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Growth and stress reactions in roots and shoots of a salt-sensitive poplar species (*Populus x canescens*)

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Abstract: To characterise saline-sensitivity of *Populus* x *canescens*, micropropagated young trees were exposed to 25 mM and 100 mM in hydroponic culture. After exposure to saline conditions the relative growth rate and biomass production was initially stimulated but declined below growth of controls after several days. After three weeks of exposure to 100 mM NaCl, leaves displayed severe injury indicated by chlorophyll loss and significant electrolyte leakage as compared with controls or plants grown in 25 mM NaCl. Roots of poplar grown in the presence of 100 mM NaCl contained elevated concentrations of malondialdyde indicating that high salinity caused lipid peroxidation. Superoxide dismutase and catalase activities in roots were enhanced immediately after exposure to saline conditions. With increasing exposure time superoxide dismutase displayed a further increase, which was not accompanied by corresponding increases in catalase activities, thus, suggesting that oxidative degradation of membranes was caused by an imbalance of protective systems after long-term NaCl exposure.

Resumen: Con el fin de caracterizar la sensibilidad a la sal de *Populus* × *canescens*, árboles jóvenes obtenidos por micropropagación fueron expuestos a 25 mM y 100 mM en cultivo hidropónico. Después de la exposición a las condiciones salinas, la tasa relativa de crecimiento y la producción de biomasa estuvieron estimuladas inicialmente, pero declinaron hasta llegar por debajo del control después de varios días. Al cabo de tres semanas de exposición a 100 mM de NaCl, las hojas mostraron un daño severo, indicado por la pérdida de clorofila y una pérdida significativa de electrolitos en comparación con los controles o las plantas que crecieron en una concentración de NaCl de 25 mM. Las raíces de los álamos que crecieron en la concentración de 100 mM de NaCl tuvieron concentraciones elevadas de malondialdeido, lo que indica que la salinidad alta causó una peroxidación de los lípidos. Las actividades de la dismutasa y la catalasa del superóxido en las raíces se intensificaron inmediatamente después de la exposición a las condiciones salinas. Conforme aumentó el tiempo de exposición, la dismutasa del superóxido mostró un incremento adicional, el cual no estuvo acompañado por los correspondientes incrementos en las actividades de la catalasa; esto sugiere que la degradación oxidativa de las membranas fue causada por un desbalance en los sistemas protectivos, después de una exposición de largo plazo al NaCl.

Resumo: Para caracterizar a sensibilidade ao sal da *Populus x canescens*, expozeram-se plantas juvenis micropropagadas a cultura hidropónica com concentrações de 25 mM e 100 mM. Depois de expostas a condições de salinidade, a taxa relativa de crescimento e a produções de biomassa foi inicialmente estimulada mas decresceu abaixo do crescimento das plantas controlo depois de vários dias. Depois de três semanas de exposição a uma concentração de 100 mM de NaCl, as folhas revelavam injúrias severas que se traduziam em perdas de clorofila e uma perda significativa de electrólitos quando comparadas com as plantas controlo e com as que se encontravam submetidas a uma concentração de 25 mM de NaCl. As raízes dos choupos

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crescendo em regime de 100 mM de NaCl continham elevadas concentrações de malondialdeido indicando que a elevada salinidade causou uma peroxidação lipídica. As actividades da dismutase superóxida e da catalase nas raízes foram imediatamente aumentadas depois da exposição a condições de salinidade. Com o aumento de tempo de exposição a dismutase superóxida mostrou um novo aumento que não foi acompanhado pelo correspondente aumento nas actividades da catalase, sugerindo, assim, que a degradação oxidativa das membranas foi causada por um desequilíbrio dos sistemas protectivos depois de uma longa exposição ao NaCl.

Key words: Antioxidative enzymes, oxidative stress, poplar, salt, salinization, sodium chloride, tree.

Introduction

In their environment, plants are exposed to various stress factors of natural or anthropogenic origin. Salinity is an environmental factor to which specialised species, like halophytes, are well-adapted to, but which limits development and growth of most plant species. In many areas of the world, problems due to salinity increase because use of fertilisers, artificial irrigation and high evaporation result in rising concentrations of salt in the soil. It has been estimated that about 20 % of the irrigated land is already affected by secondary salinization (Flowers & Yeo 1995). In many tropical countries progressive salinization of agricultural land is an important environmental problem. For example, in Indonesia, especially on Java, during the last four decades dams and artificial irrigation systems were constructed to irrigate expanding agricultural areas. These practises are currently leading to increasing salt concentrations in the soil with negative consequences for agricultural yield and other types of vegetation.

