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ORIGINAL PAPER

Paxillus involutus mycorrhiza attenuate NaCl-stress responses in the salt-sensitive hybrid poplar *Populus*×*canescens*

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Abstract In order to characterise the effect of ectomycorrhiza on Na^+-responses of the salt-sensitive poplar hybrid *Populus*×*canescens*, growth and stress responses of *Paxillus involutus* (strain MAJ) were tested in liquid cultures in the presence of 20 to 500 mM NaCl, and the effects of mycorrhization on mineral nutrient accumulation and oxidative stress were characterised in mycorrhizal and non-mycorrhizal poplar seedlings exposed to 150 mM NaCl. *Paxillus involutus* was salt tolerant, showing biomass increases in media containing up to 500 mM NaCl after 4 weeks growth. Mycorrhizal mantle formation on poplar roots was not affected by 150 mM NaCl. Whole plant performance was positively affected by the fungus because total biomass was greater and leaves accumulated less Na^+ than non-mycorrhizal plants. Energy dispersive X-ray microanalysis using transmission electron microscopy analysis of the influence of mycorrhization on the subcellular localisation of Na^+ and Cl^- in roots showed that the hyphal mantle did not diminish salt accumulation in root cell walls, indicating that mycorrhization did not provide a physical barrier against excess salinity. In the absence of salt stress, mycorrhizal poplar roots contained higher Na^+ and Cl^- concentrations than non-mycorrhizal poplar roots. *Paxillus involutus* hyphae produced H_2O_2 in the mantle but not in the Hartig net or in pure culture. Salt exposure resulted in H_2O_2 formation in cortical cells of both non-mycorrhizal and mycorrhizal poplar and stimulated peroxidase but not superoxide dismutase activities. This shows that mature ectomycorrhiza was unable to suppress salt-induced oxidative stress. Element analyses suggest that improved performance of mycorrhizal poplar under salt stress may result from diminished xylem loading of Na^+ and increased supply with K^+.

Keywords Ectomycorrhiza · Oxidative stress · Salinity · Poplar hybrid · Subcellular element localisation

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Introduction

Inappropriate irrigation practices and high evaporation in semi-arid regions result in salt accumulation in the soil destroying the land for plant production. Estimates suggest that, worldwide, about 25% of the irrigated land is damaged by salt and that these areas are increasing (http://www.fao.org/docrep/T0667E/t0667e0a.htm). In the humid climates of middle and northern Europe, salinisation of agricultural land is not a problem but application of de-icing salt in winter alongside roads results in the accumulation of sodium. Since excess salinity injures plants, control and removal of NaCl-damaged trees and re-planting causes enormous economic losses in the public sector. Elevated salinity is also a problem in land reclamation following oil mining in boreal forests (Renault et al. 1999).

At the cellular level, high salinity disrupts plant ion homeostasis leading to secondary effects such as oxidative stress, growth arrest, and even death (Zhu 2001; Tester and Davenport 2003; Munns 2005). To date, most research into the mechanisms of salt stress has focused on herbaceous model plants such as *Arabidopsis*, halophytes from salt marshes, or important crop species such as tomato, pea, maize, etc. (Al-Karki 2000; Feng et al. 2002; Rabie et al. 2005). Much less attention has been devoted to the analysis of salt-responses in tree species.

We have recently shown that the hybrid of *Populus tremula*×*Populus alba* (=*P.*×*canescens*) is very salt-sensi-

tive (Bolu and Polle 2004). Exposure to 100 mM NaCl in hydroponic nutrient solutions resulted in the loss of root and shoot elongation growth, caused severe leaf loss, and increased electrolyte leakage and lipid peroxidation (Bolu and Polle 2004). Salt susceptibility varies, however, between different poplar species (Sixto et al. 2005). In natural environments, poplar roots are normally colonised by mycorrhizal fungi (Cripps and Miller 1993; Baum and Makeschin 2000). In addition to improving nutrient supply, a main function of these symbiotic interactions is protection from soil-borne stress (Smith and Read 1997; Hall 2002; Polle and Schützendübel 2003). While there are several studies on the role of arbuscular mycorrhiza in modulating salt responses in herbaceous plants (for a review, see Sridhar and Beena 2001), only a few studies have investigated the function of ectomycorrhiza in salt protection of trees. Basidiomycetes, to which many ectomycorrhizal fungi colonising trees in temperate climates belong, are generally considered to be salt-sensitive (Tresner and Hayes 1971). However, *Laccaria* and *Hebeloma* species, as well as several *Pisolithus* strains, were recently identified as relatively salt-tolerant (Chen et al. 2001; Kernaghan et al. 2002). Muhsin and Zwiazek (2002) showed that ectomycorrhiza significantly inhibited sodium uptake in salt-exposed *Hebeloma crustuliniforme–Picea glauca* and improved nutrition and transpiration compared with non-inoculated seedlings.

In contrast to conifers, poplar is a fast-growing species typically planted as wind-break alongside roads, where it is exposed to de-icing salt. We have recently identified *Paxillus involutus* strains forming mycorrhiza with a poplar hybrid (*P.*×*canescens*) under axenic conditions (Gafur et al. 2004). In order to find out whether this fungus can grow under saline conditions and form mycorrhiza that can increase the tolerance of hybrid poplar against excessive salinity, we characterised *P. involutus* (strain MAJ) growth in the presence of up to 500 mM NaCl and studied the effect of mycorrhization on mineral nutrition in mycorrhizal and non-mycorrhizal poplar seedlings exposed to NaCl. Because salt is thought to act via oxidative stress and because antioxidant systems may be involved in mediating salt tolerance (Zhu 2001), the influence of salt on the metabolism of reactive oxygen species was determined in pure fungal cultures and in mycorrhizal and non-mycorrhizal plants.

