ORIGINAL PAPER

# Adventitious sprouting of *Pinus leiophylla* in response to salt stress

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**Abstract**
· *Context* Although adventitious shoots are produced in many tree species in response to injury, little is known about the effects of salinity on sprouting.
· *Aims* The main objective was to examine the sprouting capacity of *Pinus leiophylla* seedlings in relation to injury and physiological changes induced by NaCl.
· *Methods* Seedlings were grown in controlled-environment growth rooms and treated with 0, 100, 150, and 200 mM NaCl. Numbers of adventitious shoots were recorded and growth and physiological parameters measured after 64 days of treatments and following a 30-day recovery period.
· *Results* NaCl treatments triggered sprouting of adventitious shoots, mainly in the lower parts of the stems. However, fewer sprouts were induced by 200 mM NaCl compared with the lower concentration treatments. These changes were accompanied by needle necrosis, decreased chlorophyll concentrations, seedling dry mass, and stem diameter. Stomatal conductance, net photosynthesis, and root hydraulic conductance decreased with increasing NaCl concentrations and did not return to the control levels after 30 days of stress relief.
· *Conclusions* Pinus leiophylla has the regeneration potential when exposed to salinity. However, very high salt concentrations induce severe physiological impairments and, consequently, a decrease of this regeneration potential.

## 1 Introduction

Conversion of forests to agricultural lands used for annual crop production has intensified in the recent years in Central Mexico (Masera 1996; Alix-García et al. 2005). Between 1970 and 1990, agricultural lands increased by 39 % and the area dedicated to pasture more than doubled, while forests declined by 13 % (Segura 2000). Land conversion has had a major impact on hydrological cycle and salinization. It also increased soil compaction and erosion, decreased soil fertility, diminished natural plant regeneration, and promoted the establishment of undesirable weedy plants (Bettenay 1986; Ezcurra 1991; Rengasamy 2006). Earlier reports estimated the salinization of agricultural soils in Mexico at about 1.5 million hectares (Ortiz 1993). These areas are considered unsuitable for intensive agriculture and are frequently abandoned. Intensive reclamation efforts which include selection of salt resistant species are required prior to their subsequent reforestation.

*Pinus leiophylla* Schl. et Cham. is a widely distributed tree species in Mexico with high potential for the reclamation of degraded land due to its adaptability to different environments and its tolerance of stress conditions (Eguiluz-Piedra 1978; Musálem and Martínez-García 2003). *P. leiophylla* has been used in experimental plantations for erosion control and soil conservation purposes in Asia, Africa, Caribbean, South America, and Oceania (Rodríguez-Franco 2002).

Unlike many other pine species, *P. leiophylla* develops sprouts from the root collar and along the trunk and branches in response to defoliation, fire, pruning, and other stress

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factors (Lanner 2002; Rodríguez-Trejo and Fulé 2003). This attribute is considered an important survival strategy under conditions which cause injury to aerial parts of trees and this strategy may increase tree survival in disturbed areas (Del Tredici 2001).

In trees, sprouts can be derived from epicormic buds, which fail to flush during the growth season and remain dormant in the bark, or from adventitious buds, which may be formed from different living tissues as a result of injury (Quine 2004; Moreira et al. 2009). Although the origin of sprouts in *P. leiophylla* is not known, they have been reported to form mostly at the root collar and to decrease with tree height (Del Tredici 2001) suggesting that they are of adventitious origin, produced as a result of wounding and environmental stimuli. The formation of adventitious buds may depend on factors such as the extent of injury, hormonal status, and accumulation of carbohydrates in the parenchyma cells of sapwood (Lanner 2002; Del Tredici 2001; Climent et al. 2004). Mature parenchyma cells may resume division in response to these external factors and revert to an undifferentiated meristem which gives rise to adventitious shoots in various parts of the plant (Esau 1977; Bowes 1996).