The response of sensitive plants to excess salt is complex and involves changes in morphology, physiology and metabolism. Major effects of salt (NaCl) in plants are disturbance of the cellular water relations, i.e., osmotic stress, disturbance of nutrient relations and toxic effects on metabolic processes (Kreeb 1996). Since salinization is a world-wide growing problem, intensive research attempts are underway to improve salt tolerance of plants. However, the commercial success of increasing salt tolerance by traditional breeding, has still been very limited, even when halophytic species exist in a gene pool, like for tomato (Bolarin *et al.* 1991). A major obstacle to successful breeding of new salt-tolerant genotypes turned out to be the apparent existence of negative linkages between yield traits and salt-tolerance (Neumann 1997). A possibility to circumvent these problems is offered by genetic engineering. Recently, some genes associated with increased salt-tolerance have been identified (Kasuga *et al.* 1999; Pardo *et al.* 1998; Serrano *et al.* 1999). First attempts to improve salt tolerance in tomato were promising. For example, ectopic expression of a yeast gene (*HAL1*) facilitating K^+/Na^+ selectivity, minimised salt-induced reductions in fruit yield and increased salt-tolerance (Gisbert *et al.* 2000; Rus *et al.* 2001).

Apart from agricultural crops, traits for salt-tolerance are also important in trees. Salt-resistant tree species such as *Populus euphratica*, which exist in the presence of up to 2 % sodium chloride, are currently used for afforestation on alkaline and saline soils for stabilisation of sand dunes and in agriculture shelter belt constructions in north-west China (Wang *et al.* 1996). However, a major draw-back of these trees is their slow growth. The genus *Populus* contains many fast-growing species (Bradshaw *et al.* 2000). Obviously, it is a desirable goal to combine traits of fast growth with those for salt tolerance. However, traditional breeding targeted at the production of such poplar hybrids has not been successful (Wang, personal communication). Approaches using methods of genetic engineering may be promising. Fast-growing poplar hybrids, e.g., *P. tremula x alba* (= *P.* x *canescens*) have been introduced as model trees for genetic engineering (Bradshaw *et al.* 2000). However, *P.* x *canescens* is susceptible to oxidative stress (Foyer *et al.* 1995; Strohm *et al.* 1998). Oxidative stress is also an important factor in the suite of reactions leading to salinity-induced injury (Hasegawa *et al.* 2000). We suspected,

therefore, that *P.* x *canescens* would be sensitive to saline conditions. However, this has not yet been tested.

The major goal of the present study was to find out whether *P.* x *canescens* is a suitable tree for studies of saline sensitivity, which can serve in future investigations as model for amelioration by genetic engineering. To characterise plant performance under salt-stress, young micropropagated poplar trees were exposed in hydroponic culture to two levels of sodium chloride and analysed for shoot and root growth. Chlorophyll degradation and electrolyte leakage were determined as indicators for leaf injury. To investigate whether the trees suffered oxidative stress, soluble protein concentrations, oxidatively modified proteins and malondialdehyde concentrations as well as antioxidative defence enzymes (SOD, CAT, GuPX) were determined in roots.

Materials and methods

Plant culture and harvest

Hybrid poplar (*P. tremula* x *alba*, INRA clon 717-1B4) were multiplied by micropropagation (Leplé *et al.* 1992). Rooted plantlets of a height of about 4 cm were transferred from sterile conditions into aerated hydroculture with modified Long Ashton medium (Hewitt & Smith 1975). The plants were grown for 4 weeks under controlled conditions in a phytochamber (16 hours light, 150 µmol photosynthetically active radiation [Phillips TL 65W/25RS], temperature of 20 °C and relative air humidity of 70%). The young plants were transferred into aerated 20 l boxes (8 saplings per box) and acclimated to ambient greenhouse conditions (air temperature from 18 to 24 °C, relative air humidity about 50%) for two weeks before exposure to saline stress. The nutrient solutions were changed twice a week. The light phase was extended to 16 hours by additional irradiation as above. Growth parameters (shoot height, root lengths and leaf formation) were determined regularly. Plants (n = 3 per sampling date and treatment) were harvested immediately before addition of saline medium (25 and 100 mM NaCl) and after 1, 7, 14, 21 and 24 days of exposure to salinity.

The plants were separated into above- and below ground biomass. Roots tips (about 2 to 3 cm) and aliquots of leaves were frozen for biochemical analysis. Dry mass was determined after 72 hours at 80 °C. At the final harvest, aliquots of fresh leaves were used for the determination of electrolyte leakage.

Biochemical analysis

Pigments were extracted in 80 % acetone and determined spectrophotometrically after Lichtenthaler & Wellburn (1983). Root tissues were extracted as described previously (Schützendübel *et al.* 2001) and used to measure total soluble protein (with a commercial kit, Pierce, Oud Beijerland, Netherlands), carbonylated proteins (Levine *et al.* 1990), malondialdehyde (Peevers & Higgins 1989), and enzyme activities of SOD (McCord & Fridovich 1969), CAT (Aebi 1983) and peroxidases with guaiacol as substrate (GuPX, Polle *et al.* 1990).

