Sodium and calcium uptake, transport and allocation in *Populus euphratica* and *Populus* x *canescens* in response to salinity

Dissertation

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List of abbreviations

µ	Micro (10^{-6})
°C	Degree Celsius
A	Area
ABA	Abscisic acid
AFLP	Amplified fragment-length polymorphism
ANOVA	Analysis of variance
ATPase	Adenosine triphosphatase
Bq	Becquerel
c	Centi (10^{-2})
cDNA	Complementary desoxyribonucleic acid
CaM	Calmodulin
CBL	Calcineurin B-like protein
cpm	Counts per minute
CTAB	Hexadecyltrimethylammonium bromide
d	Diameter
ddH_2O	Double destilled water
DM	Dry mass
DNA	Desoxyribonucleic acid
EDTA	Ethylene diamine tetra acetic acid
EST	Expressed Sequence Tag
FAE	Formaldehyde-acetic acid-ethanol
FAO	Food and Agriculture Organization
FM	Fresh mass
g	Gram
h	Hour
ha	Hectar
ICP	Inductively Coupled Plamsa
HKT	H^+/K^+ transporter
k	Kilo (10^3)
KIRC	K^+ inward rectifying channel
KORC	K^+ outward rectifying channel
l	Litre
LCT	Low-affinity cation channel
LSC	Liquid scintilation counter
m	Meter
m (prefix)	Milli (10^{-3})
M	Molar
min	Minutes

MPa	Mega Pascal
MOPS	3-(N-morpholino) ethane sulfonic acid
n	Amount of substances
n	nano (10^{-9})
NCBI	National Center for Biotechnology Information
NSCC	Nonselective cation channels
OD	Optical density
PAR	Photosynthetically active radiation
PCR	Polymerase chain reaction
PS	Photosystem
PVPP	Polyvinylpolypyrrolidone K 30
qRT-PCR	Quantitative real time polymerase chain reaction
RGR	Relative growth rate
RNA	Ribonucleic acid
Rnase	Ribonuclease
rpm	Rotations per minute
RT	Room temperature
s	Second
SD	Standard deviation
SDS	Sodium dodecyl sulfate
SOS	Salt overly sensitive
SSTE	Sodium chloride SDS Tris HCl EDTA
t	Time
Taq	*Thermus aquaticus*
tRNA	Transfer ribonucleic acid
Tris	Tris-(hydroxymethyl)-amino methane
URL	Uniform resource locator
v	Velocity
V	Volume
W	Watt

1 Introduction

1.1 Soil salinity in the environment

Salinization is a problem for agriculture worldwide, occurring in almost all climatic regions from the humid tropics to the polar regions and on all populated continents (Singh and Chatrath 2001). The total area of saline soils is around 397 million ha and that of sodic soils is around 434 million ha of land worldwide which is more than 6 % of the world's total land area (FAO 2005). Every minute three hectares of arable land worldwide are lost because of soil salinity (FAO 2005). The problem of salinization is increasing often due to bad agricultural practise (Tester and Davenport 2003), leading to inefficient resource management. Since soil salinization is the major reason of land degradation, salinity is one of the main problems for agriculture nowadays. This work focuses on NaCl as the cause of salinity. For this reason, the word salinity will interchangeably used with NaCl in this thesis. Pitman and Läuchli (2002) proposed that solutions to soil salinization are of two kinds: one being the "engineering" of plants to increase their salt tolerance, the other engineering the environment, thus implementing a sustainable agricultural management. Because of the growing population worldwide and its increasing food demand, sustainable land use and efficient resource management are major challenges for agriculture practises to fulfil the enhancing demand on food production.

Improved practises are necessary because the replacement of perennial, deep-rooted native vegetation by annual crops is often a reason for secondary salinization (Munns *et al.* 2005). Secondary salinization can result from human activities that interfere in the water balance in the soil between applied water and water used by crops (Munns 2005) and resulting in rising water tables. If the ground water is saline, salt reaches the soil surface and accumulates as water evaporates (Munns *et al.* 2005). Because this process cannot be stopped by cultivating annual, shallow-rooted crops, a strategy against salinization is to plant deep-rooted, salt tolerant trees (Pitman and Läuchli 2002). In combination with high transpiration rates, planting trees can eventually lead to lower water tables in the environment (Munns *et al.* 2005). For this reason, there is a strong interest in the salinity tolerance of trees and the improvement of this trait to use trees for the reclamation of land that has become saline (Munns *et al.* 2005).

1.2 Salt tolerance in poplar

The genus *Populus* L. is a member of the *Salicaceae*. Poplars are distributed in many climatic areas and adapted to diverse conditions, resulting in a rich source of variation in tree morphology, anatomy, physiology and response to biotic and abiotic stress (Bradshaw *et al.* 2000). Combined with its rapid growth, its small genome and other factors (Bradshaw *et al.* 2000), *Populus* was chosen as a model tree for angiosperm trees. In 2005, the poplar genome project (http://genome.jgi-psf.org/Poptr1/Poptr1.home.html) released the DNA sequence of *Populus trichocarpa* (Tuskan *et al.* 2006), a species chosen because of its economic significance.

