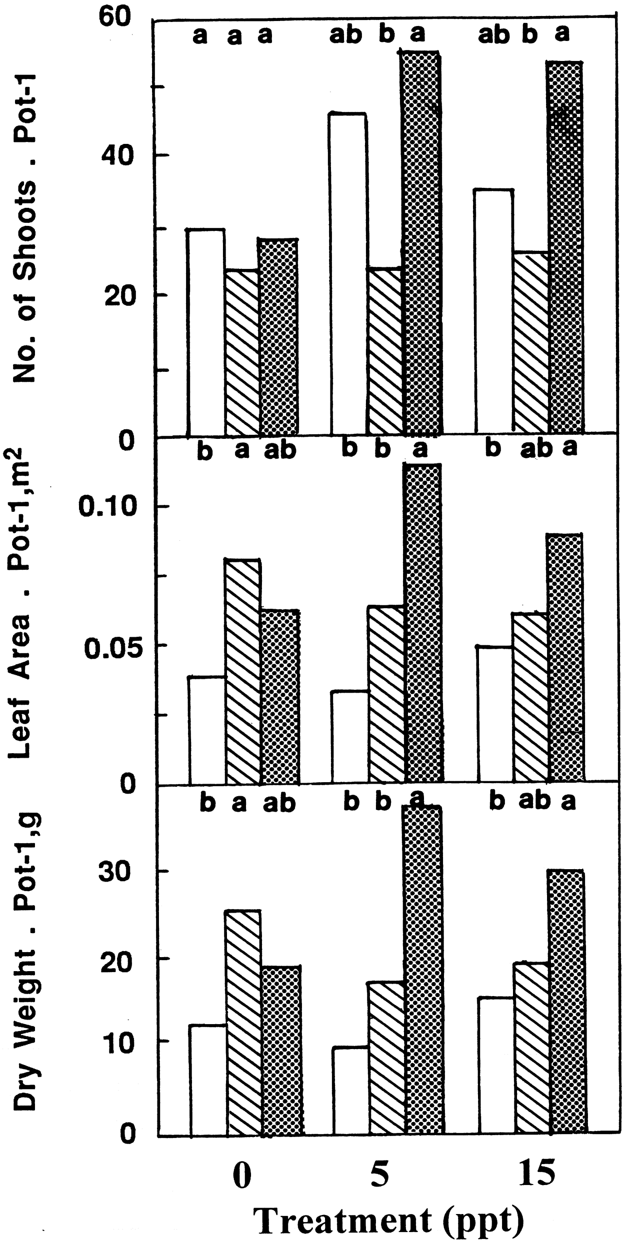

Figure 2. Changes in number of shoots, leaf area and dry weight in three populations of *Spartina patens*, Ferblanc (open bars), Clovelly (hatched bars) and Lake Tambour (dotted bars) under salinity treatment of 0 ppt, 5 ppt, and 15 ppt. Data were collected at the conclusion of the experiment (Day # 84), and represent the difference between final values minus initial values (using the means obtained from destructively harvested subsample of 12 plants per population at the initiation of the treatment) for each respective parameter. Bars within each treatment not marked with the same letter are significantly different ($p<0.05$) according to the Duncan's Multiple Range Test.

All populations had comparable g within the various treatments (Figure 1); however, Clovelly and Lake Tambour populations exhibited significantly ($p < 0.05$) greater A under the 5 ppt treatment compared to the Ferblanc population. All population had comparable A in 15 ppt treatment. The Clovelly population maintained a significantly greater dry weight and leaf area than the Ferblanc population under 0 ppt salinity treatment (Figure 2). In 5 and 15 ppt treatments, the Lake Tambour population maintained the greatest number of shoots, dry weight, and leaf area among the study populations (Figure 2).

Comparison of means among treatments showed that in the Ferblanc population, there was no significant ($p <0.05$) reduction in g or A in response to salinity at 5 and 15 ppt compared to 0 ppt treatments. Over the same range of salinity changes, the Clovelly population had no significant reduction in g but displayed a significant reduction in A ($p < 0.05$) when salinity increased to 15 ppt as compared to 0 and 5 ppt salinities. In the Lake Tambour population, there were no significant changes in g or A in response to the elevated salinities.

