Response of *Populus* x *canescens* (*Populus tremula* x *alba*) to high concentration of NaCl stress

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Abstract: *Populus* x *canescens* was cultivated on solid substrate and treated by salt (150 mM NaCl). The growth parameters including new leaf formation, height increment, diameter at the base increment, fresh and dry mass of leaf, stem, coarse root, and fine root were determined. The nutrient elements in leaves of samples under salt stress and the control, and the chlorophyll fluorescence of plants separated dark and light, initial fluorescence (F_o), and maximum fluorescence (F_m) were measured. Results showed that 150 mM NaCl treatment resulted in growth reduction of *Populus* x *canescens*. Nutrient element contents in the foliage of plants under salt stress were different from that of control. The foliar N-concentrations of plants under salt stress were not affected. Contents of Na under salt stress were 120 times as much as that under control. However, contents of S, K, P, Ca, Mg, Fe, Mn under salt stress were less than that under control. Salt stress caused damage in the PSII reaction centers, i.e. photo-inhibition couldn't be repaired under dark situation. The yield of chlorophyll fluorescence showed that several parameters associated with PSII functions, e.g. F_v/F_o, F_v/F_m were not influenced at the first stage of salt stress treatment. However, after a period of time, PSII functions were significantly inhibited, which led to the decrease of carbon assimilation. These results suggest that salt stress (150 mM NaCl) did not affect photosynthetic chlorophyll fluorescence of *Populus* x *canescens* immediately. After four day of salt stress, PSII reaction centres were seriously damaged during photo-inhibition.

Key words: Growth analysis; Salt stress; Photosynthesis; Chlorophyll fluorescence yield; Nutrient elements; *Populus* x *canescens*

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Introduction

Salinity is a major factor in inhibiting plant growth and decreasing forest productivity. Up to 1997, the total area affected by salinity in the world had amounted to 930 million hm^2 (FAO), and is still increasing. A global study of land use over 45 years found that 6% of land irrigated had become saline (Ghassemi *et al.* 1995). NaCl is a major factor in limiting plant production, since it affects almost all plant functions (Greenway and Munns 1980). Tree death due to de-icing salt (NaCl) application is a major problem in urban landscapes in colder climates (Dobson 1991). Under excess salinity, tree shows the symptoms of crown dieback, lesions on the stem or trunk, and leaf scorch. What is worse, some symptoms may aggravate. For example, tip burn of conifer leads to necrosis of needles that can cause dieback of limbs and tree death. High salinity disrupts plant ion homeostasis, which has secondary effects on plant growth such as oxidative stress, growth arrest, even death. Salt-tolerance is mediated by salt-exclusion mechanisms, enhancing protection against hyper-osmotic stress and increasing detoxification of reactive oxygen species (Zhu 2001). To date, most research on the mechanisms of salt tolerance of plant under salt stress mainly focused on herbaceous model plants such as *Arabidopsis,* or halophytes from salt marshes, or important crop species such as rice, tomato, pea, and wheat (Zhu 2002). However, less attention has been devoted to the analysis of responses of tree species exposed to salt stress.

The growth response and mechanism of physiological response of woody plant under salt stress are still unclear, especially on photosynthetic system damage and nutrient elements absorption and accumulation.

Chlorophyll fluorescence emitted by green plants reflects photosynthetic activities in a complex manner. Recent improvement in techniques of measuring fluorescence has made the fluorescence method be an important tool in basic and applied plant physiology research. The use of fluorescence from intact plant leaves has increased as a unique nonintrusive method of monitoring photosynthetic events and judging the physiological state of the plant (Maxwell and Johnson 2000). Each quantum of light absorbed by a chlorophyll molecule introduces an electron from the ground state to an excited state.

Over the last 15 years a number of studies on chlorophyll fluorescence have been done, and have the rapidly growing application of fluorescence in detection and analysis of stress effects on plants (Krause and Weis 1991). Interpretation of the fluorescence expressions provided an insight into mechanisms of salt damage on plant. Based on reductions in the performance of fluorescence yield, the damaged extent of plant could be measured.

However, the plants mentioned above are mainly crops (Hernandez *et al.* 1993, 2000). The stress situations mainly focused on drought stress, cold stress, enrichment of CO_2, heavy metal pollution, or special light (Ögren 1990; DeEll *et al.* 1999; Tognetti *et al.* 1999; Baccio *et al.* 2003). Few studies on the photosynthesis of woody plants under salt stress are conducted.

It is not known whether this species can endure and grow under saline conditions. To reveal these questions, *P.* x *canescens*

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seedlings were planted in solid substrate in the presence of up to 150 mM NaCl, the nutrient elements of plant, the effect of salt on nutrient relations in poplar seedlings exposed to salinity, and several parameters associated with PSII functions and PSII damage and recovery were studied. Aims of this study were to determine whether *Populus* x *canescens* is salt sensitive or salt tolerant and thereby provide information as to its suitability for planting in areas where de-icing salts are applied.