Populus x *canescens* is a hybrid poplar of *Populus tremula* and *Populus alba*. In previous experiments it was shown that *P.* x *canescens* is a salt sensitive poplar species (Bolu and Polle 2004). Since *P.* x *canescens* was the first tree that was transformed, it is often used as a model tree for molecular analysis.

Populus euphratica was named by OLIVIER after its occurrence at the Euphrat River (Schütt 2000). Naturally, *P. euphratica* covers semiarid areas in Asia and Africa (Wang *et al.* 1996). It can be found in Kazakhstan, Mongolia, Iran, as well as in China. Because of its NaCl tolerance, *P. euphratica* is able to grow in saline environment, but its distribution is restricted to river-banks or areas with deep water tables (Hukin *et al.* 2005).

Cevera *et al.* (2005) showed in a phylogenetic analysis of *Populus* species that *P. euphratica* and *P.* x *canescens* are phylogenetically different (value: 0.37 ± 0.02) based on AFLP-GS (amplified fragment-length polymorphism – genetic similarity) and at different ends of the dendogram. In this dendogram, *P. trichocarpa* is placed in between both (value: *P. euphratica*: 0.48 ± 0.03; *P.* x *canescens*: 0.44 ± 0.04, both compared to *P. trichocarpa*).

P. euphratica was used for afforestation in semi-arid areas of India and China (Fung *et al.* 1998) where it functions as a sand stabilizer (Fung *et al.* 1998). In addition to its function for reforestation, its wood is used for house construction and as fuel wood and its leaves are used as fodder (Schütt 2000).

It was recently shown that *P. euphratica* had considerably lower rates of Na^+ net root uptake and Na^+ transport to the shoot under salinity, compared to NaCl sensitive *Populus tomentosa* (Chen *et al.* 2003) and that *P. euphratica* is more sensitive in reacting to soil salinity than other poplars (Chang *et al.* 2006). Gene expression analysis in *P. euphratica* showed that its genome does not contain different genes, compared to the sequenced *P. trichocarpa*, but that the regulation of gene expression is different in response to salinity compared to *P.*

trichocarpa (Brosche *et al.* 2005). In response to soil water deficit less than 1.5 % of the genes on the array displayed significant changes in transcript levels (Bogeat-Triboulot *et al.* 2007). Because of these attributes, *P. euphratica* is used as a model tree for NaCl tolerance in trees. However, little is known about the adjustments in response to salinity in roots of *P. euphratica*, the primary site of NaCl uptake, and the distribution of Na^+ within the plant.

1.3 Na^+ toxicity in plants

For most plants, sodium (Na^+) is not an essential nutrient. Only few C4 photosynthetic plants require Na^+ for their enzyme synthesis and activity (Flowers *et al.* 1977, Marschner 1995). The growth of many plants is increased at a low Na^+ concentration in the soil, because Na^+ might function as an osmoticum in cell vacuoles and it can reduce the plants need for potassium (K^+) (Marschner 1995).

1.3.1 General strategies against Na^+ toxicity

Plants are divided in two categories, depending on their behaviour under salinity: the halophytes and the glycophytes respectively. Halophytes are salt tolerant plants that can cope with salt concentrations in the soil solution higher than 400 mM NaCl. In contrast, glycophytes are salt sensitive plants, often showing growth reduction at low NaCl concentrations (Flowers *et al.* 1977).

Greenway and Munns (1980) divided plants in “excluder” and “includer”, depending on their strategy to cope with salinity. Typical excluders try to avoid high internal Na^+ and Cl^- concentrations by preventing the uptake of external NaCl. Enhanced synthesis of organic solutes and a decreased Na^+ root influx are, e.g., adaptation processes in these plants. In contrast, includers accumulate larger amounts of Na^+ and Cl^-. An increase in tissue tolerance, for example by Na^+ compartmentation in vacuoles or synthesis of compatible solutes, as well as an avoidance of high ion concentration by an enhanced translocation of toxic ions in the plant are mechanisms of typical includer.

1.3.2 Effects of Na^+ toxicity on plants

Under salinity plants have to deal with two effects: first, the plants ability to take up water is impaired, due to high external concentrations of Na^+ and Cl^- in the soil solution. This is called the osmotic effect. Second, Na^+ and Cl^- ions enter the plant through the transpiration stream leading to toxic internal Na^+ and Cl^- concentrations. This has been called the salt-specific or ion-excess effect (Munns 2005, Munns *et al.* 2005).