Materials and methods

Cultivation of plants and fungi, mycorrhiza synthesis, and salt exposure

Plantlets of the hybrid *P. tremula*×*P. alba* (INRA clon 717-1B4) were multiplied by micropropagation (Leplé et al. 1992). To acclimatise the plants to ambient conditions, rooted plantlets were cultivated in hydroponic LN-nutrient solutions with low nitrogen supply [modified after Matzner et al. (1982): 300 μM NH_4NO_3, 30 μM KH_2PO_4, 200 μM K_2SO_4, 100 μM Na_2SO4, 60 μM $MgSO_4$, 130 μM $CaSO_4$, 10 μM $MnSO_4$, 7.8 μM Fe-ethylene diamine-di(o-hydroxyphenylacetate), 5 μM H_3BO_3, 0.1 μM $NaMoO_4$, 0.1 μM $ZnSO_4$, 0.1 μM $CuSO_4$, pH 3.9] for 14 days, initially in a climatised growth room (21°C, 50–60% relative air humidity, light: 150 μmol photons m^{-2} s^{-1} of photosynthetic active radiation, 16 h), then 2 days before transfer to pots, in greenhouse conditions (20 to 24°C, 40 to 70% relative air humidity, natural daylight supplemented to 16 h light by lamps yielding up to 300 μmol photons m^{-2} s^{-1} of photosynthetic active radiation).

Paxillus involutus (Bartsch.) (strain MAJ in the Göttingen stock collection, initially collected in France under a poplar tree) was grown on 2% modified Melin-Norkrans agar medium [0.5 g KH_2PO_4, 0.25 g $(NH_4)_2SO_4$, 0.15 g $MgSO_4 \times 7H_2O$, 0.05 $CaCl_2$, 0.025 g NaCl, 100 μg thiamine×HCl, 0.01 g $FeCl_3$, 10 g glucose, 5 g malt extract per 1 l]. For liquid culture, agar was omitted and the medium was buffered with citrate (Ott et al. 2002). Mycelium from agar plates was homogenised (ca. 1/4 plate corresponding to a hyphal area of ca. 16 cm^2, Ultraturrax, three times, 3 s at 8,000 rpm), transferred into 150 ml of liquid culture medium in Erlenmeyer flasks and maintained in an acclimatised room (22°C, 67% relative air humidity). After two to three passages in liquid culture, 10 ml of homogenised fungi corresponding to about 0.13 g fungal mass were transferred to fresh liquid culture medium and incubated for 10 days on a rotary shaker (100 rpm). Sterile-filtered NaCl solutions were added to give final concentrations of 100, 200, and 500 mM NaCl, respectively. Water was added to controls. The fungi were cultured for a further 14 days and harvested at regular intervals.

Liquid culture (after three passages) was used for mycorrhization. The upper clear supernatant of the culture medium was discarded, mycelium of *P. involutus* of ca. 1 g in 50 ml was homogenised and used to inoculate rooting medium. The rooting medium consisted of two parts peat, eight parts fine sand (grain size: 0.3–0.3 mm), and 10 part coarse sand (grain size 1.2–2.0 mm). Prior to use, the sand was washed with tap water and demineralised water, air dried, and mixed with the peat. The mixture was sterilised in an autoclave (120°C, 20 min, Zirbus HST666, Harz, Germany). Six hundred sixty milliliters of this mixture was inoculated or not with 50 ml of the *Paxillus* homogenate and mixed additionally with small pieces of mycelium from Agar plates (half a plate of 90 mm diameter, 4 weeks old). Rooting mixtures were incubated for 4 days under foil before use. Poplar plantlets were planted in inoculated or non-inoculated rooting mixture in growth tubes which contained a nylon mesh at the bottom (diameter 5 cm,

height 41 cm). The potted plants (n=60) were randomised twice a week. The plants were automatically irrigated twice a day for 1 min with LN-nutrient solution (Matzner et al. 1982). After 4 weeks prior to the salt treatment, two plants were sampled to evaluate the degree of mycorrhization.

Half of the mycorrhizal and non-mycorrhizal plants were exposed to 150 mM NaCl added to LN-nutrient solution. Height, stem diameter 2 cm above the root neck and leaf formation were measured twice a week. After 18 days of salt treatment, the plants were harvested, separated into leaves, stem and coarse and fine roots.

Degree of mycorrhization

Root systems of NaCl-stressed and non-stressed *P. involutus*-inoculated plants were spread in Petri dishes with LN-nutrient solution, and a total of about 200 lateral roots per plant were counted under a binocular for mycorrhizal root tips (12 plants per treatment). No mycorrhiza was observed in root systems of non-inoculated plants.

Biomass and element composition

Plant tissues were dried at 60°C for dry mass determination and subsequently milled to a fine powder. After HNO_3 extraction, element composition was determined by atomic absorption spectroscopy–inductively coupled plasma analysis (Heinrichs et al. 1986). Carbon and nitrogen were determined using a C/N analyser (Elemental Analyzer EA1108, Carlo Erba Strumentazione, Rodano, Milan, Italy).

Biochemical measurements

Plant or fungal tissues were homogenised in liquid nitrogen in a mortar. Frozen tissue powder (100 mg) was suspended in 1.5 ml extraction buffer (100 mM KH_2PO_4/K_2HPO_4, pH 7.8; 100 mg polyvinylpyrrolidone) and centrifuged (desk top centrifuge, 20,000×g, 4°C). The supernatant was passed through Sephadex G-25 (NAP-5 columns, Pharmacia, Breitscheid, Germany) and used for analysis of superoxide dismutase (SOD) and guaiacol peroxidase (POD) as described previously (McCord and Fridovich 1969; Polle et al. 1990).

Histochemical detection of H_2O_2

For detection of hydrogen peroxide, fine roots from each experimental treatment were stained with 3,3′-diaminobenzidine (DAB) (Thordal-Christensen et al. 1997). The rootlets were vacuum-infiltrated for 30 min in a 0.4% DAB solution (0.1 g DAB solved in 4 ml 1 M HCl, then further diluted with 24 ml H_2O to a final volume of 25 ml). DAB-infiltrated roots were further incubated in DAB solution in the dark for 4.5 h at room temperature. Roots were then transferred to 70% ethanol to stop the DAB reaction and to wash out non-polymerised DAB. Cross sections (30 μm) from DAB-stained roots were cut about 3–4 mm from the root tip using a freezing microtome (Jung, Heidelberg, Germany). Cross sections were mounted in 50% glycerol, observed under a light microscope (Zeiss Axioplan, Zeiss, Oberkochen, Germany) to detect brown precipitation of polymerised DAB and photographed with a digital camera (Nikon Coolpix 4500, Tokyo, Japan). To control staining specificity, some roots were infiltrated with catalase before DAB staining. Under these conditions, typical brown precipitates for H_2O_2 formation were not observed.