Materials and methods

Plant material and treatments

Hybrid poplar (*P. tremula* x *alba*) was multiplied by micropropagation (Leplé *et al.* 1992). To acclimate the plants to ambient conditions, rooted plantlets were cultivated in hydroponic LN-nutrient solutions with low nitrogen supply for 14 days initially in a climatized growth room (21°C, 50%–60% relative air humidity, light: 150 μmol photons·m^{-2}·s^{-1} of photosynthetic active radiation, 16 h) and 2 days before the transfer to pots in the greenhouse under growth conditions of 20°C to 24°C, 40% to 70% relative air humidity, and natural day light in addition to growth lamps (16 h light) yielding up to 300 μmol photons·m^{-2}·s^{-1} of photosynthetic active radiation.

Sterile-filtered NaCl solutions were added to final concentrations of 150 mM NaCl (Bolu and Polle 2004). Water was added to the controls.

The prepared sand mixture was incubated for four days before planting poplar plantlets into growth tubes which contained a nylon mesh at the bottom (diameter: 5 cm, height: 41 cm). Plants were potted into the prepared sand mixture. The potted plants (n = 60) were rotated frequently. The plants were automatically irrigated twice a day for 1 min each time with the LN-nutrient solution (Brumme *et al.* 1992). After four weeks prior to the salt treatment, plants were exposed to 150 mM NaCl added with the nutrient solutions at the same time.

Growth analysis

The number of new leaf formation, stem length, height of plants, and diameter at base of stem (SDB) were also measured twice a week. Before the beginning of NaCl stress treatments four plants were sampled immediately to determine the initial biomass, then the samples exposure to excess salt (150 mM NaCl) was maintained till the rapidly reduction of photosynthetic yield of leaves, and the plants were harvested. Each sampled plant was washed free of sand and vermiculite and then separated into leaves, stem, coarse roots, and fine roots used for further analysis. Each part of fresh weight and dry weight were obtained.

Biomass and element composition

The tissues were oven-dried at 60°C and milled to fine powder. After HNO_3 extraction, the element composition was determined by AAS-ICP analysis (Heinrichs *et al.* 1986). Carbon and nitrogen were determined by using a C/N analyzer.

Chlorophyll fluorescence yield

Chlorophyll fluorescence yield was measured and automatically recorded each day to monitor changes of photosynthetic capacity under salt stress by Mini-PAM fluorometer (Walz GmbH, Germany). Samples were placed in dark for 30 min before measurement, and then given a strong flash beam on dark condition. Chlorophyll fluorescence values of F_o, F_m, F_v/F_o, F_v/F_m were recorded. Normally in healthy leaves the yield of chlorophyll fluorescence, $F_v/F_m = (F_m-F_o)/F_m$, is always close to 0.8, regardless of the plant species studied.

Statistical analysis

Analysis of variance (ANOVA) was performed by the software of STATGRAPHICS.

Results

Growth parameters

After 4 weeks, the samples exposed to salt displayed significant loss of leaves starting at the bottom and moving towards the top. It is obvious that the new leaf formation was influenced by salt stress, which shows a considerable decrease at the end of this experiment compared with the control (Table 1). For stressed plants, salt treatment caused severe suppression in height growth in *Populus* x *canescence* (Table 1). The increase of SDB was small during the measuring time and affected lightly by salt treatment (Table 1).

Table 1. Increment of height, Diameter at base of stem and new leaf formation of *Populus* x *canescence*

Treatments	Height Increment (cm)	Diameter at base of stem (cm)	New leaf Formation (piece/ plant)
Control	3.46 ± 0.54c	2.89 ± 0.35a	4.01 ± 0.63c
150 mM NaCl	0.63 ± 0.12c	2.87 ± 0.46a	0.4 ± 0.08c

Note: Data are mean of 12 individual replicates (±SE). Different letters indicate significant differences with $P \leq 0.05$.

The samples exposed to high concentration of salt took on severe decreases not only in fresh biomass formation but also in dry biomass formation, which are caused by leaf loss, leaf area reduce, height growth decrease, and loss of root elongation growth. The leaf mass, stem mass, coarse root mass, fine root mass, the total fresh mass, and the total dry mass of plants treated by salt stress showed remarkable variations comparing with those of the control (Table 2). The total fresh mass and dry mass of the samples showed obviously decreases comparing with those of the control.

