Effects of sodium chloride salinity on root growth and respiration in oak seedlings

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Abstract – Root and shoot biomass of oak seedlings were reduced after 9 days of watering with a nutrient solution containing either 50 or 250 mM NaCl. Both moderate and high salinity treatment strongly altered root elongation. In contrast, specific respiration of roots was unaffected by the moderate salinity treatment while it was reduced by 62 % after 9 days of watering with a nutrient solution containing 250 mM NaCl. Na⁺ content strongly increased in all plant tissues with increasing NaCl concentration in the nutrient solution. Na⁺ contents in leaves and in twigs were lower than in roots at 50 mM NaCl in the nutrient solution while they were similar at 250 mM. Prevention of Na⁺ translocation in shoot in moderately stressed oak probably requires extra energy, which may be provided by an increase in maintenance respiration. At higher salinity (250 mM), root respiration was strongly inhibited, which might explain the inability of severely stressed oak seedling to prevent Na⁺ translocation to the shoot. An increase in the respiratory cost for maintenance, for active ion transport and/or for growth processes in oak root encountering sodium chloride salinity is therefore consistent with the occurrence of a high rate of root respiration while growth rate was reduced. (© Inra/Elsevier, Paris.)

growth / oak / respiration / root / salinity

Résumé – Effets de la salinité (NaCl) sur la croissance et la respiration des racines de semis de chêne. La biomasse racinaire et aérienne de semis de chêne est réduite après 9 j d’arrosage avec une solution nutritive contenant 50 ou 250 mM de NaCl. Les traitements salins modérés et élevés altèrent fortement l’elongation des racines. Au contraire, la respiration spécifique des racines reste inchangée pour le traitement salin modéré, alors qu’elle est réduite de 62 % après 9 j d’arrosage avec une solution nutritive contenant 250 mM de NaCl. Le contenu en Na⁺ augmente dans tous les tissus lorsque la concentration en NaCl augmente dans la solution nutritive. Les contenus des feuilles et des tiges en Na⁺ sont plus faible que celui des racines à 50 mM de NaCl alors qu’ils sont similaires à 250 mM. Cette faible translocation du sodium dans les parties aériennes des chênes modérément stressés a probablement un coût énergétique compensé par une augmentation de la respiration de maintenance. Pour une salinité plus forte (250 mM), la respiration racinaire est fortement inhibée. Ceci explique peut-être l’incapacité des chênes fortement stressés à s’opposer à une translocation de Na⁺ dans les parties aériennes. Une augmentation du coût respiratoire des processus d’entretien, des transports ioniques actifs et/ou du métabolisme associé à la croissance, est donc susceptible d’expliquer le maintien d’une intensité respiratoire racinaire inchangée alors que la croissance des racines est inhibée. (© Inra/Elsevier, Paris.)

croissance / chêne / respiration / racine / salinité

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1. INTRODUCTION

Salt stress limits growth and development of non-halophytes [12]. To date, studies have mainly focused on plants which naturally grow in natural saline environments or on crop plants which may encounter salinity induced by agricultural practices like irrigation. There is less information concerning temperate tree species since forest soils are rarely salt-affected. However, the use of a deicing agent along motorways may promote salt accumulation in poorly-drained soils of roadside ecosystems [11]. The effects of snow melt have been documented for wetland ecosystems [14] but little is known for forests even if rather high sodium contents (up to 0.4 mol kg\textsubscript{DW}\textsuperscript{-1}) are measured in leaves of trees growing in the vicinity of a highway [11, 13]. In another context, rural changes may promote natural or artificial afforestations of abandoned areas encountering excessive salt concentrations.

Many studies have focused on shoot growth responses and associated physiological processes. However, the root is the first organ of the plant exposed to soil salinity. The root controls delivery of salt to the shoot by its ability to exclude or sequester salts [19, 23]. As highlighted by Neumann et al. [18], the inhibition of root growth reduces the explored soil volume and may therefore limit growth by an additional alteration of uptake of nutrient and water, or by a reduction of the synthesis and the supply of growth regulators to the shoot. Moreover, the development of the root system is crucial for the establishment of tree seedlings and then for their further growth and development.