We have earlier demonstrated that direct toxicity and needle injury were largely responsible for the reduction of photosynthetic rates in *P. leiophylla* subjected to salt stress (Jimenez-Casas and Zwiazek 2013). However, shoot apical meristems in *P. leiophylla* appeared to be protected from salt injury even in the highest studied NaCl concentrations. Therefore, it is plausible that *P. leiophylla* may have effective mechanisms allowing the trees to regenerate and survive potentially lethal exposures to salinity. Tree regeneration through adventitious bud flushing requires a functional root system and vascular tissues and is more common in trees suffering from mechanical injury and traumatic events which affect hormonal distribution in trees (Del Tredici 2001). Although salt injury is mostly manifested in foliage it is usually systemic and affects different tissues and organs (Hasegawa et al. 2000). Therefore, in addition to hormonal and nutritional balance, salt injury may affect sprouting potential by affecting the processes required to support the growth of adventitious buds. We expected that sprouting and growth of adventitious shoots would be reduced in plants severely injured by salt.

In the present study, we investigated the effects of NaCl treatment concentrations on growth, needle injury, and physiological parameters in *P. leiophylla* seedlings, including gas exchange and root hydraulic conductance in relation to sprouting. We examined the hypotheses that (1) NaCl-induced injury would promote the formation of adventitious shoots in *P. leiophylla* seedlings and (2) severe impact on physiological processes by high concentration NaCl treatments would diminish the sprouting capacity of seedlings.

## 2 Materials and methods

### 2.1 Plant material and growth conditions

Seeds of *Pinus leiophylla* Schl. et Cham. were collected from open-pollinated trees growing in Tlaxcala State-Mexico (19°19′ N and 98°00′ W). The seeds were soaked in running water for 24 h and placed at 4 °C for another 24 h to enhance germination. The seeds were germinated in the dark at 26 °C in Petri dishes containing moist sand. After radicle emergence from the seed coat, seedlings were transferred to Spencer-Lemaire root trainers (170 cm$^3$ vol, Spencer-Lemaire Industries Ltd., Edmonton, AB, Canada) filled with a mixture of peat moss and sand (2:1 by volume). The seedlings were grown for 14 months in a controlled-environment growth room set to 75 % relative humidity, 24/18 °C (day/night) temperature, 18-h photoperiod, and photosynthetic photon flux (PPF) of approximately 300 μmol m$^{-2}$ s$^{-1}$ at the seedling level. Seedlings were watered twice per week and fertilized every 2 weeks with modified Hoagland's solution (Epstein 1972), containing 224 mg L$^{-1}$ N, 235 mg L$^{-1}$ K, 160 mg L$^{-1}$ Ca, 62 mg L$^{-1}$ P, 32 mg L$^{-1}$ S, 24 mg L$^{-1}$ Mg, 1.77 mg L$^{-1}$ Cl, 0.27 mg L$^{-1}$ B, 0.11 mg L$^{-1}$ Mn, 0.131 mg L$^{-1}$ Zn, 0.032 mg L$^{-1}$ Cu, 0.05 mg L$^{-1}$ Mo, and 1.12 mg L$^{-1}$ Fe. The nutrients were supplied by placing the containers with seedlings in 25-L tubs and soaking the soil for 4 h. After 14 months, the plants were transferred to 4-L pots using the same substrate and growth conditions as described above.

### 2.2 NaCl treatments

Sixteen-month-old dormant *P. leiophylla* plants were subjected to 0 (control), 100, 150, and 200 mM NaCl treatments (0.71, 10.3, 15.1, 19.6 dS m$^{-1}$ electrical conductivity, respectively). All treatments were prepared in distilled water. The treatments were applied by soaking the pots for 24 h every 3 days. All plants were fertilized every 15 days with the modified Hoagland's solution as above. Leachate electrical conductivity of randomly selected pots was checked every 8 days, and the soil was flushed with distilled water every 10 days to prevent salt accumulation. The treatments were applied randomly among the blocks of four plants and were replicated three times resulting in 12 plants per treatment for the total of 48 plants. The treatments were carried out for 64 days followed by a recovery period of 30 days. At the start of the recovery period, the soil was flushed with distilled water and all plants were irrigated with water and supplied with modified Hoagland's solution without NaCl. During the recovery period, soil electrical conductivity varied between 0.80 and 1.03 dS m$^{-1}$.

### 2.3 Determinations of biomass, growth, and adventitious shoot sprouting

Stem diameter at the root collar and terminal shoot lengths were measured at the end of NaCl treatments and after the recovery period. Adventitious shoots greater than 2-cm in length were counted at the end of the recovery period. The plants were harvested at the end of the recovery period, their roots and shoots were separated, and new needles (grown after the onset of NaCl treatment) were removed from the stems. The tissues were then dried at 70 °C (24 h for roots and 48 h for stems and needles) for dry mass determinations.