Under salinity, the plant's ability to take up water is reduced, because the osmolarity of the external solution is higher than that of the plant-internal water potential. To maintain water uptake, plants need to keep the internal water potential below the potential of the external solution. As the osmotic potential of the external solution is decreasing with increasing salt concentration, plants face water stress, leading to inhibition of water uptake. Under these conditions the rate of cell expansion is also decreased, because turgor pressure is a driving force for cell expansion (Xiong and Zhu 2002). This effect can diminish the growth of leaves (Hu and Schmidthalter 1998) and roots (Jeschke 1984), though the ion concentration in the growing cells is below toxic concentrations. Stomatal conductance and thereby photosynthesis are also decreased, due to osmotic stress (Munns 1993). Additionally the uptake of nutrients, such as P, Fe and Zn and the growth of mycorrhizal fungi can be inhibited by the osmotic effect (Tester and Davenport 2003).

As Na^+ and Cl^- ions reach the plants shoot through the transpiration stream and mass flow, these ions accumulate in the shoot of plants, inducing the salt specific effect (Munns 2005). Especially in old leaves, the concentration of Na^+ ion is increasing, because of continued Na^+ transport into transpiring leaves (Munns 2005). If the transport of Na^+ to the leaves is continued, this will lead to an increase in the cytosolic Na^+ concentration and a decrease in the cytosolic K^+/Na^+ ratio, due to interactions between Na^+ and K^+ ions. Low cytosolic K^+/Na^+ ratio inhibit the activity of enzyme in the cytoplasm, where more than 50 enzymes are activated by K^+ (Bhandal and Malik 1988). Protein synthesis is also disturbed by high Na^+ concentration in the cytoplasm (Blaha *et al.* 2000). High K^+ concentrations are necessary for binding of tRNA to ribosomes, and this may be prevented in the case of high cytosolic Na^+ concentrations. Na^+ ions can also accumulate in the cell wall, leading to dehydration of the cell (Munns and Passioura 1984, Flowers and Yeo 1986, Munns 2002). Necrosis in leaves of plants can occur with ongoing salinity, leading to leaf death. Growth and yield reduction is a result of this process, which may lead to plant death, as the development of new leaves cannot compensate the loss of older leaves (Munns 2005).

1.3.3 The movement of Na^+ in the plant

Na^+ influx into the root from the environmental solution and into the cytoplasma of cortical cells is passive (Cheeseman 1982, Apse and Blumwald 2007) and the exclusion of Na^+ from the initial entry is suggested to be important for plant salt tolerance (Schubert and Läuchli 1990) (Fig. 1.1). Plants exclude between 94 to 98 % of Na^+ in the soil solution from entry into the plant (Munns 2005).