Table 2. Masses of the *Populus* x *canescens* under 150 mM NaCl stress and the control (g·plant^{-1})

Treatments	Fresh mass					Dry mass				
	Leaf	Stem	Coarse root	Fine root	Total	Leaf	Stem	Coarse root	Fine root	Total
Control	1.9525 ±0.021c	1.2182 ±0.031c	1.2573 ±0.040c	1.8690 ±0.029c	6.2970	0.4427 ±0.012c	0.4990 ±0.009b	0.3592 ±0.010 b	0.2262 ±0.006ab	1.5271
150 mM NaCl	1.1743 ±0.016c	0.7572 ±0.009c	0.7212 ±0.027c	1.1705 ±0.021c	3.8232	0.3539 ±0.013bc	0.3172 ±0.009c	0.2517 ±0.008b	0.1643 ±0.007ab	1.0871

Note: Data are mean of 12 individual replicates (±SE). Different letters indicate significant differences with $P \leq 0.05$.

Elements

The influences of salt stress on nutrient elements contents in the leaves were selective (Table 3). Sodium content in the samples leaves exposed to salt stress indicated an extremely high level. Contents of potassium and sulphur decreased comparing with those of the control. However, the contents of foliar nitrogen and phosphorus of the samples under salt stress were not affected. Moreover, the contents of K, Ca, Mg, Mn, and Fe in samples exposed to salt stress also decreased. The ratio of Na/K became higher than that of the control and the ratio of Na/Ca increased as well. However, the ratio of Na/K was lower in comparison with that of Na/Ca. Increased ion leakage under salt stress signified the damage of cell membranes. Raised Na-concentration in the soil hindered the uptake of other nutrients like Ca, and therefore impeded root growth.

Table 3. Contents of nutrient elements in leaves of *Populus* x *canescens* treated with salt stress and the control

Treatments	P	N	K	S	Ca	Mg	Fe	Mn	Na
Control	1.74±0.18a	1.69±0.20a	16.27±1.68a	3.33±0.33ab	12.89±0.28b	4.94±0.26c	1.00±0.06c	0.73±0.05c	1.02±0.03a
NaCl	1.31±0.07a	1.70±0.21a	12.67±1.19ab	2.61±0.17a	10.27±0.98a	3.92±0.27b	0.88±0.09bc	0.64±0.05bc	120±4.32a

Note: Data ($g \cdot kg^{-1}$ dry mass) are mean of 4 individual replicates (±SE). Different letters indicate significant differences with $P \leq 0.05$.

Chlorophyll fluorescence

Chlorophyll fluorescence yield

Generally, PSII is damaged when the fluorescence yield decreased rapidly and it can't recover after dark recovering. This phenomenon is also observed in this study (Figs. 1, 2).

The response of photosynthetic chlorophyll fluorescence yield in leaves showed a progressive decrease of the photosynthetic activity with increasing salt exposure time. In 4 d of salt treatment, the yield of chlorophyll fluorescence displayed almost the same result as that of the control. However, after 4 d of salt treatment, the yield of chlorophyll fluorescence began to decline. On the 8th d it showed a very rapid decrease in the maximum yield of PSII in response to salt exposure in the light (Fig. 1). Full recovery in darkness did not occur (Fig. 2), whereas the PSII activity of the control was almost no change (Fig. 2). This indicates that 150 mM NaCl of salt stress for *Populus* x *canescens* have damaged the PSII reactive center after 4 d of exposure to salt stress.

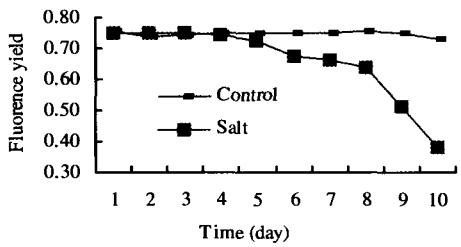

Fig. 1 Chlorophyll fluorescence yield of leaves of *Populus* x *canescence* in the light

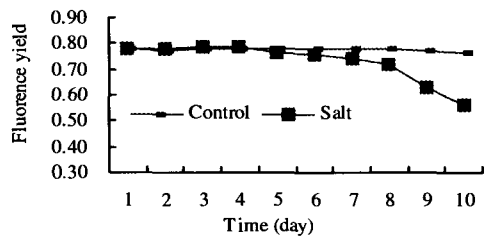

Fig. 2 Chlorophyll fluorescence yield of leaves of *Populus* x *canescence* in the dark

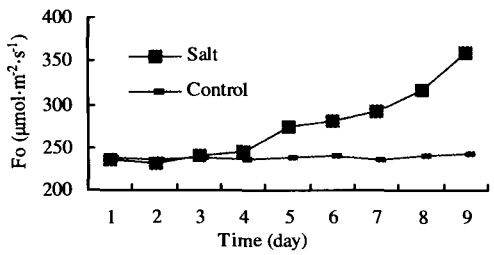

Fig. 3 The effect of salt stress on F_o of *Populus* x *canescens* leaves

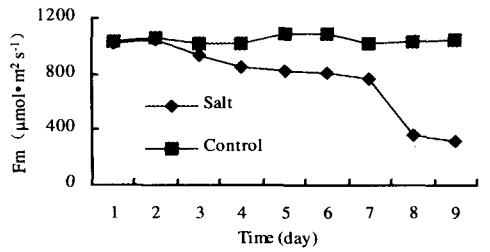

Fig. 4 The effect of salt stress on F_m of *Populus* x *canescens*

Initial fluorescence (F_o) and maximum fluorescence (F_m)