### 2.4 Determinations of survival, needle injury, and chlorophyll content

Needle injury and seedling survival were monitored daily. At the end of the recovery period, necrotic parts of the needles were separated from the green, healthy, tissues. The necrotic and green needle parts were then dried at 70 °C for 24 h and weighed. The proportion of needle necrosis was calculated from the dry weights of green and necrotic needle tissues. Plants were considered dead when needle necrosis was above 70 %, shoot apical meristems were necrotic and there was no new needle growth.

Needle chlorophyll contents in all plants were determined at the end of NaCl treatments according to Hiscox and Israelstam (1979). Six needles were randomly sampled from each part of the shoot (top, middle, and bottom), cut into small pieces and mixed. Tissue samples, each containing 100 mg of needles, were extracted with 7 ml DMSO at 65 °C for 25 min. The extracts were diluted with 7 ml DMSO and the total chlorophyll concentrations were determined spectrophotometrically. Chlorophyll a and b were calculated using the Arnon's equation (Arnon 1949).

### 2.5 Measurements of gas exchange and root hydraulic conductance

Net photosynthesis ($P_n$) and stomatal conductance ($g_s$) were measured every 8 days during 64 days of NaCl treatments and after the recovery period of 30 days. For the measurements, six seedlings per treatment were randomly taken, and four fascicles per seedling were measured. Each fascicle with five fully grown needles was selected approximately 3 cm below the terminal shoot apex. Prior to the measurements, the pots with plants were soaked overnight in water to the field capacity. The measurements started two hours after the onset of photoperiod and were taken with a portable infrared gas analyzer equipped with the conifer sample chamber (LCA4, Analytical Development Company Limited, Hoddesdon, UK). During the measurements, leaf temperature in the

**Table 1** Root, stem, needle, and total dry mass of *P. leiophylla* seedlings subjected to NaCl treatments for 64 days and control plants (0 mM NaCl). Means ($n=12\pm$SE) with different letters in the same column indicate significant differences at $\alpha=0.05$ level as determined by the Duncan's standardized range test

| [NaCl] mM | Roots (g) | Stems (g) | Needles (g) | Total (g) |
|---|---|---|---|---|
| 0 | 39.8±11.3 a | 14.8±2.36 a | 46.8±4.82 a | 101.4±8.86 a |
| 100 | 21.9±3.14 b | 9.64±1.72 b | 37.9±6.36 b | 69.6±5.35 b |
| 150 | 15.6±3.35 c | 6.25±0.86 c | 30.9±3.56 c | 53.0±5.76 c |
| 200 | 10.7±1.95 c | 5.96±0.55 c | 24.2±4.37 d | 40.9±3.47 d |

sample chamber was $24\pm1$ °C and PPF was 300 µmol m$^{-2}$ s$^{-1}$. Needle surface areas were obtained after computer scanning and calculated using Sigma Scan Pro 5.0 software (SPSS, Jandel Scientific, San Rafael, CA, USA).

After the recovery period, the pots with plants were soaked overnight in water to minimize the effects of possible soil moisture differences for the maximum hydraulic conductance and the next morning, they were moved to the laboratory for the measurements of root hydraulic conductance ($K_r$) (Tyree et al. 1995). For $K_r$ measurements, the shoots were excised about 3 cm above the root collar, and the roots were connected through the cut stem to the high-pressure flow meter (HPFM) via a piece of flexible high-pressure tubing. The
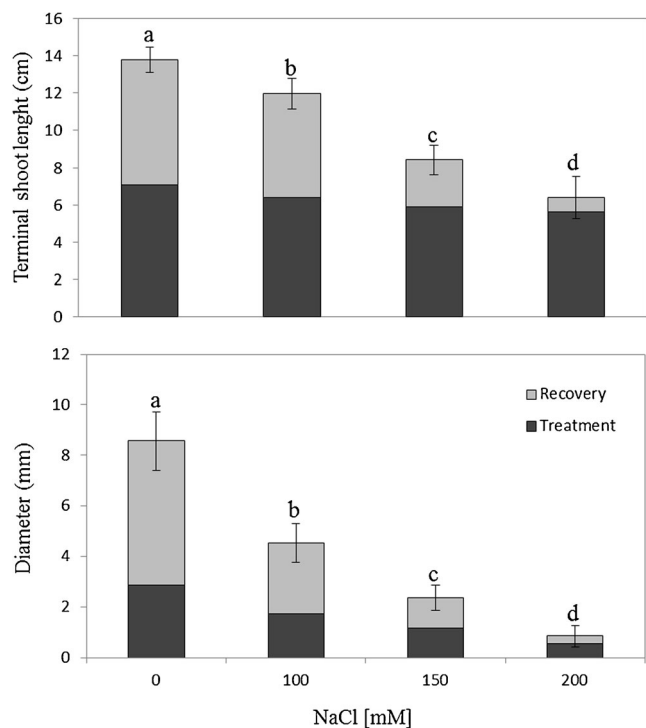