Root growth results from both cell production at the root tip level and turgor-dependent cell expansion, which may be altered by either the osmotic effects of salt and/or salt-induced changes in cell wall extensibility [15, 18]. These changes in cell wall properties could increase the respiratory cost of root growth. Additional active ion transports and increased turnover of proteins to cope with salt-induced damages can increase the respiratory cost of maintenance processes [23]. Therefore, the capacity of the respiratory system may become limiting, especially if ion accumulations alter both the amount and the activity of respiratory enzymes.

The objectives of the present work were to examine the effects of sodium chloride salinity on the non-halophyte but drought-tolerant woody species Quercus robur. We focused our attention on the growth of the root system and attempted to investigate the relationship between the inhibition of root growth and changes in specific root respiration. In addition, we discussed whether the inhibition of root growth is due to the decrease in the osmotic potential of the rooting medium or to the toxic effects of salts.

2. MATERIALS AND METHODS

2.1. Plant material and growth conditions

Oak acorns (Quercus robur L.) were soaked in aerated deionized water for 48 h and germinated on wet vermiculite in the dark at room temperature for 7 days. The seedlings were transplanted in 4 L transparent Plexiglas tubes (50 cm high) filled with a 1:1 (v/v) mixture of perlite and vermiculite. The tubes were held at a 30° angle from vertical and covered with a black plastic sheet. Seedlings were grown in a growth chamber with a day/night temperature of 20/30 °C, day/night relative humidity of 40/60 %, and a 14 h photoperiod with a photon flux density at the height of the first leaves of about 180 μmol m\textsuperscript{-2} s\textsuperscript{-1}. Plants were watered daily with distilled water during the first week and then with the following nutrient solution: 2.5 mM NO\textsubscript{3}\textsuperscript{-}, 0.5 mM NH\textsubscript{4}\textsuperscript{+}, 2 mM K\textsuperscript{+}, 1 mM Ca\textsuperscript{2+}, 0.5 mM Mg\textsuperscript{2+}, 0.05 mM Fe-EDTA, 5 μM Mn\textsuperscript{2+}, 0.5 μM Zn\textsuperscript{2+}, 0.5 μM Cu\textsuperscript{2+}, 1 mM Cl\textsuperscript{-}, 0.55 mM SO\textsubscript{4}\textsuperscript{2-}, 0.5 mM PO\textsubscript{4}\textsuperscript{3-}, 1.5 μM B\textsuperscript{3-}, 0.1 μM MoO\textsubscript{4}\textsuperscript{2-}. Salinity treatment began 24 days after sowing. NaCl was added to the nutrient solution to a final concentration of 0, 50 and 250 mM. The highest NaCl concentration was reached in three daily steps of 50, 150 and 250 mM. Five seedlings per treatment were randomly distributed in the growth cabinet and the location of the seedlings was randomly changed every day. Leaf predawn water potential was measured with a pressure chamber at the end of the dark period just before measuring root respiration and harvesting the plants.

2.2. Measurement of root growth

The roots visible through each tube were traced onto acetate sheet every 2 or 3 days at the end of the night period with fine waterproof markers of different colours. Root length produced between two successive measurements was calculated by summing the length of all root segments, and represented root production as root loss did not occur. Root growth rates were calculated by dividing root production by the time interval between two successive measurements. Tap and lateral roots were distinguished.