To determine electrolyte conductivity, 20 leaf disks (diameter 8 mm) were cut from fresh leaves and flooded on 20 ml distilled water (leaf upside up) in a phytochamber with light, temperature and humidity maintained as above. Electrolyte conductivity was determined at the beginning ($E_{t=0}$) and after 24 hours ($E_{t=24}$) with a conductometer (WTW, Weilheim, Germany). To determine maximum electrolyte conductivity (E_{max}) the leaf disks were boiled in a sealed vessel for 30 min to avoid evaporation of water and cooled to room temperature. Relative electrolyte leakage (%) was calculated as:

$$[(E_{t=24}) - (E_{t=0})] \times 100 / E_{max}$$

To determine relative electrolyte concentration, the electrolyte conductivity E_{max} of controls measured under these conditions was set as 100 % and E_{max} from saline treatments was calculated as:

$$E_{max\ (saline\ treatment)} \times 100 / E_{max\ (control)}.$$

Statistical analysis

The experiment was replicated twice with similar results. All data shown are from the second experiment. Per treatment and sampling date three individual plants were analysed. Data are means ± SD. To determine whether effects of salt treatment were statistically significant, multivariate analysis of variance was performed (Statgraphics, STN, Inc., St Louis MO, USA) and calculated P-values are indicated in figures. Where appropriate, a multiple range test was applied and significant differences with $P \leq 0.05$ are indicated with different letters.

Results

Growth of poplar under saline conditions and injury

After transfer of poplar to saline conditions the plants showed wilting. Poplar exposed to 25 mM NaCl recovered in about 3 hours, whereas those exposed to 100 mM NaCl within about 12 hours. Visible symptoms of leaf injury, such as necrotic leaf areals, were apparent in poplar exposed to high NaCl after about one week and loss of foliage occurred after two weeks. Some initial visible symptoms of foliar injury were apparent on leaves of poplar exposed to low NaCl concentrations after about three weeks towards the end of the experiment.

Low salinity had no effect on height growth of the shoot or on formation of new leaves during the experimental period of 24 days (Figs. 1A & B). By contrast, high salinity resulted in significant growth inhibition and no new leaves emerged after two weeks of salt exposure (Figs. 1A & B). Although necrotic spots appeared on leaves of high salinity-exposed plants within one week, no significant effects were apparent on chlorophyll or carotenoid concentrations in this period of time (Figs. 2A & B). Interestingly, exposure to low salinity initially caused more severe pigment loss than high salinity (Figs. 2A & B). At the end of the experiment, controls contained highest, low salinity-exposed plants intermediate and high salinity-exposed plants lowest pigment concentrations (Figs. 2A & B).

High salinity inhibited length growth of roots immediately, whereas the influence of low salinity was more subtle resulting in significant inhibition only after about three weeks of exposure to NaCl (Fig. 1C). It is important to note that these inhibitory effects of salinity on elongation growth did not correspond to loss in biomass production (Figs. 3A & B). Most notably, low salinity stimulated root and shoot biomass formation resulting in increased biomass as compared to controls over the whole experimental period (Figs. 3A & B). After exposure to high salinity root length growth was immediately inhibited (Fig. 1C), but root biomass was initially maintained and declined below controls after about two weeks (Fig 3B). Shoot biomass of plants exposed to high salinity dropped below that of controls after about two weeks (Fig. 3A).

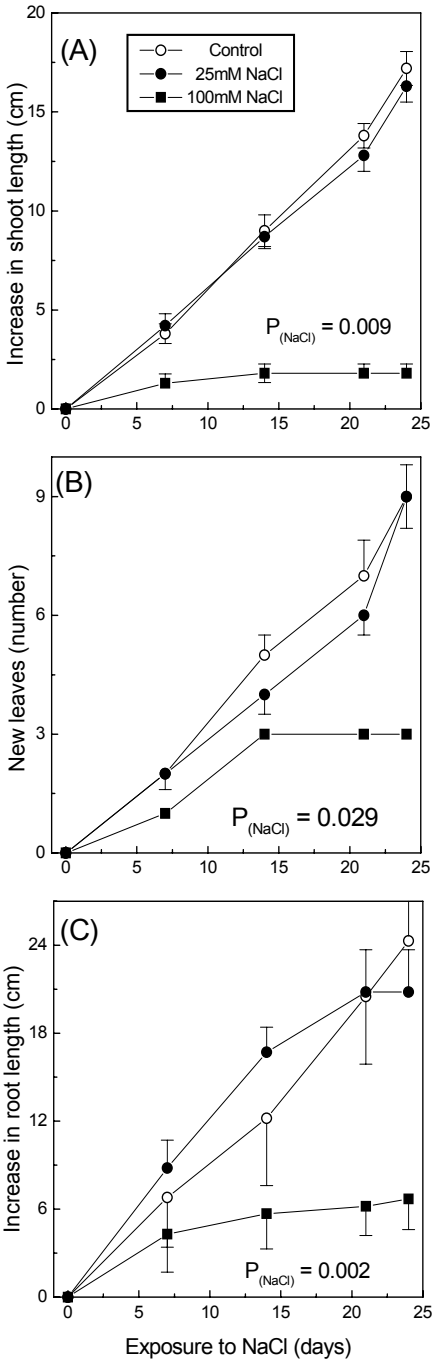

Fig. 1. Increase in shoot length (A), leaf number (B) and root length (C) in *P. x canescens* grown for 24 days in hydroponic media (controls = open circle) or after addition of 25 mM (black circles) and 100 mM NaCl (black squares). Before addition of NaCl, shoot and root length as well as leaf number were determined for each plant and the increment was determined weekly. Data represent means (n = 3 ± SD).