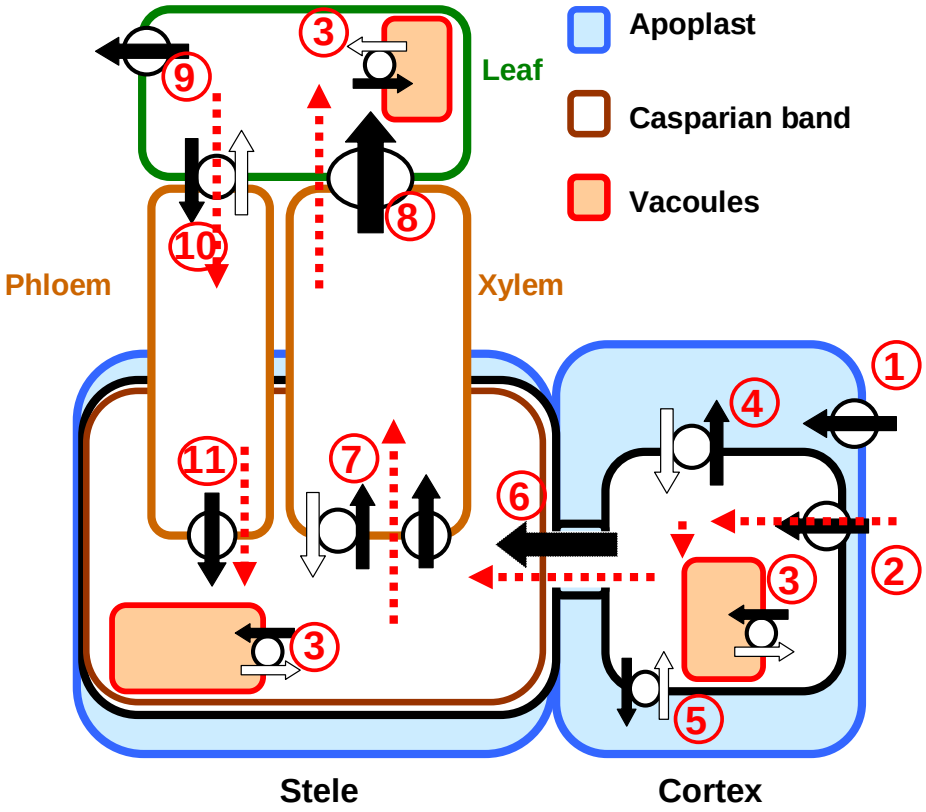

Fig. 1.1: Schemes of Na^+ movement through the plant. (1) Na^+ diffuses into the apoplast. (2) Under salinity, Na^+ enters the plant cell through different channels (NSCCs, KIRCs, KORCs, HKTs, LCP1) into cortical cells. To maintain low cytosolic Na^+ concentration, the ions are (3) incorporated into vacuoles via NHX-type transporters, (4) transported into the apoplast using Na^+/H^+ antiporter or (5) plasma-membrane transporter (SOS1). (6) The Casparian band protects the stele against ion leakage and functions in a higher selectivity of Na^+ transport into and out of the stele. (7) Na^+ is transported into the xylem via a plasma-membrane transporter (SOS1, NSCCs, HKTs) or "leaks" into the xylem. (8) Na^+ xylem unloading into the leaves is supposed to be passive, because of the electrochemical gradient, or Na^+ can be transported via Na^+ selective uniports (HKT1;1, Nax2, NSCCs). (9) The exclusion of Na^+ through salt glands is observed for few halophytes. (10) The mechanism of Na^+ phloem loading and (11) Na^+ phloem unloading are both unknown, but HKT1;1 is supposed to function in this process (modified after Davenport *et al.* 2007, Apse and Blumwald 2007).

High external Na^+ concentrations lead to electrochemical potential differences between the plant tissue and the external solution. This will favour the passive transport of Na^+ from the environment through the symplast of Na^+ into cortical cells (Apse and Blumwald 2007) and the Na^+ influx into the apoplast and into cortical cells (Yeo *et al.* 1987). It has been suggested that this apoplastic "leakage pathway" contributes to Na^+ uptake under salinity, although its mechanism is not fully understood and its significance for Na^+ uptake varies between plant species (Yeo and Flowers 1985, Garcia *et al.* 1997).

The bulk of Na^+ that enters the cell is transported through nonselective cation channels (NSCCs) that are located in the plasma membrane (Amtman and Sanders 1999, Tester and Davenport 2003, Horie and Schroeder 2004). Additionally, Na^+ uptake is attributed to low Na^+ permeability through K^+ transport systems (Amtman and Sanders 1999, Maathius and Amtman 1999). Examples for these transport systems are inward rectifying K^+ channels (KIRCs) and outward rectifying K^+ channels (KORCs) (Maathius and Amtman 1999, Blumwald *et al.* 2000). High-affinity K^+ transporters (HKTs) and low-affinity cation transporter (LCT1) also contribute to cellular Na^+ influx (Amtman and Sanders 1999, Maathius and Amtman 1999, Tyerman and Skerrett 1999, Tester and Davenport 2003, Munns 2005). The role of each type of transporter can vary within species and growth conditions (Apse and Blumwald 2007). Because there is a lower initial entry of Na^+ into roots of halophytes compared to glycophytes it is suggested that halophyte plants are more effective in controlling initial entry of Na^+ (Cheeseman *et al.* 1985) and in regulating Na^+ transport than glycophytes (Tester and Davenport 2003). In glycophytes, the influx of Na^+ ion has been described as an "accidental" process (Demidchik *et al.* 2002) because transport mechanisms are often not Na^+ selective.

For plant cells it is crucial to maintain low cytosolic Na^+ concentration under salinity. However, it is unknown how the salt is sensed. Plasma membrane proteins, ion transporters or Na^+ sensitive enzymes have been hypothesized as sensors of toxic Na^+ concentrations in extracellular and intracellular sites (Sairam *et al.* 2006). One way to establish low cytosolic concentration is to incorporate Na^+ in vacuoles of the cell. This process is catalysed by vacuolar Na^+/H^+ antiporters, whose expression is increased by the addition of Na^+ (Blumwald *et al.* 2000). These vacuolar Na^+/H^+ antiporters are members of the NHX-type gene family of transporter located at the tonoplast membrane (Apse *et al.* 2003). It was shown that an overexpression of the vacuolar Na^+/H^+ antiporter NHX1 increased salt tolerance of *Arabidopsis* (Apse *et al.* 1999) and tomato plants (Zhang and Blumwald 2001).

As Na^+ is accumulated in the vacuoles under salinity, the osmotic potential in the cytoplasm must be balanced, which is achieved by organic solutes accumulation (Hu *et al.* 2000). An advantage of this osmotic adjustment is that many organic solutes act as so-called compatible solutes as they do not inhibit biochemical reaction, in contrast to the osmotic adjustment established by ions. A disadvantage is the high energy costs for the production of organic solutes (Yeo 1983).