**Fig. 1** Effects of 0 (control), 100, 150, and 200 mM NaCl on stem diameters and terminal shoot lengths of seedlings after 64 days of treatments and after the recovery period. *Bars* are means ($n=12$)±SE. Different *letters* indicate significant differences at $\alpha=0.05$ determined by the Duncan's test

**Table 2** Numbers of adventitious shoots per plant and percentages of plants with adventitious shoots (in brackets) present at the root collar, main stem, and branches; and dry weights of new needles in seedlings subjected to 0 (control), 100, 150, and 200 mM NaCl. Means ($n=12\pm$SE) in each column followed by different letters are significantly different at $\alpha=0.05$ level as determined by the Duncan's standardized range test

| [NaCl] | Number of adventitious shoots | | | | New needles |
|---|---|---|---|---|---|
| mM | Root collar | Main stem | Branches | Total | (g) |
| 0 | 1.58±1.13 b (75) | 2.61±2.15 b (67) | 0.333±0.651 b (25) | 4.60 | 15.6±1.57 a |
| 100 | 7.33±1.71 a (100) | 11.6±2.11 a (100) | 4.58±1.56 a (100) | 23.5 | 9.66±1.14 b |
| 150 | 7.83±2.12 a (100) | 12.7±1.93 a (100) | 4.33±1.49 a (100) | 24.9 | 4.93±1.13 c |
| 200 | 7.16±2.75 a (100) | 3.63±5.31 b (33) | 1.50±2.46 b (33) | 12.2 | 0.969±0.42 d |

measurements were carried out in the transient mode with the roots pressurized up to 0.5 MPa (Calvo-Polanco et al. 2009).

### 2.6 Statistical analysis

The data analysis was performed with SAS-GLM procedure, and means of treatments were compared by Duncan's range test. $P_n$ and $g_s$ were analyzed by repeated measurements ANOVA using the split-plot design with time (weeks) as a subplot effect. The analysis was conducted using the PROC MIXED-SAS/PC for Windows version 8.2 (SAS institute Inc. 2001).

## 3 Results

### 3.1 Biomass, growth, and adventitious shoot sprouting

After 64 days of treatments, shoot and root dry mass of plants treated with 100 mM, 150 mM, and 200 mM NaCl was significantly lower compared with the control, untreated plants (Table 1). Shoot and root dry mass of treated plants decreased with increasing NaCl treatment concentrations (Table 1).

Terminal shoot length and stem diameter growth of treated plants significantly decreased with increasing NaCl concentration compared with control plants (Fig. 1).

Plants treated with NaCl showed a significant increase in the adventitious shoot production (Table 2). Sprouting of adventitious shoots in plants was first observed 40 days after the start of NaCl treatments. The first adventitious shoots were produced at the base of the stem near the root collar (Fig. 2), while sprouting of adventitious shoots from branches occurred towards the end of NaCl treatments. About 33 % of plants treated with 200 mM NaCl produced adventitious shoots compared with 100 % of seedlings that were treated with 100 and 150 mM NaCl (Table 2). Sprouting density was higher in the upper parts of the terminal shoot compared with the base of the terminal shoot or branches (Table 2). The production of needles was significantly reduced by increasing NaCl concentrations. The total dry mass of new needles was reduced by as much as 94 % in the 200 mM NaCl treatment (Table 2).

