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ScienceDirect
Environmental and Experimental Botany 62 (2008) 357–363
Environmental and Experimental Botany
www.elsevier.com/locate/envexpbot

Responses of ectomycorrhizal *Populus tremuloides* and *Betula papyrifera* seedlings to salinity

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Received 20 June 2006; received in revised form 23 May 2007; accepted 6 October 2007

Abstract

Roots of trembling aspen (*Populus tremuloides* Michx.) and paper birch (*Betula papyrifera* Marsh.) seedlings were inoculated with *Hebeloma crustuliniforme* or *Laccaria bicolor* and treated with 25 mM NaCl for 6 weeks. Both tree species appeared to be relatively tolerant of the applied NaCl treatment and did not develop visible leaf symptoms that are characteristic of salt injury. Salt treatment reduced total dry weights in aspen and birch, but did not significantly affect transpiration rates and root hydraulic conductance. Salt-treated ectomycorrhizal aspen maintained higher root hydraulic conductance compared with non-mycorrhizal plants. Na and Cl concentrations increased in shoots and roots of mycorrhizal and non-mycorrhizal aspen and birch in response to NaCl treatment. Roots of NaCl-treated aspen inoculated with *H. crustuliniforme* had over twofold higher concentrations of Na compared with non-mycorrhizal NaCl-treated plants. Similarly to aspen, Na and Cl concentrations increased in roots and shoots of NaCl-treated birch seedlings. However, in birch, there were no significant differences in Na and Cl concentrations between mycorrhizal and non-mycorrhizal plants. The results suggest that salt exclusion by the ectomycorrhizal associations is host-specific or/and that the processes leading to salt exclusion are activated in ectomycorrhizal plants by a threshold salt level which may vary between plant species.

Keywords: Ectomycorrhizae; Root hydraulic conductance; Salt tolerance; Salt exclusion; Transpiration

1. Introduction

Since salinity problems are commonly associated with agricultural areas, salt resistance of tree species has received relatively little attention. However, salinity can also be of major concern in natural boreal ecosystems (Purdy et al., 2005) as well as in urban areas (Lait et al., 2001) and industrial reclamation sites (Renault et al., 1999). Salt adversely affects plants by inducing osmotic imbalance, ionic toxicity, nutrient deficiencies, or a combination of the above factors (Shannon, 1997). It also upsets water balance by interfering with the activity of water channel proteins (aquaporins) which results in an increase in water flow resistance of the root system (Carvajal et al., 2000; Martinez-Ballesta et al., 2000; Apostol et al., 2002; López-Berenguer et al., 2006).

Under natural conditions, the majority of trees form ectomycorrhizal associations which help with the supply of essential nutrients and water (Smith and Read, 1997). When grown in culture, some ectomycorrhizal fungi tolerate relatively high salt concentrations (Kernaghan et al., 2002; Bois et al., 2006). Although the exact mechanisms of salt resistance in cultured fungi are not known, salt exclusion and osmoregulation have been implicated (Bois et al., 2006). Salt resistance of ectomycorrhizal fungi may be an important trait to their host plants. In our earlier studies (Muhsin and Zwiazek, 2002a; Nguyen et al., 2006), *Hebeloma crustuliniforme* and *Laccaria bicolor* reduced tissue Na and Cl concentrations and alleviated salt injury in white spruce (*Picea glauca*), black spruce (*Picea mariana*) and jack pine (*Pinus banksiana*) seedlings. The reductions in rates of salt uptake by the ectomycorrhizal plants were likely independent of the rates of water uptake since the ectomycorrhizal plants had higher transpiration rates and root hydraulic conductance than non-mycorrhizal plants (Muhsin and Zwiazek, 2002a; Nguyen et al., 2006).

As opposed to evergreen conifers which retain needles for many years, deciduous trees may remove some salt from their tissues when the leaves are shed in autumn. Therefore, the mechanisms of salt resistance in evergreen conifers and deciduous

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doi:10.1016/j.envexpbot.2007.10.008

trees may be different. However, since salt exclusion is likely one of the main processes contributing to salt resistance of cultured fungal hyphae (Bois et al., 2006), we expected that the reduction of salt uptake would be the main mechanism through which ectomycorrhizal fungi increase salt resistance in both evergreen and deciduous tree species. In the present study, we examined the effects of ectomycorrhizal *H. crustuliniforme* [(Bull. Ex St. Amans) Quel.] and *L. bicolor* [(Maire) Orton] on salt resistance of two deciduous boreal tree species, *Populus tremuloides* (Michx.) and *Betula papyrifera* (Marsh.). We studied the hypothesis that, similarly to the studied species of conifers (Muhsin and Zwiazek, 2002a; Nguyen et al., 2006), both ectomycorrhizal fungi would increase salt resistance in aspen and birch by reducing Na and Cl uptake and alleviating the effects of salt on water transport. Both *L. bicolor* and *H. crustuliniforme* are common ectomycorrhizal fungi that usually appear in the early stages of fungal succession in young forests (Smith and Read, 1997). These fungi form associations with a broad range of tree species and show high salinity tolerance in culture (Kernaghan et al., 2002).