Comparison of means among treatments indicated that the increase in salinity from 0 to 5 ppt resulted in a significant increase in shoot regeneration in the Ferblanc and Lake Tambour populations but not in the Clovelly population. In addition, the Lake Tambour population maintained a significantly greater number of shoots in 15 ppt treatment as compared to control. The increase in salinity from 0 to 15 ppt did not significantly change dry weight or leaf area per pot in either the Ferblanc or Clovelly populations. However, in the Lake Tambour population the number of shoots, dry weight per pot, and leaf area per pot increased significantly ($P < 0.05$) in response to a salinity increase from 0 ppt to 5 ppt and 15 ppt.

Discussion

All populations performed well under the salinity treatments for the duration of the study. The observed responses of the study populations to elevated salinities could be partially explained in light of field observations which indicate that the Lake Tambour and Ferblanc populations are associated with high salinities characteristic of a brackish-saltmarsh zone while the Clovelly population occupies the less saline environment of freshwater-brackish marsh zone. For instance, differences in growth, biomass production, and patterns of biomass partitioning in response to salinity treatments were evident among the populations. The Clovelly population produced a significantly greater leaf area than the Ferblanc population in the 0 ppt treatment. However; in the 5 ppt and 15 ppt treatments, the Lake Tambour population had the greatest leaf area. In addition, the Ferblanc population produced a greater number of shoots than the Clovelly population under 5 and 15 ppt salinity. One explanation is that the study populations may differ in allocation of carbohydrate to shoot growth, thereby, allowing the one with the greatest leaf area (the Lake Tambour Population) to fix more CO_2 and produce more vigorous shoots. Similar findings have been reported for three populations of *Plantago lanceolata* (Van Tienderen and Van der Toorn, 1991). Hannon and Bradshaw (1968) reported a positive correlation between salt tolerance in *Festuca rubra* and *Agrostis stolonifera* and the salinity of the site from which populations had been collected. Other studies since then have reached similar conclusions (Tiku and Snaydon, 1971; Pezeshki and DeLaune, 1991). In the present study, there was evidence of differences in salt-tolerance based on the photosynthetic and growth responses of these populations to elevated salinities. The Lake Tambour population showed a growth superior to both the Clovelly and Ferblanc populations under elevated salinity regimes (Figure 2). Traits such as leaf area and culm regeneration are important as they are closely associated with competitive characteristics and can influence the success of transplants in different environments (Silander, 1985). However, population selection should be based on many genetic strains desirable for use in revegetating various wetland sites, the soil of which may have differing physicochemical characteristics.

The success of marsh restoration projects is governed by numerous factors including hydrology, physicochemical characteristics of the soil, and plant ability to cope with such conditions. The present research aimed at evaluating the potential superior salt-tolerant populations of *S. patens* have resulted in findings which are encouraging and promising for future studies. These findings are important from the practical point of view because of the potential for selection of stress-tolerant populations of *S. patens*. The within- and between- population variation in the characteristics studied suggests three major points: First, collection of germplasm from a wide range of sites is needed to increase the likelihood of capturing the maximum salinity tolerance. Second, the need for future evaluation of US Gulf Coast populations of *S. patens* and other dominant species to elevated salinities is obvious. The variation in the characteristics studied suggests that collection of germplasm from a wide range of sites is needed to increase the likelihood of capturing the maximum salinity tolerance. Obviously, such evaluation encompasses laboratory and greenhouse studies that must be followed by field *in situ* transplanting to further compare performance of selected populations under a broad range of flooding/salinity habitats under natural conditions. Third, although certain measured parameters appeared to reflect the relative position of these populations in the natural range of *S. patens*, based on the present data salt-tolerance in this species cannot generally be inferred from the field position. Thus finding the greatest level of salt-tolerance among the population of this and other important coastal macrophyte species remains a major task.

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