### 3.2 Survival, needle injury, and chlorophyll content

Seedlings which were subjected to 0, 100, and 150 mM NaCl for 64 days showed 100 % survival compared with 67 % survival for plants treated with 200 mM NaCl (Table 3). Injury symptoms started appearing on older needles after 24 days of treatment, first as needle tip chlorosis followed by the appearance of necrotic lesions about 10 days later. Although needle injury was observed in all NaCl-treated plants, the extent of injury was the greatest in plants exposed to 200 mM NaCl with 80 % of needle weight affected by necrosis (Table 3). The seedlings treated with 200 mM NaCl also had the lowest chlorophyll needle concentrations with chlorophyll a and b reduced to approximately 42 and 13 %, respectively, of the control level (Table 3). Plants treated with 100 mM NaCl had the chlorophyll a and b concentrations reduced to 71 and 81 %



**Fig. 2** Adventitious shoots in the *Pinus leiophylla* seedling treated with 150 mM NaCl for 64 days

**Table 3** Needle chlorophyll concentration ($n=6$), needle necrosis and survival ($n=12$) of seedlings subjected to NaCl treatments for 64 days and in control plants (0 mM NaCl). Means±SE are shown. In each column, different letters indicate significant differences at $\alpha=0.05$ as determined by the Duncan's test

| [NaCl] | Chlorophyll (mg g$^{-1}$) | | | Needle necrosis (% needle weight) | Survival (%) |
|---|---|---|---|---|---|
| mM | a | b | Total | | |
| 0 | 0.720±0.019 a | 0.476±0.013 a | 1.19±0.028 a | 0 | 100 |
| 100 | 0.518±0.022 a | 0.387±0.012 b | 0.865±0.025 b | 39.9±9.23 c | 100 |
| 150 | 0.370±0.046 c | 0.180±0.012 c | 0.551±0.055 c | 63.2±7.21 b | 100 |
| 200 | 0.304±0.051 d | 0.062±0.016 d | 0.367±0.059 d | 80.4±6.96 a | 33.3 |

of the control level and with 40 % of needle necrosis (Table 3). Control plants had no needle necrosis (Table 3).

### 3.3 Gas exchange and root hydraulic conductance

Both $P_n$ and $g_s$ significantly decreased over time with increasing NaCl concentration treatments overtime (Table 4), and the most rapid decreases occurred in 150 and 200 mM NaCl compared with 100 mM NaCl (Fig. 3 a, b). At the end of the treatments, the $g_s$ values of plants treated with 100 and 150 mM were approximately 70 and 90 %, respectively, lower than those of control plants (0 mM NaCl) (Fig. 3a). The $g_s$ of plants treated with 200 mM NaCl treatment was completely inhibited at the end of treatment (Fig. 3a). At the end of the recovery period, the $g_s$ values slightly increased in NaCl-treated plants, but were still several fold lower compared with untreated controls (Fig. 4a). The $P_n$ values of treated plants followed similar patterns as those shown by $g_s$ (Fig. 3b). At the end of the NaCl treatments, the $P_n$ values of plants treated with 100, 150, and 200 mM were 42, 64, and 84 % lower, respectively, than those of control plants.

Similarly to $g_s$ $P_n$, and $K_r$ values did not return to the control levels after 30 days of stress relief (Fig. 4b, c). The recovery of $P_n$, $g_s$, and $K_r$ in plants treated with 200 mM was

**Table 4** F values in the analysis of variance of the effects of NaCl (Sal), block (B), and treatment duration (time) on net photosynthesis ($P_n$) and stomatal conductance ($g_s$) in plants treated for 64 days

| Source | DF | Gas exchange | |
|---|---|---|---|
| | | $P_n$ | $g_s$ |
| Sal | 3 | 745.8** | 901.7** |
| B | 2 | 1.1 | 4.0 |
| Sal X B | 6 | 0.9 | 3.3 |
| Time | 7 | 209.9** | 497.5** |
| Time X Sal | 21 | 42.3** | 43.6** |
| Time X B | 14 | 1.0 | 0.8 |
| Time X B X Sal | 42 | 1.1 | 0.7 |

**Significance level, $P\leq0.01$

not measured due to low seedling survival and high extent of needle injury.