Another way to establish low cytosolic Na^+ concentration is the Na^+ transport out of the cell into the apoplast. Under salinity root cells accumulate lower concentrations of Na^+ than shoot cells and tend to maintain constant Na^+ root concentration under salinity (Tester and Davenport 2003). To prevent the cells from high Na^+ concentration, a huge efflux of Na^+ from root cells under salinity is postulated (Munns 2005). Na^+ efflux from cells is a process that is probably catalysed by Na^+/H^+ transporters (Blumwald *et al.* 2000). Na^+ efflux from cells takes place against the electrochemical potential.

Salinity inducible transporters may also contribute to keep the cytosolic Na^+ concentration low. Rising cytosolic Na^+ activates a cytoplasmic Ca^{2+} signalling cascade (Zhu 2000). This signalling cascade starts activating salt overly sensitive (SOS) genes leading to upregulation of ion transporters. The SOS pathway regulates the expression level of SOS1, a gene encoding a plasma membrane Na^+/H^+ antiporter (Shi *et al.* 2000). Reconstitution of a NaCl sensitive yeast strain with antiporter rescued its NaCl tolerance. This indicates a role of SOS1 in Na^+ transport (Shi *et al.* 2002). Reconstitution of salt-sensitive *E. coli* with NHD1, another Na^+/H^+ antiporter, also increased growth under NaCl stress conditions (Ottow *et al.* 2005 a). In *Arabidopsis*, SOS1 is proposed to function for either Na^+ efflux or Na^+ influx into the cell, depending on Na^+ concentration in the environmental solution (Shi *et al.* 2002, Tester and Davenport 2003). Yang *et al.* (2007) proposed that Na^+ movement across vesicle membranes highly depends on H^+-ATPase activity.

The apoplastic transport of Na^+ into the stele is blocked by the Casparian band resulting in a high selectivity for ion transport in and out of the stele. In general, Na^+ levels in *P. euphratica* decreased inwards from cortex to the stele (Chen *et al.* 2002, Chen *et al.* 2003) but showed no differences in *P.* x *canescens* (Langenfeld-Heyser *et al.* 2007). In some halophytes, this physical barrier against Na^+ leakage into the stele is important, resulting in an increased Casparian band width in response to salinity (*Suaeda monoica* and *Suaeda fruticosa*: Poljakoff-Mayber 1975; *Suaeda maritima*: Hajibagheri *et al.* 1985).

The process of xylem loading is suggested to be important for plant salt tolerance (Termaat and Munns 1986). In a wide range of species, salt tolerance is correlated with the plants

ability of controlling xylem loading and thus excluding Na^+ from entry into the xylem, respectively into the shoot (Gorham *et al.* 1990, Schachtmann *et al.* 1992, Santa-Maria and Epstein 2001, Zhu *et al.* 2001). There is evidence for a passive Na^+ xylem loading. When plants transpire, the xylem sap has a lower electrochemical potential than the surrounding stelar cells. This would favour a passive transport of Na^+ into the xylem (Munns 1985).

In sparsely transpiring plants, Na^+ xylem loading from stelar cells is active (Tester and Davenport 2003). Na^+ concentrations in the xylem sap are similar to that found in the cytoplasm of root cells (Tester and Davenport 2003). Therefore, electrochemical differences are mainly influenced by differences in the pH values in both tissues. As stelar cells have a pH around 7 and xylem sap has a lower pH, the electrochemical potential is negative in stelar cells relative to the xylem sap (de Boer 1999, Tester and Davenport 2003). Thus, there must be an active Na^+ xylem loading against the electrochemical potential. In *Arabidopsis*, the Na^+/H^+ transporter SOS1 may play a role in active Na^+ xylem loading (Shi *et al.* 2002). "Leakage" of Na^+ into the xylem has also been proposed. Although the Casparian band plays an important role in preventing Na^+ ions from apoplastic influx into the root stele (Sairam *et al.* 2006), Na^+ can enter the stele and therefore the xylem through leaks in the endodermis (Yeo *et al.* 1999). Although the importance of Na^+ xylem loading is unquestionable, its mechanism and its control are unknown (Tester and Davenport 2003).

Before Na^+ reaches the leaves with the transpiration stream, unloading of Na^+ from the xylem sap is possible, although the mechanism of this removal is unknown. Apse and Blumwald (2007) suggested the operation of a Na^+/H^+ antiporter in xylem unloading. Different sites for this unloading are possible such as mature roots, the base of the shoot or the mature extended shoot (Tester and Davenport 2003). Davenport *et al.* (2007) showed that *AtHKT1;1* is responsible for the retrieval of Na^+ from the xylem respectively Na^+ xylem unloading. The significance of Na^+ xylem unloading for plant NaCl tolerance is unknown, although it is clear, that an exchange between xylem and phloem occurs and plays a role in the distribution of Na^+ and K^+ within the plant (Wolf *et al.* 1991).