2. Materials and methods

2.1. Plant material and fungal inoculation

Seeds of trembling aspen (*P. tremuloides* Michx.) and paper birch (*B. papyrifera* Marsh.) were collected at the University of Alberta campus (Edmonton, AB, Canada). The seeds were surface-sterilized and germinated on moist filter paper in Petri dishes. After 1 week following root emergence, the germinants were transplanted to 100 cm^3 pots containing a sterilized mixture of peat moss and sand (3:1, v/v). The seedlings were kept for 2 weeks in a growth room at 20/15 °C (day/night) temperatures and 16-h photoperiod with photosynthetically active radiation of 350 $\mu mol\ m^{-2}\ s^{-1}$. The pots were watered every 2 days and fertilized weekly to soil saturation with modified Hoagland's nutrient solution (Epstein, 1972).

The aspen and birch seedlings were inoculated with fungal cultures 3 weeks after transplanting. In preparation for fungal inoculation, the seedlings of each species were divided into three groups, each with 50 plants. One group served as non-mycorrhizal control, the second group was inoculated with *H. crustuliniforme* [(Bull. Ex St. Amans) Quel., UAMH isolate no. 5247], and the third one with *L. bicolor* [(Maire) Orton] (UAMH isolate no. 8232)]. The cultures were obtained from the University of Alberta Microfungus Collection and Herbarium and grown in modified Melin–Norkan's nutrient solution (Mason, 1980) for 2 weeks before the inoculation. The inoculum consisted of 30 ml of homogenized liquid fungal culture which was injected into the soil of experimental seedlings with a sterile syringe. The seedlings were grown for four more weeks in the growth room before the commencement of NaCl treatment and their roots were inspected weekly for mycorrhizal colonization. Just before the NaCl treatment, the seedlings were randomly selected and their roots microscopically examined for the presence of fungal mycelia and morphological changes. For both tree and fungal species, the mycelia were present in at least 95% of the examined seedlings and the inoculated seedlings had numerous enlarged and bifurcate short roots.

2.2. NaCl treatment

After 4 weeks of inoculation, all inoculated and non-inoculated aspen and birch seedlings were divided into two groups. The first group was treated with 25 mM NaCl solution which was added to the soil to saturation every second day for 4 weeks. The other half received distilled water and served as treatment control. Salt accumulation in the soil was minimized by flushing the soil with de-ionized water before every third NaCl application. The salinity level generated by this treatment produced mild to moderate injury in the studied conifer seedlings (Muhsin and Zwiazek, 2002a; Nguyen et al., 2006).

2.3. Measurements of dry weights, transpiration rates and root hydraulic conductance

For all dry weight measurements, eight seedlings per treatment per species combination were taken ($n = 8$). Shoot and root dry weights were determined after drying in an oven at 70 °C for 48 h.

Transpiration rates (E) were measured 4–8 h following the onset of photoperiod with a LI-1600 steady-state porometer (LI-COR Inc., Lincoln, NE, USA) on the uppermost fully developed leaves (Renault et al., 2001). The measurements were conducted in the growth room where the plants were grown and under the same temperature and light conditions as listed above ($n = 6$). Leaf areas were measured using a LI-3000 leaf area meter (LI-COR).

Root hydraulic conductance (K_r) was measured with a high pressure flow meter (HPFM, Dynamax Inc., Houston, TX) as previously described (Muhsin and Zwiazek, 2002b). For K_r measurements, the shoot of each potted seedling was excised about 1.5 cm above the root collar and the root connected to the HPFM. The root system was gradually pressurized up to 0.5 MPa to obtain a pressure-flow relationship (Tyree et al., 1995).

2.4. Tissue salt and nutrient analysis

Tissue concentrations of Na and Cl were determined in roots and shoots (six seedlings per treatment) and K, PO_4, Ca and Mg in shoots of non-mycorrhizal seedlings and seedlings inoculated with *H. crustuliniforme*. For the analysis, roots and shoots were briefly washed in deionized water and frozen at −85 °C. The tissue samples were freeze-dried, pulverized and extracted with hot water for Cl and PO_4 analysis (Apostol et al., 2002) or concentrated HNO_3 for Na, K, Ca and Mg (Renault et al., 1999). The extracts were analyzed using a DI 300 ion chromatograph (Dionex; Sunnyvale, CA, USA) and an inductively coupled plasma optical emission spectrometer (Vista-PRO RL; Varian Analytical Instruments, Victoria, Australia).