Element analysis by energy dispersive X-ray microanalysis using transmission electron microscopy

To analyse element distribution at tissue and subcellular levels, root tips and ectomycorrhiza were harvested as described previously (Ottow et al. 2005), freeze-dried under vacuum, and infiltrated by a non-aqueous method developed to avoid redistribution of elements during sample preparation (Fritz 1989). Cross sections of 1 μm were mounted for transmission electron microscopic analysis (EM 420, Philips, Eindhoven, The Netherlands) and used

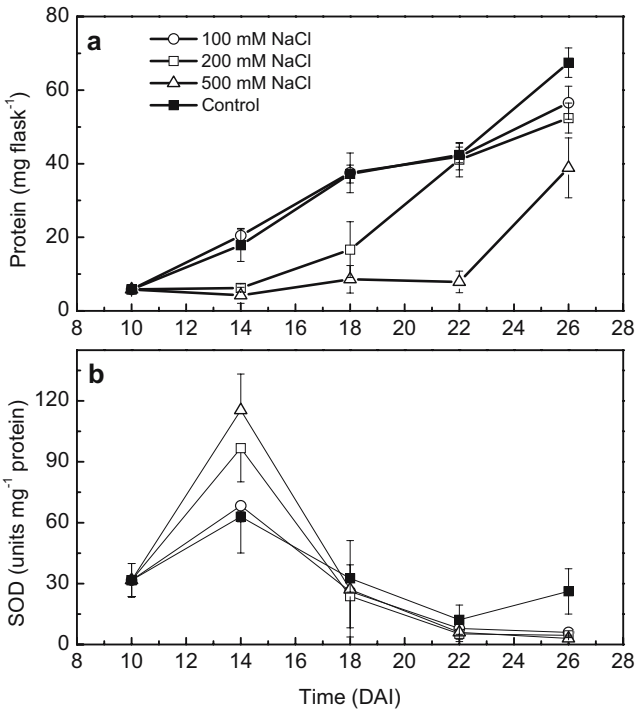

Fig. 1 Growth of *Paxillus involutus* in liquid culture in the presence of increasing concentrations of NaCl. At the indicated days after inoculation (*DAI*), four flasks were harvested per treatment. The experiment was repeated three times. Data are means (±SE) of a typical experiment

Table 1 Perfomance of *Populus*×*canescens* after 4 weeks of growth in rooting medium inoculated and non-inoculated with *Paxillus involutus*

	Inoculated poplar	Non-inoculated poplar	*P*
Number of leaves	19.6±3.6	18.8±3.3	0.434
Stem height (cm)	15.5±2.7	19.1±3.0	0.605
Stem diameter (mm)	2.50±0.28	2.42±0.26	0.517

Figures are means (n=24±SD). *P* indicates calculated *P* value

to measure element distribution by EDX (EDAX-DX4, International, Mahwah, NJ, USA) according to the method of Fritz and Jentschke (1994). The width of the electron beam was set to 200 nm for high resolution. For area measurements, the beam diameter was expanded to 40 μm.

Statistical analysis

If not reported otherwise, 5–10 individuals were analysed. Data are indicated as means±SD. Statistical analyses were performed with STATGRAPHICS Plus using analysis of variance (Statistical Graphics, St. Louis, MO, USA), followed by a multiple range test.

Results

Influence of salt on growth and antioxidant enzyme activity of *P. involutus*

Growth of *P. involutus*, estimated by protein amounts, was not affected by 100 mM NaCl (Fig. 1a), a concentration