Na^+ xylem unloading in the leaves of plants supposed to be passive (Tester and Davenport 2003). Because of electrochemical differences, Na^+ is removed from the xylem mass flow passively. This transport can be conducted by Na^+ selective uniport (Nax2) or by NSCCs (Apse and Blumwald 2007). Na^+ accumulates mainly in older leaves of plants under salinity, since older leaves have a higher Na^+ and Cl^- concentration compared to younger leaves at any given time (Colmer *et al.* 1995). The protection of young leaves is proposed to be crucial for salt tolerance of plants because in these cells the number of vacuoles is lower compared to

mature leaves. This leads to a decrease of storage capacity for sequestering Na^+ ions (Jeschke 1984). Additionally, increased Na^+ concentrations disturb protein synthesis processes that are intensified in growing tissue, respectively young leaves (Tester and Davenport 2003). Na^+ distribution in the plant through the transpiration stream is not the only mechanism of Na^+ transport. Other Na^+ transport mechanism must occur, since Wolf *et al.* (1991) described a transport of Na^+ towards target leaves in barely protecting special shoot areas. This leads to a non-uniform distribution of Na^+ and K^+ within the shoot under salinity whereas young leaves are protected from high Na^+ concentration.

For a few species, a recirculation of Na^+ from the leaves in the phloem has been reported (lupine: Munns *et al.* 1988, sweet pepper: Blom-Zandstra *et al.* 1998, maize: Lohaus *et al.* 2000). Perez-Alfocea *et al.* (2000) described an increased recirculation of Na^+ in the phloem in salt tolerant tomato species compared to salt sensitive species. For NaCl tolerant reed (*Phragmites communis*), an increased Na^+ transport from the leaves via the phloem was observed compared to rice (Matsushita and Matoh 1991). Berthomieu *et al.* (2003) discussed a function of *AtHKT1;1* in the recirculation of Na^+ from the shoot into the phloem and an unloading via the roots and Rus *et al.* (2006) suggested a role of HKT1;1 in the regulation of Na^+ homeostasis. A recent publication by Davenport *et al.* (2007) questioned HKT1;1's function in Na^+ recirculation. The significance of recirculation for plant NaCl tolerance is unknown or seems to be too low to contribute to salt tolerance (Tester and Davenport 2003, Davenport *et al.* 2007). Whether Na^+ recirculation in *P. euphratica* and *P.* x *canescens* contributes to their NaCl tolerance is unknown and needs to be determined.

Another strategy against salinity is the development of leaf succulence (Tester and Davenport 2003). Some plants respond to high NaCl concentration with an increased number of cells or cell volume in leaves, leading to an increase in leaf water content per unit area. Because of this, leaves sequester large amounts of solutes without adversely increasing cell osmotic pressure (Suarez and Sobrado 2000). Ottow *et al.* (2005 b) described the development of leaf succulence and the apoplastic Na^+ accumulation in response to salinity in *P. euphratica*.

Finally, some halophytes posse salt glands to increase their salt tolerance (Flowers *et al.* 1977). Salt glands are microhair-based glands existing of just two cells (Marcum 1999). These cells contain Na^+ extruding pumps in the plasma-membrane, pumping Na^+ into the apoplast of the hair and onto the surface of leaves. The mechanism helps to establish a steady balance of Na^+ in leaves of halophytes (Ball 1988). Mangrove trees, salt cedar (*Tamarix* spp.), salt bush (*Atriplex* spp.) (Atkinson *et al.* 1967) and *Populus euphratica* (Lichtenberg 2006) are plants using this strategy.

Since both poplar species differ in their response to salinity, Na^+ movement and Na^+ distribution in *P. euphratica* and *P.* x *canescens* needs to be analysed to determine differences in Na^+ uptake and Na^+ transport between both species functioning in NaCl tolerance.