## 4 Discussion

In our study, adventitious sprouting was observed at the stem base, main stem, and branches of all NaCl-treated *P. leiophylla* seedlings. This points to the ability of this species to sprout in response to salt injury similarly to trees damaged by fire (Perry 1991) and herbivory (Jasso-Mata and Jiménez-Casas 2001). Sprouting is considered as part of the plant regeneration strategy after damage caused by biotic and abiotic factors (Vest and Westoby 2004; Wan et al. 2007). Sprouting of adventitious shoots is thought to be related to apical dominance with auxins and cytokinins likely involved in the control of these processes (Cline 1991; Wan et al. 2006). On the other hand, the sprouting ability of sugar maple, red alder, giant sequoia, Canary Islands pine, and other tree species has been explained by the carbohydrate reserves present in sapwood parenchyma (Mooney and Hayes 1973; Harrington 1984; Climent et al. 2004; O'Hara et al. 2008).

Both 100 and 150 mM NaCl were more effective than 200 mM NaCl in stimulating sprouting of adventitious shoots from the lateral branches and from the upper parts of the main stem, but the number of adventitious branches that were produced from the stem base was similar for all NaCl treatments (Table 2). The sprouting from the stem base also took place in seedlings which suffered extensive damage to the shoots. Similar findings were reported for *Arbutus arizonica* where sprouting capacity decreased as a result of shoot injury severity that was caused by fire (Barton 2005). However, in *Sequoia gigantea*, sprouts were more frequent and larger on more severely pruned trees (O'Hara et al. 2008). These results indicate that the frequency and number of sprouts vary according to the severity and type of stress. The inhibition of photosynthesis by salt stress may severely reduce the stored carbohydrates. The declines in tree productivity may take a long time to recover as evidenced in the present study. The slow photosynthetic recovery is likely partly due to the effect

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**Fig. 3** Changes in net photosynthesis ($P_n$) and b stomatal conductance ($g_s$) in seedlings during 64 days of 0 (control), 100, 150, and 200 mM NaCl treatments. Each *point* represents mean ($n=6$)±SE
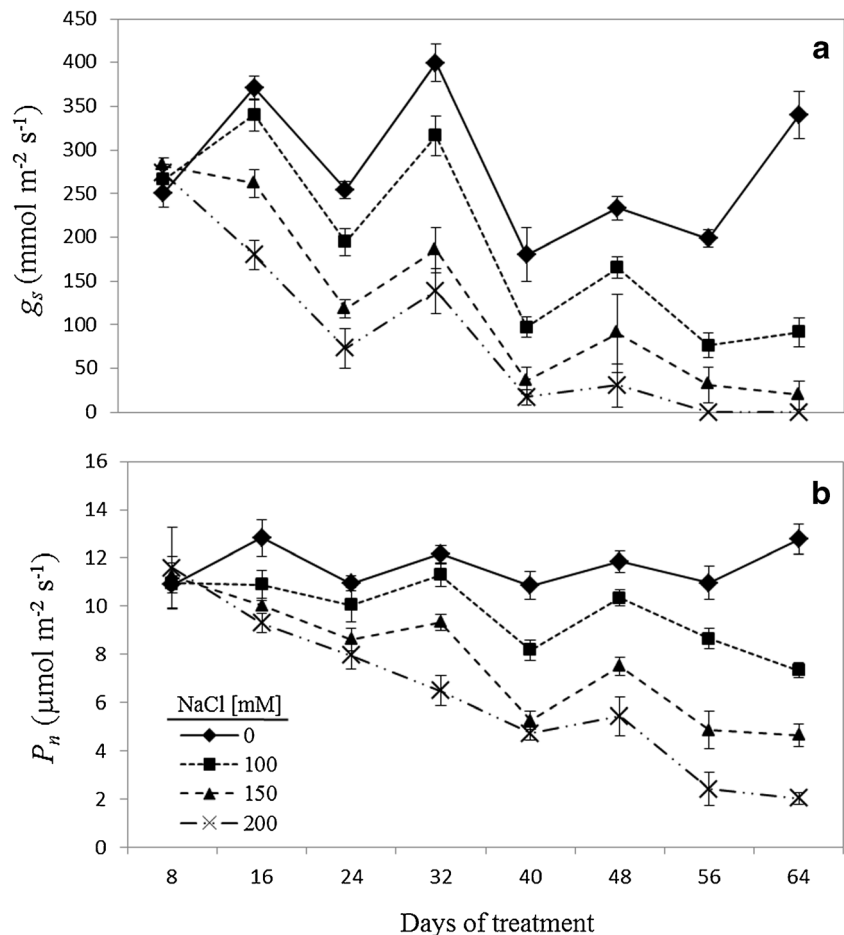