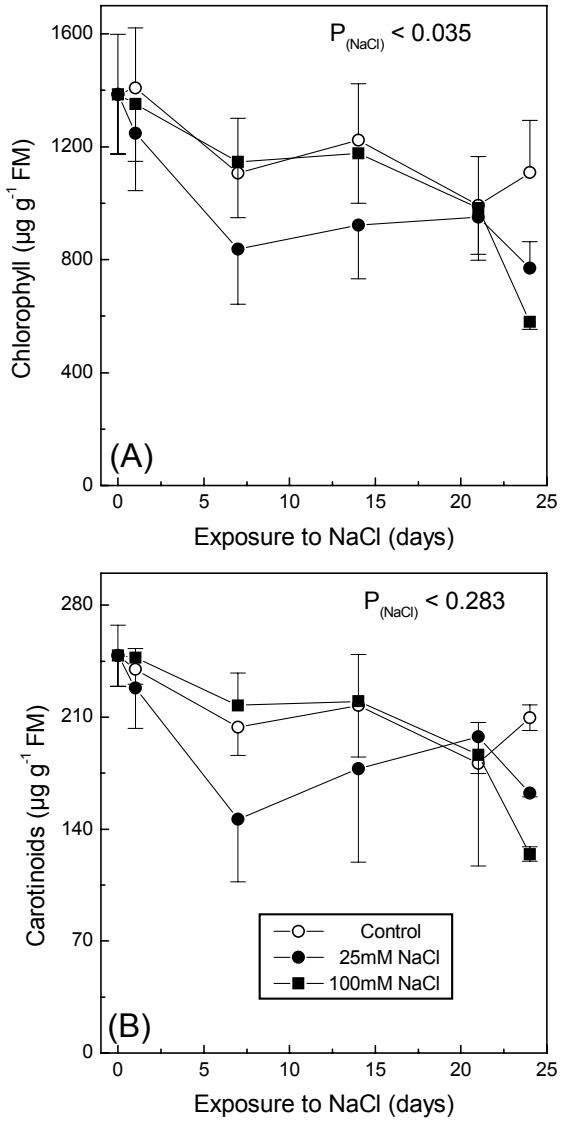

Fig. 2. Chlorophyll (A) and carotenoid concentrations (B) in leaves of *P. x canescens* grown for 24 days in hydroponic media (controls = open circle) or after addition of 25 mM (black circles) and 100 mM NaCl (black squares). Data represent means (n = 3 ± SD).

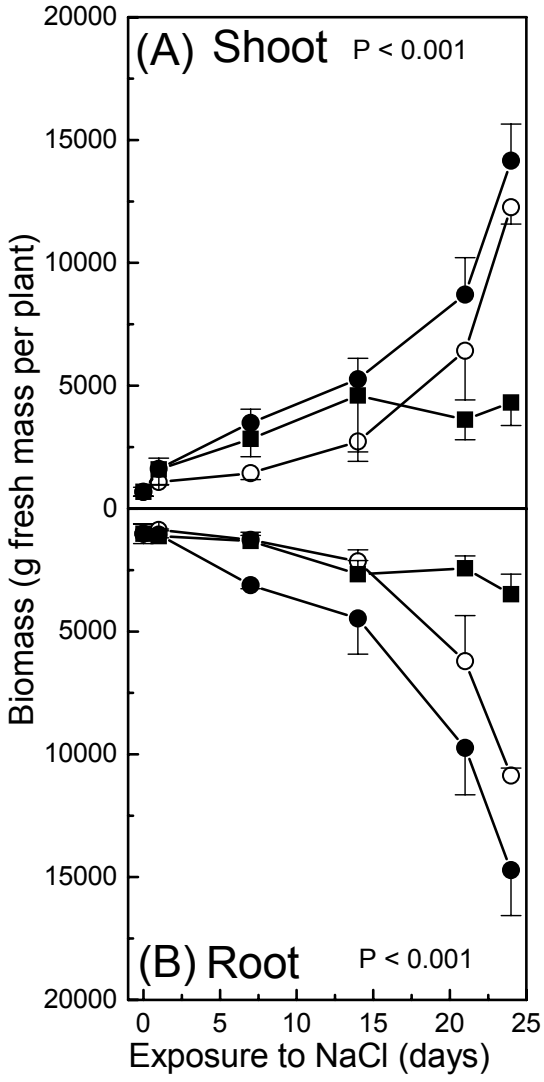

Fig. 3. Development of above-ground (A) and below-ground biomass (B) of *P. x canescens* grown for 24 days in hydroponic (controls = open circle) or after addition of 25 mM (black circles) and 100 mM NaCl (black squares). Data represent means for fresh mass (n = 3 ± SD).

Analysis of the relative growth rate [calculated as (dry mass (t_2) – dry mass (t_1) / dry mass (t_1)] indicated that growth of controls was initially low and increased consistently throughout the experiment (Fig. 4). Exposure to salinity instantaneously stimulated the relative growth but these enhanced growth rates were not maintained and declined throughout the exposure time to NaCl below those of controls (Fig. 4). Poplar grown at low salinity maintained elevated growth for about 10 days and thereafter growth rates declined to about half those of controls (Fig. 4). After an initial growth stimulation, trees exposed to high salinity displayed growth rates similar to those of controls for about two weeks, but after three weeks growth was completely inhibited (Fig. 4).