1.4 Significance of calcium in plant nutrition and salt tolerance

1.4.1 Ca^{2+} functions and transport in plants

Calcium (Ca^{2+}) is an essential element in all plants (Marschner 1995). Ca^{2+} concentrations in the cytoplasm are very low (Clarkson and Hanson 1980, Marschner 1995, Cramer 2002). This is a precondition for Ca^{2+} acting as a second messenger in plant cells. Environmental signals can activate calcium channels in the plasma membrane that mediate Ca^{2+} efflux from the cytoplasm and Ca^{2+} influx and thereby influence cytosolic Ca^{2+} concentration (Bush 1995, Marschner 1995). In plant cells, calcium is most commonly stored in the endoplasmatic reticulum and in the vacuoles (Marschner 1995). Under high calcium supply, Ca^{2+} is bound as calcium oxalate crystals in the vacuoles and in the apoplast to maintain low cytosolic free Ca^{2+} concentration (Marschner 1995). Additionally, calcium and oxalate can act as a counter ion for inorganic and organic anions in the cation-anion balance within the cell (Kinzel 1989). Calcium enters plants cells through Ca^{2+} permeable ion channels in the plasma membrane (White 1998, White and Broadley 2003). The removal of Ca^{2+} from the cytosol is catalysed by Ca^{2+}-ATPase and H^+/Ca^{2+} antiporter (White and Broadley 2003). These channels are required to keep free cytosolic Ca^{2+} at very low concentration (Bush 1995, Marschner 1995). Ca^{2+}-signalling is involved in activation of stress acclimation. Environmental stress triggers a signal transduction pathway which activates Ca^{2+} channels. This results in increasing cytosolic free Ca^{2+} concentrations that are supposed to be an universal response to stress. Free cytosolic Ca^{2+} acts through Ca^{2+}-modulated proteins (Bush 1995) known as calmodulins (CaM) or calcineurin B-like proteins (CBLs) (Knight and Knight 2001). Changes in cytosolic Ca^{2+} concentration can initiate different cell processes like cell division (Bush 1995) or initiate the plants response to drought (Knight *et al.* 1997) or salinity stress (Halperin *et al.* 1997, Knight *et al.* 1997).

Furthermore, significant amounts of apoplastic Ca^{2+} are bound to the cell walls, at the exterior surface of the plasma membrane and in the middle lamella. In plant cells, Ca^{2+} is a structural

component related to its capacity for coordination of intermolecular linkages, mainly in cell walls and at the plasma membrane (Marschner 1995). It strengthens cell wall structures and is a major cation in the protein-pectin "cement" (Clarkson and Hanson 1980) where it is exchangeable bound to R-COO groups (Clarkson and Hanson 1980, Marschner 1995). Therefore, a typical symptom of calcium deficiency in plant tissue is the disintegration of cell walls, leading to collapse of the affected tissues (Marschner 1995). Ca^{2+} also plays a structural role in membrane integrity (Cramer *et al.* 1985).

Ca^{2+} uptake from soil solution by the plant occurs through the roots. Ca^{2+} uptake is restricted to the extreme root tip and to regions in which lateral roots are being initiated (White 2001) while Ca^{2+} delivery to the xylem is maximal in the apical zone of the root (White 2001).

The delivery of Ca^{2+} to the xylem is not completely understood. It has been suggested that both apoplastic and symplastic pathways contribute to the Ca^{2+} delivery to the xylem (White and Broadley 2003), but the relative contributions of both pathways are unknown (White 2001). One pathway for Ca^{2+} transport to the xylem is the symplastic transport. Plants transport Ca^{2+} to the xylem symplastically through Ca^{2+} permeable channels, Ca^{2+} ATPase or Ca^{2+}/H^{+} antiporters (White 1998, de Boer 1999, White 2000). The Casparian band is present in the apical zone of the root and restricts the apoplastic Ca^{2+} movement to the xylem (Clarkson 1984, Marschner 1995, White 2001). Therefore, Ca^{2+} must bypass the Casparian band symplastically. As there is a significant effect of transpiration on Ca^{2+} delivery to the shoot (Lazaroff and Pitman 1966, Marschner 1995), it has been suggested that Ca^{2+} can also enter the stele via the apoplastic pathway (White 2001). This may take place in regions where the Casparian band is absent or disrupted or it takes place through unsuberized endodermal cells (White and Broadley 2003). In the xylem sap Ca^{2+} is translocated upward with the transpiration stream (Mengel and Kirkby 1992). As Ca^{2+} is immobile in the phloem, it cannot be mobilized from older tissue and redistributed in the plant (Mengel and Kirkby 1992, Marschner 1995, White and Broadley 2003). Therefore, Ca^{2+} xylem transport and Ca^{2+} tissue accumulation mainly depends on the transpiration of the respective organ (Marschner 1995, White and Broadley 2003).

1.4.2 Na^+/Ca^{2+} interactions

The ameliorative effects of Ca^{2+} on Na^+ toxicity and its positive effect on plant growth under salinity have been described for many plants (Epstein 1961, Greenway and Munns 1980, Läuchli and Schubert 1989), but differences in the response to supplemental Ca^{2+} under salinity for different genotypes were observed in rice (Yeo and Flowers 1985), maize (Maas and Grieve 1987) or sorghum (Grieve and Maas 1988), respectively.