2.3. Measurement of root respiration

At the end of the experiment, the shoot was cut, the cut-edge covered with mastic and the head of the Li 6000-09 (LiCor Inc., Lincoln, NE, USA) was tightly sealed to the top of the Plexiglas tubes. The increase of the CO\textsubscript{2} concentration within the closed system was recorded with the Li 6250 infrared gas analyser (LiCor Inc., USA) for
2 min. Three measurements were made to check that the CO₂ flux was stabilized. Whole root respiration rates (R, μmol s⁻¹) were calculated as:

\[ R = V \frac{d[CO_2]}{dt} \]

V being the volume of air inside the closed system (mol), and \( d[CO_2]/dt \) the rate of increase in the CO₂ concentration (μmol mol⁻¹ s⁻¹). Specific root respiration rates were whole root respiration rates divided by root dry weights (kg). The CO₂ concentration within the system ranged between 550 and 650 μmol mol⁻¹ during measurements. Measurements were done at the end of the dark period. At this time, root zone temperature (15 cm depth) was 21 °C. Two tubes filled with the same substrates and watered with the same nutrient solutions but without seedlings were used to check for an eventual heterotrophic respiration due to unwanted microbial colonization of the tubes. In fact, no background respiration was detected.

2.4. Final harvest and chemical analysis

At the end of the experiment, the seedlings were harvested and separated into leaves, twigs, tap and lateral roots. Roots were washed with deionized water. Whole plant leaf areas were measured with a leaf area meter (Li 3000, LiCor Inc., USA). Dry weights were determined after oven drying at 60 °C for 140 h. Then, each part was finely ground in a mill using a 1 mm mesh. A subsample (0.1 to 0.5 g) was ignited on a muffle furnace. The remaining ash was then dissolved in 1.5 mL of concentrated HNO₃. The solution was made up with distilled water to a final volume of 50 mL. Lanthanum oxide was added to a final concentration of 5 mM. Determinations of K⁺, Na⁺, Mg²⁺ and Ca²⁺ were done by atomic absorption spectrophotometry (Model 3110, Perkin Elmer Corp., Oak Brook, Ill, USA).

2.5. Statistics

Statistical analyses were based on one-way analysis of variance. The effects of salinity treatments were considered statistically significant when \( P < 0.05 \). In these cases, the Fisher least significant differences (LSD) were calculated and are given in the tables and figures. Five replicates per treatment were used.

3. RESULTS

3.1. Water potential, biomass and leaf area

After 9 days of watering with a nutrient solution containing 50 and 250 mM NaCl, leaf predawn water potential dropped to −0.30 (± 0.03) and −1.43 MPa (± 0.27) respectively, while it remained at −0.14 MPa (± 0.02) in control seedlings. These values are in agreement with the expected osmotic potentials of the nutrient solutions. Both root and shoot dry weights were affected by the presence of NaCl in the nutrient solution (−22 % at 50 mM and −59 % at 250 mM for the shoot, and −20 % at 50 mM and −41 % at 250 mM for the root, table I). After 9 days, leaves of severely stressed seedlings (250 mM NaCl) showed typical NaCl-induced necroses. The mean leaf area per seedlings was also decreased by NaCl treatments (−21 % at 50 mM and −62 % at 250 mM, table I). More biomass was allocated to roots in severely stressed seedlings than in moderately stressed or control seedlings (40 and 31 %, respectively, calculated from table I). This increased allocation to roots happened to the detriment of leaves. In contrast, leaf mass per unit area was unaffected by NaCl treatments (data not shown).

3.2. Root elongation

The elongation rates of roots are shown in figure I for plant watered with nutrient solutions containing 0, 50 and 250 mM NaCl. The root length of control seedlings increased by 0.6–0.8 mm h⁻¹ for tap roots and by up to 3 mm h⁻¹ for the whole lateral roots. Salinity strongly altered root elongation. Reduction in root growth rates was already evident after 4 days of severe salinity treatment (250 mM NaCl in the nutrient solution), for both tap and lateral roots. Moderate salinity (50 mM) altered the elongation rates of tap roots after 6 days (9 days for lateral roots). At the end of the experiment (day 9), the elon-