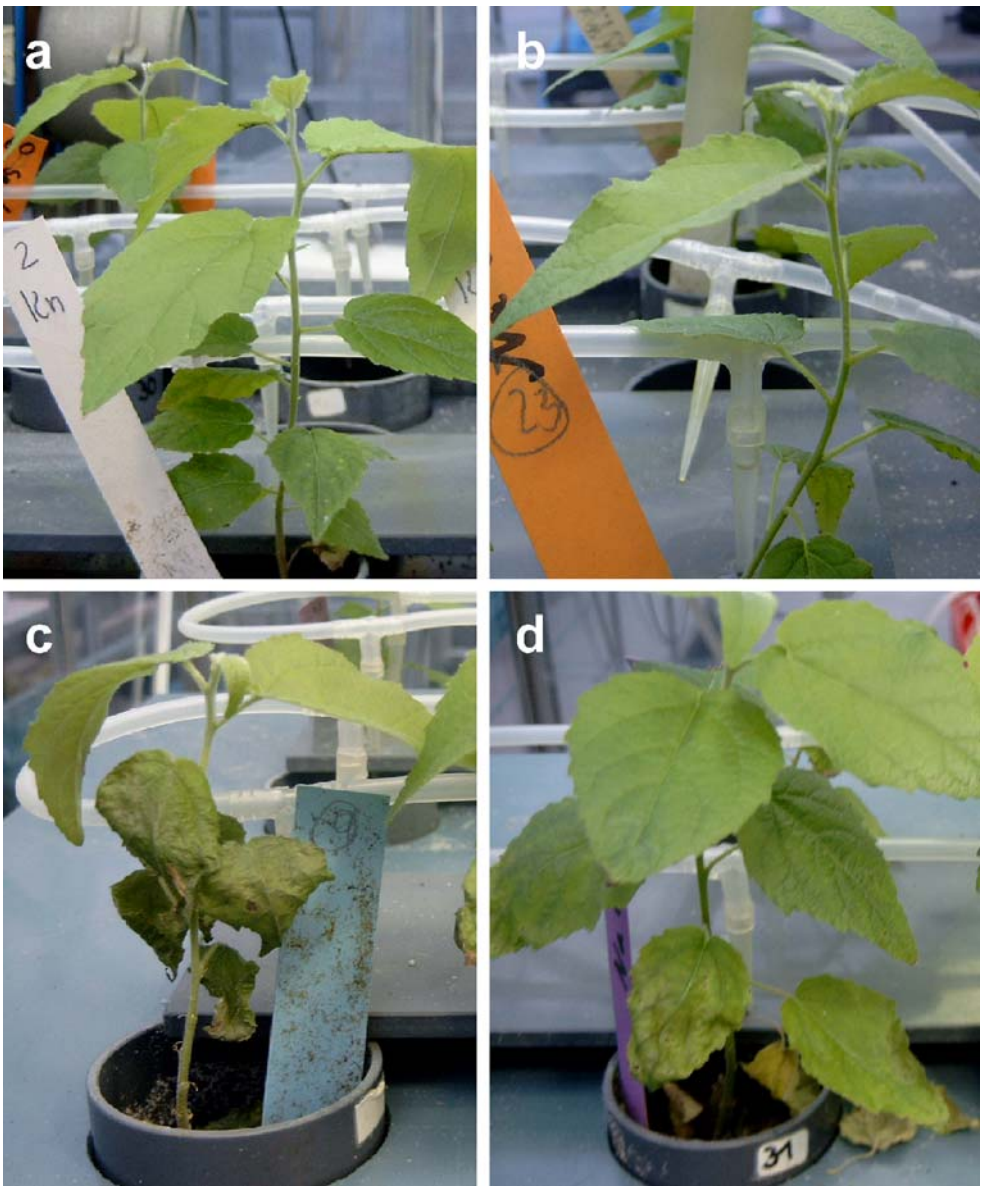

Fig. 2 Performance of *Populus*×*canescens* under salt stress. Non-mycorrhizal control plant (**a**), mycorrhizal plant (**b**), non-mycorrhizal (**c**) and mycorrhizal plant (**d**) after salt exposure (150 mM NaCl for 18 days)

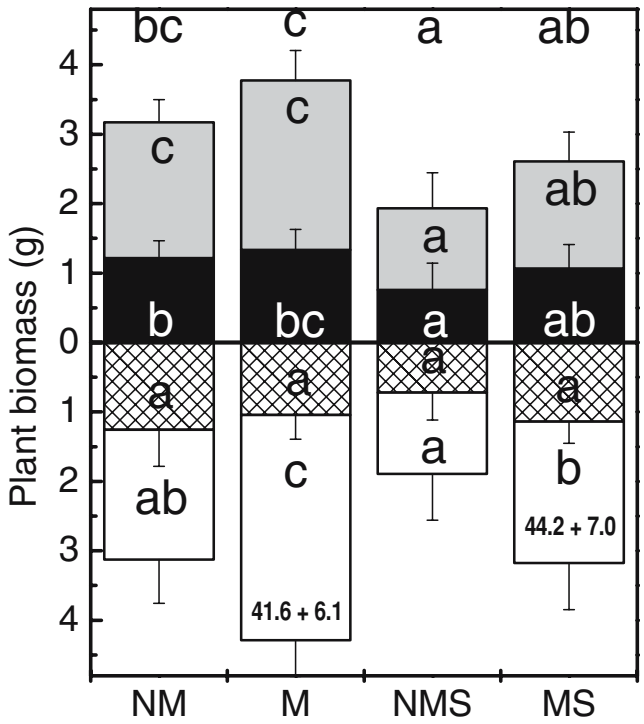

Fig. 3 Biomass of mycorrhizal (*M*) and non-mycorrhizal *P.*×*canescens* (*NM*) plants in the absence or presence of salt stress (*S*). Biomass of leaves (*grey*), stem (*black*), coarse roots (*hatched*) and fine roots (*white*). *Numbers inside bars* indicate degree of mycorrhization. *Different letters inside the bars* indicate significant differences at $P \leq 0.05$ for leaves, stem and coarse and fine roots, respectively. *Different letters outside bars* refer to whole plant biomass. Data indicate means of five plants per treatment (±SE)

previously shown to cause severe injury to *P.*×*canescens* (Bolu and Polle 2004). Higher salt concentrations delayed the growth of *P. involutus*. The negative effect of 200 mM NaCl was, however, moderate, and after acclimation for about 3 weeks, growth was observed even at 500 mM NaCl (Fig. 1a).

Exposure to NaCl caused transient increases in the activities of fungal SOD corresponding approximately to the concentration of salt (Fig. 1b). Controls also showed a moderate increase, which was probably caused by opening of the flasks and addition of water instead of salt. Neither H_2O_2 formation nor guaiacol POD activities were detected in fungal cultures in the absence or presence of additional NaCl (not shown).

Influence of salt on growth, mycorrhization, and element accumulation in *P.* × *canescens*

Poplars were grown in inoculated and non-inoculated sand mixtures and supplied with nutrient solution. After 4 weeks of growth in the peat–sand mixture, roots of *P. involutus*-inoculated poplar showed 39% mycorrhization, and exposure to excess salt (150 mM NaCl) was started. At this time point, inoculated poplar had leaf numbers, heights and stem diameters similar to those of non-inoculated plants (Table 1). During the time course of NaCl-exposure, salt-exposed plants displayed a loss of leaves starting at the bottom and moving towards the top. Leaf loss was, however, less pronounced in mycorrhizal compared with non-mycorrhizal plants (Fig. 2).

Mycorrhization resulted in higher whole-plant biomass production under both salt-stressed and non-stressed conditions compared to corresponding controls (Fig. 3). Fine lateral root formation was especially stimulated by mycorrhization (Fig. 3). Exposure to salt caused severe decreases in biomass but the trend of increased biomass and significantly higher fine root formation was maintained in