of NaCl on needle chlorophyll concentrations which may need more time to recover compared with the stomatal responses. In our study, accumulation of Na and Cl in tissues and the injury level in plants treated with 200 mM NaCl likely affected the ability of plants to mobilize carbohydrates which, in turn, reduced the number of sprouts in stems and branches but not in the root collar section. Root collar is a storage place of starch reserves in many conifers (White 1981; Chomba et al. 1993). Therefore, buds close to the root collar had likely higher concentrations of available carbohydrate reserves.

The effects of salinity on plants are associated with ion toxicity and low osmotic potential which triggers stomatal closure and decreases photosynthetic rates (Allen et al. 1994; Hasegawa et al. 2000; Ashraf and Harris 2004). The reduction of $P_n$ and $g_s$ in NaCl-treated *P. leiophylla* plants increased with increasing NaCl concentrations and progressed over time. It is interesting that $P_n$ values in plants treated with 200 mM NaCl for 56 and 64 days were very low, but still positive and $g_s$ was near 0. Although this could be explained by minor instrument calibration issues, the changes in $P_n$ and $g_s$ showed a clear NaCl concentration-dependent pattern of decline in both parameters over time. Similar responses were observed in plants of *P. pinaster*, *Arbutus unedo*, *and Aster tripolium* subjected to a NaCl gradient where $P_n$ and $g_s$ also

was reduced progressively by NaCl in a concentration-dependent manner (Loustau et al. 1995; Robinson et al. 1997; and Navarro et al. 2007).

Compared with other studied conifers (Fostad and Pedersen 2000; Apostol and Zwiazek 2003; Franklin and Zwiazek 2004; Renault 2005), *P. leiophylla* seedlings showed a high degree of salt tolerance with no seedling mortality in NaCl concentrations as high as 150 mM after 64 days of treatment. Needle chlorosis and necrosis in *P. leiophylla* were likely related to Na and Cl tissue concentrations. In tamarack and jack pine treated with NaCl, the increase in needle concentrations of Na and Cl corresponded to the applied NaCl concentrations and were correlated with the extent of needle injury (Apostol et al. 2002; Renault 2005; Calvo Polanco et al. 2008).

The reduced levels of $P_n$, $g_s$, and $K_r$ in NaCl-treated plants at the end of the recovery period indicate that seedlings may require a relatively long time to fully recover from the effects of salt and this may affect the ability of plants to produce new roots and needles to replace the biomass loss caused by salinity. The loss of root tissue was likely among the main factors responsible for the reduction of $K_r$. Contrary to drought, salinity effects on gas exchange and water transport may be more persistent due to Na uptake. The principal mechanism of salt tolerance in many woody plants is Na