<table>
<thead>
<tr>
<th>NaCl in the nutrient solution (mM)</th>
<th>0</th>
<th>50</th>
<th>250</th>
<th>LSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Root biomass (g)</td>
<td>0.80</td>
<td>0.64</td>
<td>0.47</td>
<td>0.20</td>
</tr>
<tr>
<td>Tap root</td>
<td>0.63</td>
<td>0.50</td>
<td>0.37</td>
<td>0.17</td>
</tr>
<tr>
<td>Lateral root</td>
<td>0.17</td>
<td>0.14</td>
<td>0.09</td>
<td>0.06</td>
</tr>
<tr>
<td>Shoot biomass (g)</td>
<td>1.75</td>
<td>1.37</td>
<td>0.71</td>
<td>0.35</td>
</tr>
<tr>
<td>Twigs</td>
<td>0.51</td>
<td>0.36</td>
<td>0.23</td>
<td>0.14</td>
</tr>
<tr>
<td>Leaves</td>
<td>1.24</td>
<td>1.01</td>
<td>0.48</td>
<td>0.23</td>
</tr>
<tr>
<td>Leaf area (dm²)</td>
<td>2.99</td>
<td>2.35</td>
<td>1.12</td>
<td>0.57</td>
</tr>
</tbody>
</table>

Values are means of five replicates. The Fisher least significant difference (LSD) is given when the effects of salinity treatments were considered statistically significant (one-way analysis of variance, \( P < 0.05 \)).
gation rates of tap and lateral roots of seedlings grown in 50 mM NaCl were reduced by 52 and 58 %, respectively. At higher salinity levels, reductions were stronger (77 and 90 %). For both salinity levels, root elongation rates did not stabilize at the end of the experiment. It would have been interesting to continue the experiment some days more to see whether the root growth would stop; however, the root system would have reached the bottom of the rhizotron.

3.3. Root respiration

The mean respiration rate of oak roots was 15 μmol kg⁻¹ s⁻¹ on a dry weight basis for unstressed seedlings. After 9 days of watering with a nutrient solution containing 250 mM NaCl, the specific respiration rate of the root was reduced by 62 % while it was unaffected by the mildest salinity treatment (figure 2A). The slight decrease in whole root respiration rate at 50 mM NaCl (−18 %) was related to a lower root biomass in moderately stressed than in control seedling (figure 2B). In contrast, the large decrease in whole root respiration rate at 250 mM NaCl (−81 %) was the consequence of both a decrease in root biomass and a decrease in specific respiration rate.

**Figure 1.** Tap (A) and lateral (B) root elongation rates of young oak seedlings watered with nutrient solutions containing either 0 ●, 50 ○ or 250 mM △ NaCl. Salinity treatments began 24 days after sowing (dotted lines). Values are means of five replicates. Vertical bars denote the Fisher least significant differences (LSD) when the effects of salinity treatments were considered statistically significant (one-way analysis of variance, P < 0.05).

**Figure 2.** Effects of salinity on specific respiration (A) and whole respiration (B) of the entire root systems of young oak seedlings watered for 9 days with nutrient solutions containing either 0, 50 or 250 mM NaCl. Values are means of five replicates. Vertical bars denote the Fisher least significant differences (LSD) when the effects of salinity treatments were considered statistically significant (one-way analysis of variance, P < 0.05).
3.4. Chemical composition

Na\(^+\) contents strongly increased in all plant tissues with increasing NaCl concentration in the nutrient solution (Table II). Na\(^+\) contents in leaves and in twigs were lower than in roots at moderate salinity, whereas they were similar at 250 mM. K\(^+\) content was decreased by 50 to 70% in roots of stressed seedlings. In contrast, twig K\(^+\) content was only slightly decreased by salinity, while leaf K\(^+\) content strongly increased (+100% and +190% in 50 and 250 mM NaCl, respectively). Then, as expected from Table II, the Na\(^+\)/K\(^+\) ratio remained lower than 1 in leaves of stressed oaks while strong increases in Na\(^+\)/K\(^+\) ratio were observed in twigs and roots in response to salinity. Ca\(^{2+}\) and Mg\(^{2+}\) contents in roots and twigs were unaffected by salinity. In contrast, leaf Ca\(^{2+}\) and leaf Mg\(^{2+}\) contents were decreased by about 30% under moderate NaCl concentration. The highest NaCl level did not induce any change in leaf Ca\(^{2+}\) and leaf Mg\(^{2+}\) contents.