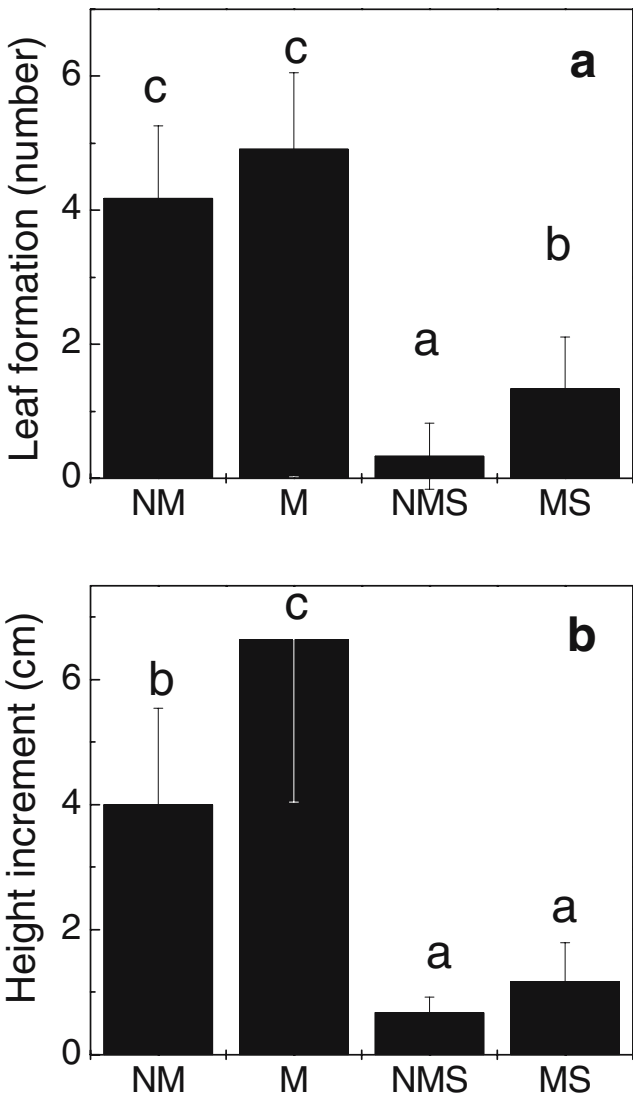

Fig. 4 Leaf formation (**a**) and stem elongation (**b**) of mycorrhizal (*M*) and non-mycorrhizal *P.*×*canescens* (*NM*) plants in the absence or presence of salt stress (*S*). Data indicate mean number of leaves and mean increment of stem height (cm) formed during the time of salt exposure ($n=5$ plants per treatment, ±SE). *Different letters* indicate significant differences at $P \leq 0.05$

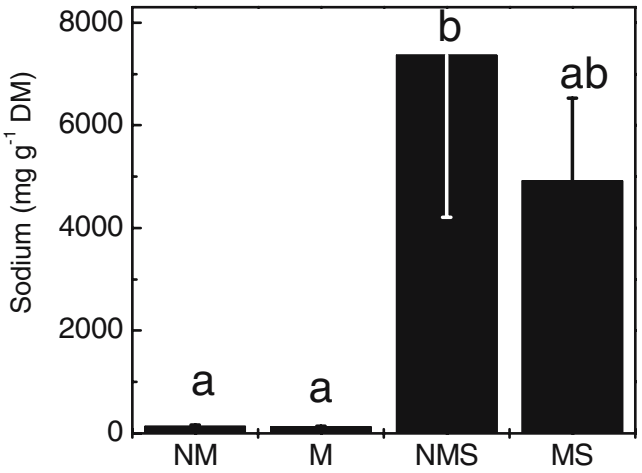

Fig. 5 Sodium concentrations in leaves of mycorrhizal (*M*) and non-mycorrhizal *P.×canescens* (*NM*) plants in the absence or presence of salt stress (*S*). Data indicate means of five plants per treatment (±SE). *Different letters* indicate significant differences at $P \leq 0.05$

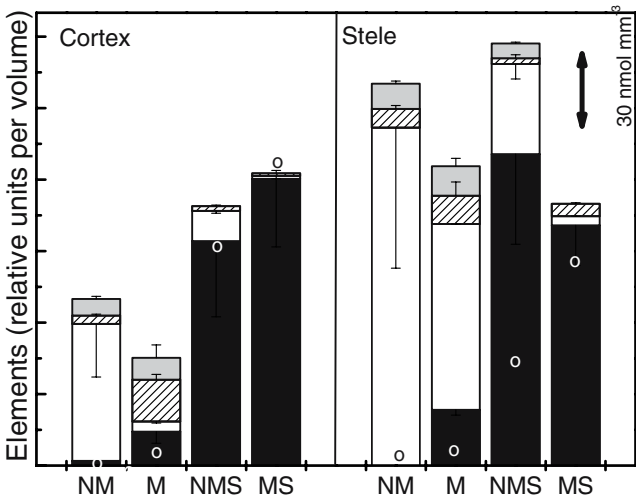

Fig. 6 Cation and chloride concentrations in roots of mycorrhizal (*M*) and non-mycorrhizal *P.×canescens* (*NM*) plants in the absence or presence of salt stress (*S*). Energy dispersive X-ray microanalysis using transmission electron microscopy measurements were conducted on cross sections of roots in the cortex and the stele in areas of 1,256 μm^2. Five to 20 spectra were averaged in each plant. *Stacked bars* indicate Na (*black*), K (*white*), Ca (*hatched*), and Mg (*grey*). Chloride concentrations are indicated by *circles*

mycorrhizal plants (Fig. 3). The degree of mycorrhization was about 40% and was not influenced by salt stress (Fig. 3).

To characterise the influence of mycorrhization and salt stress on plant performance, the number of new leaves formed during the period of salt stress was measured (Fig. 4a). Leaf formation of non-stressed mycorrhizal plants was similar to that of non-mycorrhizal plants but shoot elongation was increased (Fig. 4a,b). Salt exposure caused severe slowing down of shoot elongation in both mycorrhizal and non-mycorrhizal poplar (Fig. 4b), but mycorrhizal plants still displayed a small but significantly higher rate of leaf formation than non-mycorrhizal plants (Fig. 4a).

Analysis of sodium concentrations indicated that extremely high concentrations of Na^+ were accumulated in leaves of salt-stressed plants (Fig. 5). In mycorrhizal plants, the accumulation was about 30% lower, reaching intermediate levels between those of controls and non-mycorrhizal salt-stressed plants and suggesting a trend of restricted Na uptake under these conditions (Fig. 5).

The influence of mycorrhization and salt stress on nutrient element concentration in leaves was variable (Table 2). In the absence of salt-stress, mycorrhizal plants had higher phosphorus, potassium and sulphur concentrations than non-mycorrhizal plants, whereas foliar N concentrations were not significantly affected (Table 2). Exposure to salinity resulted in further increases in potassium but in decreases in sulphur and had no effect on phosphorus concentration in leaves of mycorrhizal plants. Mycorrhization resulted in decreased tissue concentrations of Ca, Mg, Mn and Fe in non-stressed plants. Exposure to salt caused decreases in Ca and Mg in non-mycorrhizal plants.