Initial fluorescence (F_o) of *Populus* x *canescens* leaves under salt stress was almost the same as that of the control in the beginning of treatments. After 4 d, the F_o of leaves began to increase in an increasingly rapid manner (Fig. 3). Maximum fluorescence (F_m) of samples under salt stress remained the same as that of the control at first and decreased after 2 d. But a rapid decrease of F_m by salt treatment was observed after 7 d (Fig. 4).

Generally, the increase of F_o is considered as indicator of damage or non-activity of PSII reaction center, and the decrease of F_m is considered as caused by the inhibition of electronic transfer. Therefore, it is evident that the PSII reaction center and electronic transfer of leaves of *Populus* x *canescens* under 150 mM NaCl was damaged or inhibited.

Discussion

It is clear that salt stress of 150 mM NaCl damaged *Populus* x *canescens* growth (Table 1) and physiologic metabolism, especially in ion homeostasis (Table 2) and PSII reaction center (Fig. 2, 3). Salt stress of 150 mM NaCl changed the model of plant for

elements absorption. The selective elements uptake induced Na accumulation and Ca decrease under salt stress. The contents changes of K, Mg and Ca in plant indicate the changes of osmotic regulation, transport of ion on plasma membrane.

In order to survive under extreme saline conditions, plants must maintain a high cytoplasmic K/Na ratio and therefore must be efficient at K uptake in a high Na background and be able to exclude or remove Na from the cytoplasm (Serrano and Rodriguez 2002). So it is very important to keep the ratio of K/Na in a certain level. The uptake of plant for different ions probably resulted from the higher selectivity of the plasma membrane. When external K was high, K uptake and transport from root to shoot were inhibited by exogenous Na (Peng 2004). The K/Na selectivity of potassium channels and the existence of an apoplastic barrier lead to the lateral gradient of K and Na across root tissue, resulting not only in high levels of K in the shoot but also a large Na gradient between the root and the shoot. Low K increases reactive oxygen species (ROS) activity, not only stimulating the biosynthesis of the Na, but also inducing total protein synthesis. The Na mediates epidermal growth factor receptors, which send messages to nuclei through organized cytosolic cascades of signaling events. Among those signaling events, ROS and intracellular Ca are essential second messengers. Some research approaches some channels, such as Na^+/H^+ antiporter are selective uptake for K and Na. One channel for Na uptake is blocked by external calcium and occurs via non-selective cation channels (Zhao and Zhou 2005). Therefore, the decreased uptake of Ca under salt stress undoubtedly affects the uptake of Na.

Analysis of the fluorescence characteristics, such as the nature and intensity of the emission bands, quantum yield, and induction kinetics, reflects the properties of the chlorophyll molecules and their environment. Consequently, alterations to these characteristics as a result of environmental stress can be used to study photosynthetic electron transport and associated physiological processes (Hall and Rao 1999). Chlorophyll fluorescence has proved particularly useful in salinity-tolerance screening programs (Jimenez *et al.* 1997) because the effects of salt damage can be detected prior to visible signs of deterioration (West 1986). Percival and Fraser (2001) used chlorophyll fluorescence as a diagnostic tool to identify salt-tolerant trees. Marked differences in sensitivity among species within the *Crataegus* genus were recorded. It therefore appears that chlorophyll fluorescence provides a means by which to identify salt-tolerant genotypes for forest planting and urban landscape plantings (Maxwell and Johnson 2000).

Recently many studies have shown that peroxidants and H_2O_2 were produced by PSII. These ROS not only damage protein, but also improve to a certain extent resistance of plants.

It is noticeable that the response of *Populus* x *canescens* to 150 mM NaCl treatment varied at different stages of the experiment. Very slight reductions of dry mass in foliage and whole-plant were shown at the beginning, while remarkable changes in leaf area, height increment, chlorophyll concentration, chlorophyll fluorescence yield took place at the end.

Some studies showed remarkable SDB reduction under abiotic stress (Baccio *et al.* 2003). However, this study showed that SDB increment was barely affected by the time of subjection to 150 mM NaCl (Table 1). As the period of salt treatment was not long enough in this study, we can not conclude that SDB increment reduction would occur after a long time of exposure to salt stress,

Although further studies at the biochemical and molecular levels are necessary, these results have shown that *Populus* x *canescens* is sensitive to salt stress, therefore this species is not suitable for afforesting in coastal areas or reforesting salinity areas.

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