The loss of growth found in poplar exposed to high salinity was accompanied by a severe loss of shoot water content (Fig. 5A). It is interesting to

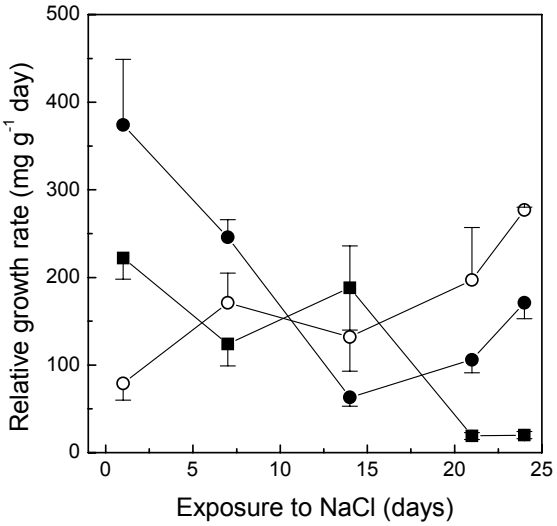

Fig. 4. Relative growth rate of *P. x canescens* grown for 24 days in hydroponic media (controls = open circle) or after addition of 25 mM (black circles) and 100 mM NaCl (black squares). Data were calculated on the basis of whole-plant dry mass and represent means (n = 3 ± SD).

note that the relative water content of roots was not affected under these conditions (Fig. 5B). Nevertheless, poplar root tips of plants exposed to high salinity showed significant decreases in soluble protein concentrations suggesting that the physiology of roots was also disturbed (Fig. 5C). Low salinity had no effect on any of these parameters (Figs. 5A-C).

At the end of the experimental treatments, the electrolyte concentrations of leaves from plants exposed to low and high salinity were determined relative to controls. Leaves of plants exposed to 25 mM NaCl contained about 1.7-fold and those from plants exposed 100 mM NaCl about 4-fold higher foliar electrolyte concentrations than controls (Fig. 6A). This increment was probably caused by accumulation of NaCl but perhaps additionally by formation of osmotically active compounds. Membrane injury, measured as leakage of electrolytes into an incubation medium during 24 hours, was not found in leaves from low salinity treated plants but was severe in leaves of poplar exposed to high salinity (Fig. 6B).

Oxidative stress and antioxidative systems in roots under saline conditions

To find out whether exposure to NaCl caused `oxidative stress in roots, carbonylated protein and

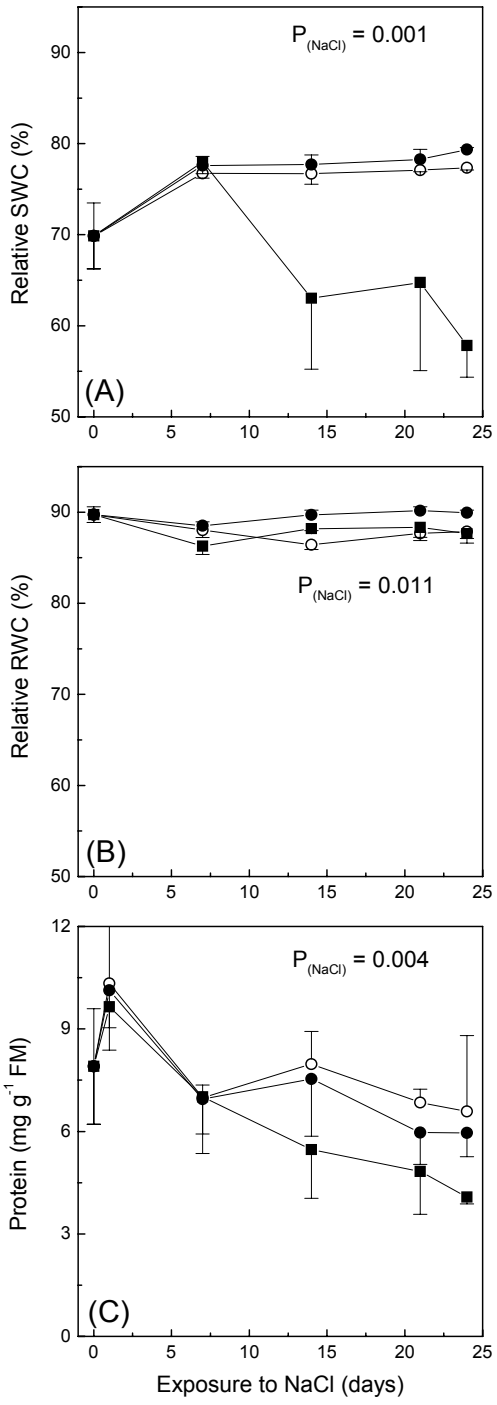

Fig. 5. Relative shoot water content (SWC, A), relative root water content (RWC, B) and total soluble protein concentration in roots (C) of *P. x canescens* grown for 24 days in hydroponic media (controls = open circle) or after addition of 25 mM (black circles) and 100 mM NaCl (black squares). Data represent means (n = 3 ± SD).