Subcellular distribution of cations and chloride in poplar roots under the influence of mycorrhiza and salinity

To know whether the hyphal sheath of *P. involutus* mycorrhiza formed a physical barrier against excess NaCl, energy dispersive X-ray microanalysis using transmission electron microscopy area measurements were conducted in cross sections of cortex and stele of roots of mycorrhizal and non-mycorrhizal plants (Fig. 6). In the absence of salt stress, vascular tissues contained higher cation concentra-

Table 2 Concentrations of nutrient elements in leaves of *Populus×canescens* as affected by mycorrhization with *Paxillus involutus* and salt stress

Treatment	P	N	K	S	Ca	Mg	Mn	Fe
NM	1.74±0.18a	1.69±0.20a	12.67±1.19a	3.33±0.33ab	12.89±0.28b	4.94±0.26c	0.73±0.05c	1.00±0.06c
M	2.83±0.51b	1.98±0.37a	17.43±1.78b	4.16±0.54b	9.75±0.21a	3.33±0.06ab	0.37±0.02a	0.26±0.04a
NMS	1.31±0.07a	1.70±0.21a	16.27±1.68ab	2.61±0.17a	10.27±0.98a	3.92±0.27b	0.64±0.05bc	0.88±0.09bc
MS	3.58±0.30b	1.88±0.12a	23.36±0.81c	2.58±0.12a	8.88±0.74a	2.78±0.28a	0.51±0.08ab	0.59±0.14b

Data (g kg^{-1} dry mass) are means of four individual replicates (±SE). Different letters indicate significant differences with $P \leq 0.05$
NM non-mycorrhizal plants, *M* mycorrhizal plants, *NMS* non-mycorrhizal plants+salt stress, *MS* mycorrhizal plants+salt stress.

tions than the cortex, and mycorrhization decreased potassium and increased Na^+ and Cl^- concentrations (Fig. 6). Under salt stress, tissues of both non-mycorrhizal and mycorrhizal roots contained considerably higher concentrations of these ions. This clearly indicates that neither mycorrhization nor the endodermis constituted a significant barrier against sodium or chloride uptake. Salt stress also caused severe decreases in calcium, magnesium, and potassium in root tissues (Fig. 6).

More detailed analyses of sodium accumulation in cell walls of the outer epidermis, the cortex, xylem parenchyma cells and secondary walls of xylem vessels in the vascular system showed a steep gradient of sodium from outside the roots to the inner part of the vascular system in the absence of salt stress, for both mycorrhizal and non-mycorrhizal plants (Fig. 7a). In the presence of salt stress, sodium concentrations in all cells were increased, but the gradient from outside to inside was less pronounced, especially in non-mycorrhizal roots (Fig. 7a). Under these conditions, outer cell walls adjacent to mycorrhizal hyphae contained significantly higher sodium concentrations than those of non-mycorrhizal plants (Fig. 7a). The subcellular distribution of chloride was similar to that of sodium (Fig. 7b). Taken together, these data suggest that mycorrhizal roots have access to more NaCl than non-mycorrhizal roots and that the hyphal mantle does not provide a physical barrier against NaCl influx.

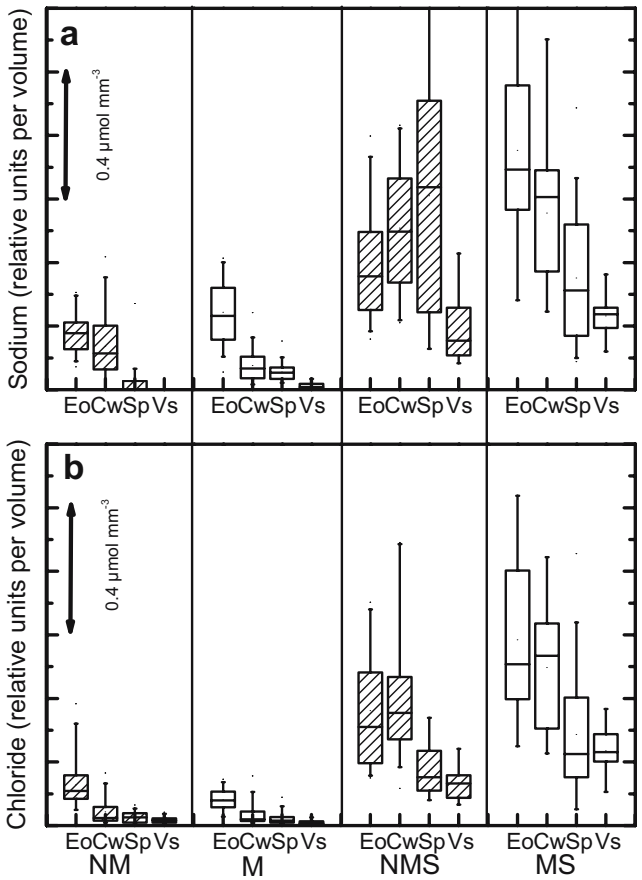

Fig. 7 Sodium (**a**) and chloride (**b**) concentrations in cell walls of different root cells of mycorrhizal (*M*) and non-mycorrhizal *P.*× *canescens* (*NM*) plants in the absence or presence of salt stress (*S*). Energy dispersive X-ray microanalysis using transmission electron microscopy measurements were conducted in cell walls of the outer epidermis (*Eo*), cortical cells (*Cw*), parenchyma cells in the stele (*Sp*) and secondary walls of xylem vessels (*Vs*). Because of scattering of the point measurements (n=20–30 measurements per plant and treatment), data are shown as box–whisker plots. *Boxes* indicate the 2nd and 3rd quartile and medium value of the data. The whisker lines indicate the range of data

Influence of salt stress on the formation of reactive oxygen species and antioxidative defences

Since both mycorrhiza (Gafur et al. 2004) and salinity (Bolu and Polle 2004) can cause oxidative stress, we investigated whether mycorrhization would increase antioxidative systems in roots, thereby constituting a preformed defence mechanism. Analysis of reactive oxygen production in roots revealed that exposure to excess sodium induced the formation of H_2O_2 in epidermal cells of non-mycorrhizal root tips (Fig. 8b), whereas in non-salt stressed plants, H_2O_2 formation was confined mainly to the outer walls (Fig. 8a). In accordance with previous data (Gafur et al. 2004), mycorrhizal roots displayed H_2O_2 formation in the hyphal sheath (Fig. 8c). Salt stress caused massive H_2O_2 production across the whole section of the tip of mycorrhizal roots (Fig. 8d).

SOD and POD activities were measured to know whether oxidative stress caused by salt and/or mycorrhiza activated antioxidative defences. Compared with roots of non-mycorrhizal plants, SOD and POD activities were decreased in mycorrhizal roots in the absence of salt stress (Fig. 9a,b). Exposure to salt stress caused increases in POD activities regardless of the presence or absence of mycorrhiza (Fig. 9b), while SOD activities remained unaffected in mycorrhizal roots and declined in non-mycorrhizal roots after salt exposure (Fig. 9a).