4. DISCUSSION

The NaCl concentrations in the rooting medium is thought to initially differ from those in the nutrient solutions since the mixture of perlite and vermiculite was previously soaked with a non-salinized nutrient solution. However, the predawn leaf water potentials at the end of the experiment are in agreement with the expected osmotic conditions imposed by nutrient solutions containing either 50 or 250 mM NaCl.

Root growth was strongly inhibited by salinity, leading to a reduction of root biomass. Shoot biomass was similarly or more reduced than root biomass, resulting in a slight increase in the root shoot ratio, a typical response to salinity for non-halophyte plants [12]. The growth rate of both the tap and the whole lateral roots of oak seedlings was significantly decreased by salinity, even at moderate NaCl concentrations. Similar results were reported for many species, like cotton [6] or maize [3]. It has been postulated that growth is first inhibited by a decrease in the osmotic potential of the root medium and then further inhibited by the toxic effects of salt [16, 17]. In oak seedlings, however, the response to salinity is rather different to that in water stress. In contrast with salinity, drought (-2.0 to -2.7 MPa) did not affect root biomass in Quercus robur seedlings [20]. An increase in root elongation was often reported for tree seedlings submitted to mild osmotic stress while only stronger osmotic stress decreased root elongation [22]. Here, a decrease in root growth rate and root biomass was evident even at the mildest salinity level.

Table II. Effects of salinity on mineral composition (mol kg\(^{-1}\)) of tap roots, lateral roots, twigs and leaves of young oak seedlings watered for 9 days with nutrient solutions containing either 0, 50 or 250 mM NaCl.

<table>
<thead>
<tr>
<th>NaCl in the nutrient solution (mM)</th>
<th>LSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>50</td>
</tr>
<tr>
<td>Tap roots</td>
<td></td>
</tr>
<tr>
<td>Na(^+)</td>
<td>0.01</td>
</tr>
<tr>
<td>K(^+)</td>
<td>0.30</td>
</tr>
<tr>
<td>Ca(^{2+})</td>
<td>0.04</td>
</tr>
<tr>
<td>Mg(^{2+})</td>
<td>0.10</td>
</tr>
<tr>
<td>Lateral roots</td>
<td></td>
</tr>
<tr>
<td>Na(^+)</td>
<td>0.03</td>
</tr>
<tr>
<td>K(^+)</td>
<td>0.30</td>
</tr>
<tr>
<td>Ca(^{2+})</td>
<td>0.14</td>
</tr>
<tr>
<td>Mg(^{2+})</td>
<td>0.20</td>
</tr>
<tr>
<td>Twigs</td>
<td></td>
</tr>
<tr>
<td>Na(^+)</td>
<td>0.02</td>
</tr>
<tr>
<td>K(^+)</td>
<td>0.34</td>
</tr>
<tr>
<td>Ca(^{2+})</td>
<td>0.08</td>
</tr>
<tr>
<td>Mg(^{2+})</td>
<td>0.13</td>
</tr>
<tr>
<td>Leaves</td>
<td></td>
</tr>
<tr>
<td>Na(^+)</td>
<td>0.01</td>
</tr>
<tr>
<td>K(^+)</td>
<td>0.28</td>
</tr>
<tr>
<td>Ca(^{2+})</td>
<td>0.12</td>
</tr>
<tr>
<td>Mg(^{2+})</td>
<td>0.22</td>
</tr>
</tbody>
</table>

Values are means of five replicates. The Fisher least significant difference (LSD) is given when the effects of salinity treatments were considered statistically significant (one-way analysis of variance, \(P < 0.05\)). ns indicates that the effects of treatments were not significant.
Alternatively, salt-induced reduction in cell wall extensibility may account for the inhibition of root growth. An increase of the yield threshold pressure and a decrease in cell wall extensibility as a consequence of cell wall hardening has been observed in salt-treated maize root tip [18]. In our study, reduced root growth was more likely a consequence of ion toxicity or ion imbalance on wall metabolism or cell plasmalemma rather than a direct effect of a salt-induced osmotic stress.