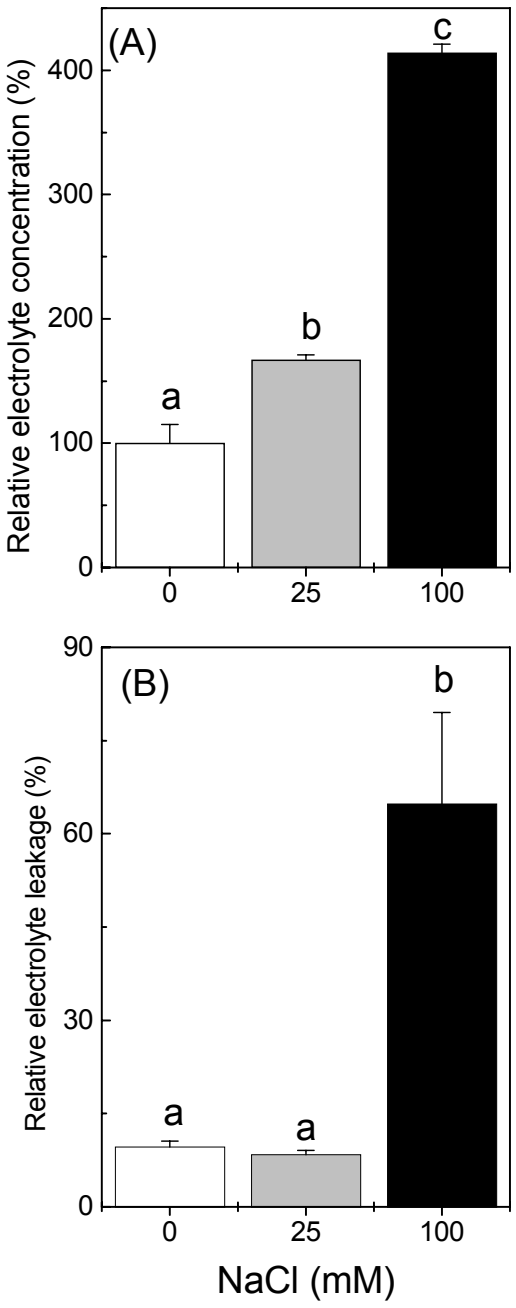

Fig. 6. Relative electrolyte concentration (A) and relative electrolyte leakage (B) in leaves of *P. x canescens* after 24 days growth in hydroponic media in the absence of NaCl (control), or in the presence of 25 mM and 100 mM NaCl. For calculation of the parameters see Materials and Methods. Data represent means (n = 5 ± SD).

malondialdehyde concentrations were determined in root tips of poplars exposed to low and high salinity. It has been shown that oxidative stress results in carbonylation of proteins, which marks these proteins for degradation (Ames *et al.* 1993; Levine *et al.* 1990). The present data show, however, that the concentrations of carbonylated proteins were highest in controls, intermediate in roots of low salinity-exposed plants and lowest in plants exposed to high salinity, except for the last harvest (Fig. 7A). It is possible that this unexpected result was caused by increased protein degradation rates, which may be inferred from loss in total soluble protein concentrations (Fig. 5C). If increased protein degradation resulted preferentially in loss of carbonylated protein, the above result would be expected.

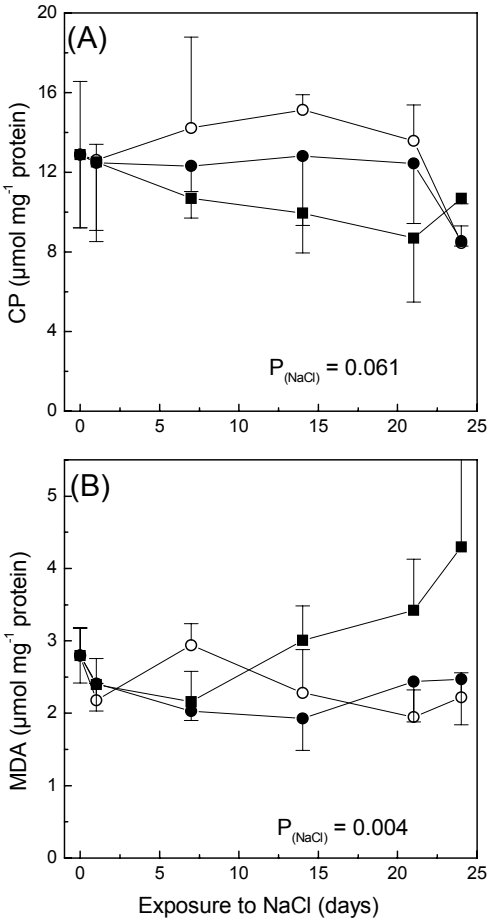

Fig. 7. Concentration of carbonylated protein (CP, A) and malondialdehyde (MDA, B) in roots of *P. x canescens* grown for 24 days in hydroponic media (controls = open circle) or after addition of 25 mM (black circles) and 100 mM NaCl (black squares). Data represent means (n = 3 ± SD).