Discussion

In this study we provide evidence that *P. involutus* (strain MAJ) is highly salt tolerant. In the plant kingdom, concentrations of sodium as high as 500 mM are tolerated only by halophytes. Empirical data have shown that in salt marshes, members of typical non-mycorrhizal plant families such as *Armeria maritima* and *Salicornia europaea* can be colonised by arbuscular mycorrhizal fungi, suggesting a possible role of these fungi in host salt tolerance (Hildebrandt et al. 2001). Information has since accumulated showing that arbuscular mycorrhizal fungi can improve the performance of plants

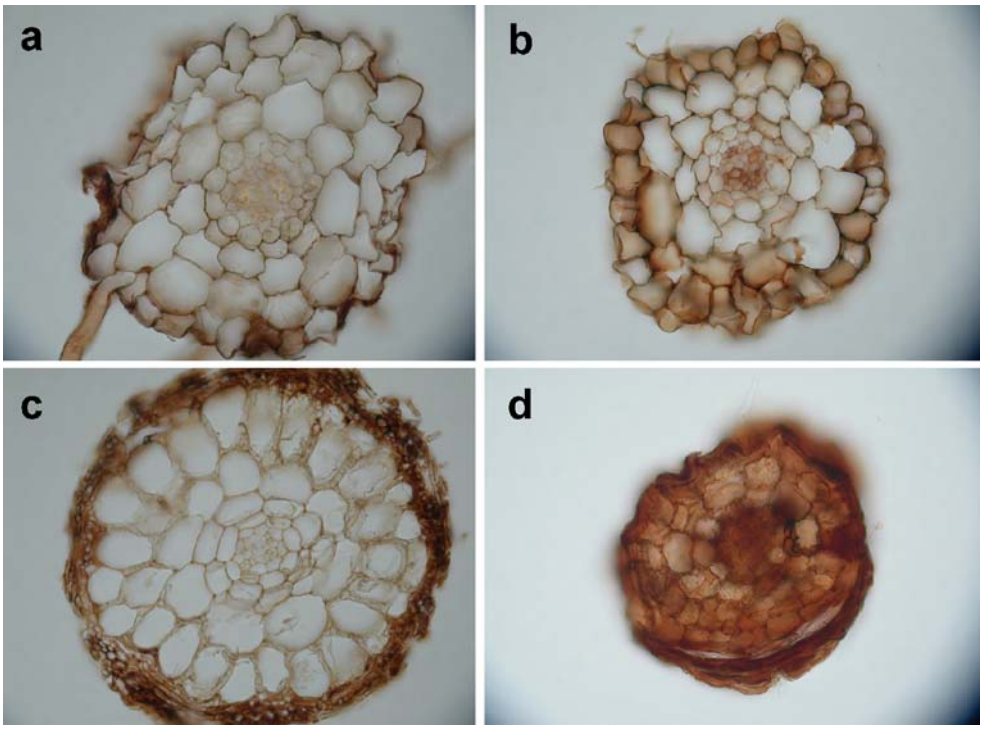

Fig. 8 Hydrogen peroxide formation in root tips of poplar (*P.*×*canescens*). **a** Non-mycorrhizal, non-stressed control; **b** salt-stressed, non-mycorrhizal root; **c** non-stressed mycorrhiza and **d** salt-stressed mycorrhiza. H_2O_2 was detected by staining with DAB

under salinity stress (Feng et al. 2002; Giri et al. 2003; Giri and Mukerji 2004; Tian et al. 2004; Asghari et al. 2005; Rabie 2005). Basidiomycetes, which form ectomycorrhizal symbioses with tree species, have been considered to be relatively salt sensitive (Tresner and Hayes 1971; Bois et al. 2006). However, there is now evidence that some basidiomycetes also display significant salt tolerance and can have a positive influence on the salt susceptibility of their host tree (Chen et al. 2001; Kernaghan et al. 2002; Muhsin and Zwiazek 2002). Chen et al. (2001) showed that most isolates of different *Pisolithus* species collected in Australia showed less than 50% growth reduction in the presence of 200 mM NaCl, indicating relatively high salt tolerance. In our study, *P. involutus* showed a delay in growth after the addition of 200 mM NaCl, but this disappeared with time, and in established symbiosis with hybrid poplar, positive effects on host performance were found.

Salt stress has been shown to lead to oxidative stress in plants (Gueta-Dahan et al. 1997; Hernandez et al. 1999, 2000). Unattended excessive formation of reactive oxygen species may cause injury, but H_2O_2 also serves as a signalling intermediate in stress responses (Polle and Schützendübel 2003). In poplar roots, salinity resulted in the formation of H_2O_2 and stimulation of POD activities, suggesting the activation of cell rescue pathways. In contrast to plant roots, pure, liquid cultures of *P. involutus* exposed to salt stress did not accumulate H_2O_2. However, the observed increases in SOD activities after the addition of salt suggest that reactive oxygen species are probably detoxified. Apparently, the ability of *P. involutus* to adapt its metabolism to environmental extremes is very pronounced because the SOD increases were found only transiently. The lack of fungal H_2O_2 production under stress was surprising and unexpected because the fungal sheath shows massive H_2O_2 accumulation in the symbiotic stage. This indicates that the enzymatic machinery for H_2O_2 formation is present in the fungus but is apparently regulated differently to that of plants.