**Fig. 4** Stomatal conductance ($g_s$) (**a**), net photosynthesis ($P_n$) (**b**), and root hydraulic conductance ($K_r$) (**c**) in seedlings subjected for 64 days to 0 (control), 100, and 150 mM NaCl treatments followed by 30 days of recovery. *Bars* are means ($n=6$)±SE. Different *letters* indicate significant differences at $\alpha=0.05$ determined by the Duncan's test
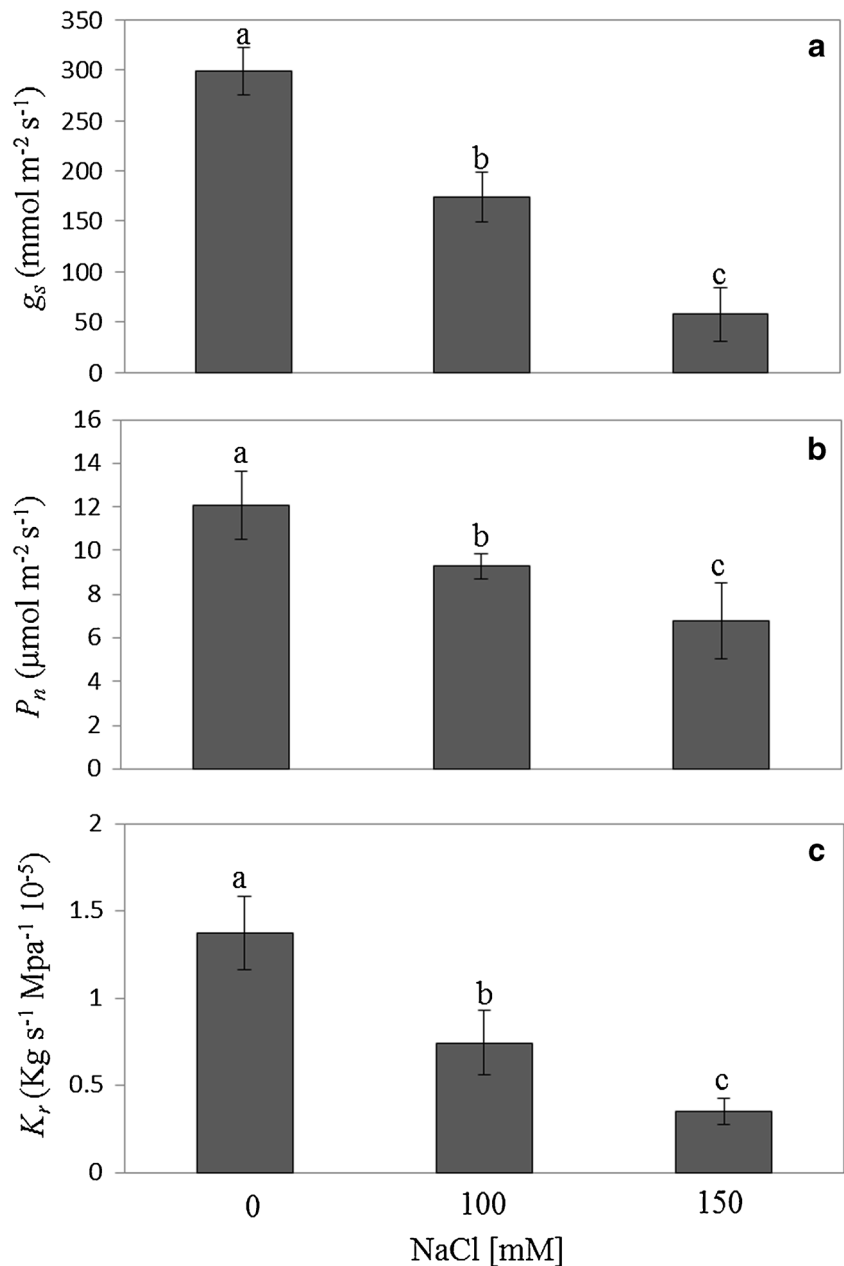


exclusion from the shoot tissues through its sequestration in roots (Renault et al. 2001; Muhsin and Zwiazek 2002). There-fore, in severely salt-stressed plants, the presence of Na in shoots hampers the gas exchange recovery processes. Al-though in plants subjected to 200 mM NaCl, the roots were too severely damaged to measure $K_r$, the injury patterns displayed in response to 100 and 150 mM NaCl indicate that the increasing NaCl concentrations reduced the ability of the root system to deliver water to the shoots. Therefore, the delivery of water and mineral nutrients could also be a restricting factor to growth of adventitious shoots in high NaCl concentrations. In less injured plants, the adventitious branch sprouting in response to NaCl may contribute to the production of new photosynthetic tissues and consequently

improve $P_n$ levels to increase the photosynthate production needed for the re-growth of plants injured by salinity.

In conclusion, NaCl treatments stimulated adventitious sprouting in *P. leiophylla* seedlings and caused reductions in growth, $P_n$, $g_s$, and $K_r$. In 100 and 150 mM NaCl treatments, the increased adventitious sprouting was associated with an increase in the extent of needle injury. However, in the highest concentration, 200 mM NaCl treatment, the production of adventitious shoots decreased in all parts of the seedlings with the exception of root collar. The results indicate that *P. leiophylla* has the potential to regenerate even after expo-sure to relatively high NaCl concentrations. However, at high levels of NaCl injury, the regeneration potential is likely to be affected by the physiological effects of NaCl including

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decreased photosynthetic productivity and the resulting depletion of stored energy.

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