In controls and in low salinity-exposed plants, the concentrations of malondialdehyde, which is formed as a product of oxidative membrane degradation, fluctuated around 2.3 ± 0.3 μmol mg^{-1} protein (Fig. 7B). Under high salinity conditions, the concentrations of malondialdehyde started to increase in roots after about one week of exposure to NaCl and were about twice higher than in controls after about three weeks of NaCl exposure (Fig. 7B).

SOD is important for the removal of superoxide radicals. In controls, SOD fluctuated around 45.5 ± 4.9 units mg^{-1} protein (Fig. 8A). In low salinity-exposed plants, SOD started to increase towards the end of experiments (Fig. 8A), when effects on root elongation growth were apparent (Fig. 1C). Under high salinity SOD activity was immediately enhanced; these elevated levels were maintained for about two weeks and showed a further rise thereafter (Fig. 8A), which corresponded to increasing malondialdehyde concentrations (Fig. 7B), protein degradation (Fig. 5C), and loss of biomass production (Fig. 3B, Fig. 4).

CAT activities, which catalyse H_2O_2 degradation, fluctuated around 3.3 ± 0.7 μkat mg^{-1} protein in roots of controls (Fig. 8B). In roots of poplar exposed to low salinity, the CAT activities were increased immediately after salt exposure (6.8 ± 1.2 μkat mg^{-1} protein), but showed no further changes over time (Fig. 8B). High salinity caused an immediate increase in CAT activities to about 4-fold higher levels than in controls (Fig. 8B). However, this increase was maintained only as long as roots maintained growth (Fig. 3B). It is also notable that high salinity stress resulted in high standard deviations of the CAT measurements. Such a scattering of the data is expected when the cellular homeostasis is significantly disturbed.

GuPX activities in roots of poplar exposed to low salinity were similar to those found in control roots (Fig. 8C). In roots of poplar exposed to high salinity, GuPX initially were moderately increased. However, towards the end of the experiments, when severe injury was apparent (Fig. 7B), GuPX activities were about 70% increased as compared with controls (Fig. 8C).

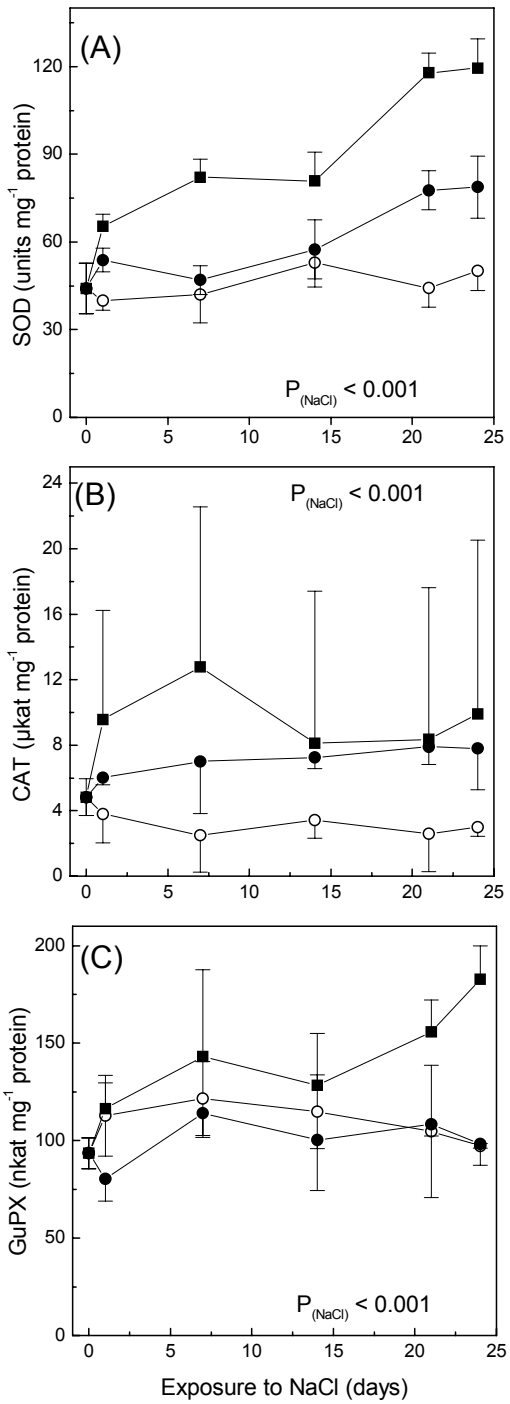

Fig. 8. Activities of superoxide dismutase (SOD, A), catalase (CAT, B) and guaiacol peroxidase (GuPX, C) in root tips of *P. x canescens* grown for 24 days in hydroponic media (controls = open circle) or after addition of 25 mM (black circles) and 100 mM NaCl (black squares). Data represent means (n = 3 ± SD).