Growth reduction may also result from a decrease in carbon uptake (decrease in both leaf photosynthesis and leaf area), a change in carbon allocation from growth processes (synthesis of wall and cellular components) to maintenance processes (turnover, repair and ion transport) or to osmotic adjustment by non-structural carbohydrates, and an increase in respiratory cost for growth. It has often been postulated that an increase in active ion transport and repair of salt damages compete with growth for available carbohydrates [8, 23] while others have calculated that the extra cost would not be quantitatively important [2].

In this study, the occurrence of a high rate of root respiration under moderate salinity while growth rate was reduced, as well as the stronger reduction in root growth than in root respiration at high salinity, suggested that respiratory cost for growth and/or maintenance processes are increased. This is in agreement with previous results showing that respiration remained high under saline conditions, the reduction of growth respiration being balanced by an increase in maintenance respiration [21]. An increase in the maintenance component of whole-plant respiration has been reported for both salt-tolerant or intolerant species such as Phaseolus vulgaris, Atriplex halimus and Xanthium strumarium [21] while maintenance respiration remain unaffected in Zea mays [21] or Plantago coronatus [2]. Whether an increase in the respiratory cost for growth or maintenance processes compete with growth for available carbon, and therefore contribute to growth cessation, is not in the scope of the present work.

Using the specific lengths of tap and lateral roots, the root dry weight at the final harvest, the specific root respiration rates and the root elongation rates measured at the end of the experiment, assuming a salt-insensitive growth coefficient for root respiration of 0.45 and that 1 mol of CO₂ is equivalent to 25 g of dry matter, the growth and maintenance respiration can be estimated [21]. With these assumptions, growth and maintenance respiration were, respectively, 6.5 and 8.5 μmol kg⁻¹ s⁻¹ in roots of control seedlings. Growth respiration was decreased by 45 % while maintenance respiration was increased by 20 % under moderate salinity (50 mM). At moderate salinity, Na⁺ content strongly increased in the root while it accumulated to a lesser extent in the shoot and leaf, indicating that Na⁺ is excluded from the shoot. Prevention of Na⁺ translocation in moderately stressed oak is probably achieved by sequestering it in the root vacuole [1, 19]. This would require extra energy, which may be supplied by an increase in maintenance respiration. At higher salinity (250 mM), root respiration was strongly inhibited presumably by Na⁺ or Cl⁻ toxicity on enzymatic activities. It is consistent with the inability of severely stressed oak seedling to prevent Na⁺ translocation to the shoot.

In our calculation, we assumed that the growth coefficient for root respiration was salt-insensitive. Schwarz and Gales [21] reported that mild salinity did not alter the slope of the respiration versus photosynthesis plots and therefore concluded that the yield of constructive growth was unaffected by salt. However, we used higher salt concentrations in this study. Therefore an increasing cost for growth processes cannot be excluded and may also account for a stronger reduction in root growth than in root respiration. Since reduced root growth may imply some kind of cell wall hardening (see earlier), a change in the respiratory cost of cell wall metabolism is not unlikely.

We conclude that oaks, which are known to be drought tolerant [9, 10], appeared to be rather salt sensitive. In particular, root elongation of pedunculate oak seedlings is inhibited even at moderate (50 mM) salinity level, probably because of the toxic effects of ion or ion imbalance on wall metabolism or cell plasmalemma. An increase in the respiratory cost for maintenance, for active ion transport and/or for growth processes is consistent with the occurrence of a high rate of root respiration while growth rate was reduced.

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