Despite the beneficial effects of *P. involutus* on plant biomass under salt stress, oxidative stress was not prevented, as indicated by increased H_2O_2 production and induction of POD activities in poplar mycorrhiza. Arbuscular mycorrhizal fungi also seem to be unable to prevent oxidative stress because soybean roots inoculated with *Glomus etunicatum* showed increased SOD activities under salt stress (Ghorbanli et al. 2004). However, in contrast to our results, salinity-induced increases in H_2O_2-scavenging enzymes were not found in the study by Ghorbanli et al. (2004). H_2O_2 activates plant defence pathways including PODs (Kovtun et al. 2000) and results in systemic acquired resistance (Alvarez et al. 1998). Since poplar root POD activities increased under salt stress but were diminished in non-salt-stressed roots surrounded by a H_2O_2-producing fungal sheath, we suggest that either H_2O_2 diffusion out of the mantle into the roots is prevented in mycorrhiza or that H_2O_2 is not directly involved as a signalling intermediate in stimulating in salt-stress-responsive PODs. POD activities are normally up-regulated when barriers against invading pathogens are being formed (Punja 2001). It has been

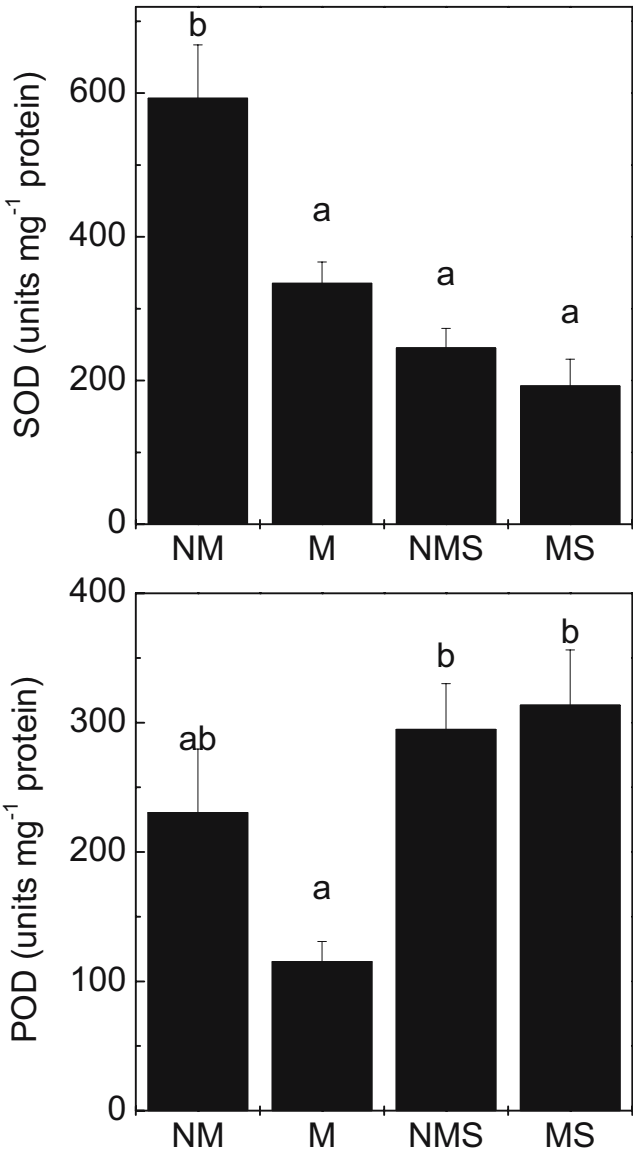

Fig. 9 Superoxide dismutase (**a**) and peroxidase (**b**) activities in roots of mycorrhizal (*M*) and non-mycorrhizal *P.×canescens* (*NM*) plants in the absence or presence of salt stress (*S*). Data indicate means of five plants per treatment (±SE). *Different letters* indicate significant differences at $P \leq 0.05$

repeatedly observed that massive defence reactions are not elicited in mycorrhiza (Gianinazzi-Pearson et al. 1996; Münzenberger et al. 1997), though the reasons are unclear. An important future task will be to investigate whether fungal H_2O_2 can activate pathways for systemic acquired resistance, thereby mediating hardening against environmental constraints.

A crucial question addressed in this study was whether mycorrhization with a salt-tolerant fungus would also protect the host from the uptake of excess NaCl. Since there was delayed leaf degradation and a trend for diminished sodium accumulation in mycorrhizal compared to non-mycorrhizal poplar plants, an obvious assumption was that the fungus might provide a physical barrier against salt uptake into the root, e.g. as suggested for mycorrhizal spruce roots (Muhsin and Zwiazek 2002). However, microanalysis in different root parts showed that this was not true. Instead, Na^+ and Cl^- accumulation in outer epidermal cell walls were higher than in non-mycorrhizal plants. Although clear Na^+ and Cl^- gradients from the outer side of roots into the vascular system were found under salinity stress, the overall concentrations of these ions in the vascular system of mycorrhizal and non-mycorrhizal roots were not different. Tolerance to salt stress can be increased in poplar by preceding exposure to sub-injurious concentrations of NaCl (Ottow et al. 2005). In this respect, it is interesting that mycorrhizal poplar not exposed to salt stress contained higher Na^+ and Cl^- concentrations in roots than non-mycorrhizal plants, which might have caused some adaptation. Higher Na concentrations in roots of mycorrhizal plants were also reported by Scheloske et al. (2004) for symbiotic associations of a salt aster with a *Glomus* sp. However, this is not a general phenomenon because, depending on plant species, some arbuscular fungi decrease salt concentrations in root cortex (Giri and Mukerji 2004; Tian et al. 2004).

In addition to an influence on NaCl concentrations, *P. involutus* mycorrhiza improved the general nutrient status of poplar, causing significant increases in phosphorus and potassium concentrations in leaves. Positive effects on biomass production and element concentrations of *Paxillus* sp. have been previously reported with other host plants (e.g. *Picea abies*, L., Jentschke et al. 2001). Excess Na^+ causes injury in plants because it competes with K^+, leading to a depletion of this essential element (Tester and Davenport 2003). Our data show that the disruption of K^+ homeostasis was prevented in mycorrhizal poplars, which even showed increases in foliar K^+ concentrations under salinity stress compared with non-stressed mycorrhizal plants. Such beneficial effects on potassium concentrations have also been occasionally reported for arbuscular mycorrhiza (Rabie 2005). We suggest that the maintenance of elevated K levels may also contribute to alleviate the negative effects of sodium. It is currently not clear how ectomycorrhizal fungi affect plant transport and uptake systems for potassium. However, with the advent of molecular tools for poplar and mycorrhizal fungi, i.e. *Populus trichocarpa* and *Laccaria bicolor*, the molecular mechanisms can be addressed in the near future.

An important conclusion of the present study is that, despite its striking salt-tolerance, *P. involutus* only appears to delay but not to prevent salt stress of its host tree. Still, the potential to retard the negative effects of salinity together with the ability of *P. involutus* to form mycorrhiza with a broad range of host tree species may render it a

useful inoculum for trees grown at sites endangered by fluctuating exposure to elevated NaCl concentrations.

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