Discussion

The present results show that *P.* x *canescens* was able to cope with about 25 mM NaCl in the nutrient solution for at least three weeks, whereas

100 mM NaCl were toxic, leading to oxidative stress and severe growth inhibition. Other poplar species displayed saline sensitivity in a similar concentrations range, e.g., mortality was found in *P. robusta, P. berolinensis* and *P. popularia* after three weeks exposure to 86 mM NaCl (0.5 %) (Fung *et al.* 1998).). In comparison to these examples, the relatively salt-tolerant species *P. euphratica* survived 250 mM NaCl in hydroponic culture (Watanabe *et al.* 2000). In an other experiment, in which salinity was gradually increased from about 70 to above 400 mM in soil, *P. euphratica* showed no visible leaf injury until three weeks when NaCl exceeded 320 mM in the soil, whereas a hybrid *of P. euphratica x Salix alba* showed leaf necrosis after 8 days when NaCl concentrations exceeded 200 mM (Chen *et al.* 2001). In comparison with *P. euphratica*, *P.* x *canescens* is a highly salt-sensitive poplar hybrid and thus suitable as a model for tree genetic engineering for enhanced salt tolerance in fast-growing tree species.

Short-term growth responses of trees to salinity have not been investigated in detail before. Our data show that a primary response of *P.* x *canescens* to low and high salt concentrations was an immediate and highly significant growth stimulation before growth was severely inhibited during the subsequent time course of the experiment (Figs. 3 & 4). It is unclear, how plants can achieve such a tremendous increase in the relative growth rate in a short period of time. Several mechanisms are feasible: the initial salt shock induced general osmotic stress as evident from leaf wilting. This stress was apparently mitigated since recovery occurred within several hours. Plants are known to produce osmoprotectants such as proline, glycinebetain etc. to adjust and maintain turgor in response to salinity (Hasegawa *et al.* 2000). However, gradual uptake of NaCl will also increase cellular osmolytes and thus increase turgor. Na^+-toxicity is prevented by exclusion. At the cellular level this is achieved by pumping Na^+ into the vacuole or the apoplastic space by an interacting system of proton pumps and Na^+/H^+ antiporters located in the tonoplast and in the plasma membrane (Apse *et al.* 1999; Barkala & Pantoja 1996; Binzel 1995). Whether or to what extent such systems work in hybrid poplar is not yet known. The functioning of the plasma membrane Na^+/H^+ antiporters will require cell wall acidification. Low cell wall pH results in loosening of cell wall texture and is a key step for growth (Rayle & Cleland 1992). We suggest, therefore, that cell wall acidification and increased turgor may result in the transient growth stimulation found in poplar grown under sub-lethal saline conditions. So far, this is a speculation, which will require further studies.

The present results show that *P.* x *canescens,* like a range of saline-sensitive herbaceous plant species (Hernandez *et al.* 1993, 1999, 2000; Savoure *et al.* 1999; Shalata & Tal 1998), suffered from oxidative stress (Figs. 5B & 7B). In contrast to most other studies, which investigated salt effects on antioxidant systems in leaves, we analysed the responses of antioxidative enzymes in roots tips. In salt-sensitive species loss or marked decreases in antioxidative enzymes have been reported (Comba *et al.* 1998; Gueta-Dahan *et al.* 1997; Lechno *et al.* 1997). Under the present conditions, where high salinity-treatment caused lethal injury as evident from severe water loss in the shoot (Fig. 5A), electrolyte leakage of leaves (Fig. 6B) and marked increase in lipid peroxidation in roots (Fig. 7B), SOD activity was still increased (Fig. 8A). Such increases in SOD activity, especially in mitochondrial SOD activity, have also been found in peas exposed to injurious NaCl concentrations (Hernandez *et al.* 1999).

However, the analysis of the time course of SOD induction in the present study indicates two separate phases: a first rapid increase when oxidative injury was not apparent and a second further increase when malondialdehyde levels started to rise. Recently, Kawano *et al.* (2001) have shown that NaCl administered above a threshold of 100 mM to tobacco suspension cultures caused activation of a NADPH-oxidase and resulted in significant $O_2^{\cdot-}$ accumulation. The induction of SOD in our system probably indicates activation of defences in response to NaCl-induced $O_2^{\cdot-}$-formation. These defences together with elevated CAT activities appeared to be sufficient to prevent oxidative degradation of membranes. The importance of SOD for mediating salt-tolerance has been shown in *Arabidopsis* mutants with altered expression of SOD and in transgenic rice overexpressing SOD, which displayed increased salt-tolerance (Tanaka *et al.* 1999; Tsugane *et al.* 1999). Despite relatively high saline-sensitivity of *P.* x *canescens*, SOD and CAT activities were apparently sufficient activated

to prevent oxidative injury to roots during the initially phase of salt exposure.

The second increase in SOD in roots occurred when shoots were severely injured and thus the nutrient supply to roots probably collapsed. It is likely that under these conditions energy for repair and maintenance was limited, thereby, leading to oxidative degradation of the system. The situation might have been aggravated since a correspnding increase in CAT activities was not observed (Fig. 8B). In conclusion, our data suggest that although roots are in primary contact with salinity, they appeared to cope better with salt stress than shoots. In other experiments with *P. euphratica*, roots have been shown to maintain lower Na^+ concentrations than leaves (Chen *et al.* 2001), which may be achieved through means of translocation and exclusion. Our data suggest that roots become susceptible to oxidative damage, when energy supply from shoots is no longer warranted.

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