There is strong evidence that the tight Na^+/Ca^{2+} interaction is the result of similar crystal ionic radii, which is 0.099 nm for Ca^{2+} and 0.097 nm for Na^+ (Allen *et al.* 1994, Cramer 2002). Therefore, excess Na^+ can outcompete Ca^{2+} transport into cells through ion channels that are permeable to both ions (White 1998). Na^+ uptake and Na^+ concentrations increase in plant tissue and plant cells and Ca^{2+} uptake and Ca^{2+} concentrations decrease in response to increasing external NaCl concentrations (Rengel 1992, Cramer 1997, Lazof and Bernstein 1999, Cramer 2002). Under salinity, the inhibition of Ca^{2+} root uptake results in a decrease of Ca^{2+} xylem loading and Ca^{2+} shoot concentration, whereas Na^+ shoot uptake and Na^+ shoot concentration in plant tissue increase (Rengel 1992, Amtmann and Sanders 1999, Zhu 2001).

Since Ca^{2+} influx is reduced under salinity (Lynch and Läuchli 1985 and 1988, Cramer *et al.* 1987, Halperin *et al.* 1997), Ca^{2+} deficiency can occur under high external Na^+/Ca^{2+} ratios (Maas and Grieve 1987, Fortmeier and Schubert 1995, Cramer 2002) resulting in decreased Ca^{2+} content in growing tissue under salinity (Lazof and Bernstein 1999). Na^+/Ca^{2+} interactions also take place at plant cell walls where Na^+ and Ca^{2+} are competing for negatively charged binding sites that have a high specificity for Ca^{2+} (Zid and Grignon 1985, Grignon and Senetenac 1991, Munns 2005). With increasing Na^+ concentration Na^+ is replacing Ca^{2+} at these binding sites leading to a decrease in cell turgor of plant cells and results in plant injury under salinity (Oertli 1968, Flowers *et al.* 1991, Munns 2005). However, exceptions have been reported. It was shown that the leaf apoplast of *P. euphratica* was the main site of Na^+ accumulation and that the leaf cells remained turgescent. This showed that the Na^+ fraction in the leaf apoplast was not active as an osmolyte leading to cell dehydration (Ottow *et al.* 2005 b).

LaHaye and Epstein (1971) proposed, to use the ability to transport Ca^{2+} under salt stress to the shoot as an index of salt tolerance in plants. Similar to that, Lynch and Läuchli (1985) and Unno *et al.* (2002) suggested that the maintenance of Ca^{2+} accumulation and Ca^{2+} distribution to the shoot under salinity are important factors associated with plant salt tolerance. Whether

the ability to transport Ca^{2+} to the shoot under salinity is the reason for differences in NaCl tolerance in poplar is unknown and needs to be investigated.

1.5 Aim of this work

The main objective of this work was to analyse the uptake, transport and allocation of Na^+ in salt tolerant poplar species *P. euphratica* in comparison with the salt sensitive species *P.* x *canescens*. Since trees may have to cope with saline conditions for extended periods of time, the focus of the present study was on the analysis of transport processes in NaCl-adapted plants.

To characterise the performance and adaptation of *P. euphratica* and *P.* x *canescens* in response to increasing salinity, growth parameters were determined in both poplar species during NaCl treatment.

As the maintenance of Ca^{2+} transport under salinity is crucial for plant NaCl tolerance, the distribution and accumulation of Ca^{2+} in both poplar species exposed to increasing external NaCl was determined, using image analysis of radioactive $^{45}Ca^{2+}$ in whole plants.

Radioactive $^{22}Na^+$ was applied to measure Na^+ uptake and its xylem transport rate. In a leaf feeding experiment Na^+ phloem transport rates and Na^+ root exudation were determined. Since HKT1;1 has been proposed to play a major role for Na^+ transport, transcript level of HKT1;1 were measured in roots and bark of both poplar species under salinity and control conditions.

During salt adaptation, modifications of the roots morphology of *P. euphratica* but not of *P.* x *canescens* were observed. The function of these novel organs for salinity tolerance were tested. For this purpose, morphological and anatomical measurements of *P. euphratica* roots treated with NaCl were performed. NaCl was also replaced by KCl, or plants were exposed to salinity under Ca^{2+} nutrient deficiency to analyse the Na^+ specificity of this effect. To investigate the influence of this modification in root morphology on the plants Na^+ uptake, the uptake of radioactive $^{22}Na^+$ in NaCl adapted and non-adapted root tips was determined. Based on the data, Na^+ uptake, xylem transport, shoot allocation and recirculation were compared for *P. euphratica* and *P.* x *canescens*.

2 Material & Methods

2.1 Chemicals

All chemicals were obtained from Merck (Darmstadt, Germany) with the exception of the following chemicals:

Tab. 2.1: List of chemicals

$^{45}CaCl_2$	PerkinElmer Life Science, Austin, Texas, USA
$^{22}NaCl$	Amershan Biosciences, Buckinghamshire, UK
Agarose	Cambrex, Rockland, USA
Bromphenol blue	Carl Roth GmbH & Co. KG, Karlsruhe, Germany
Chloramphenicol	SERVA Electrophoresis GmbH, Heidelberg, Germany
Hexadecyltrimethylammonium bromide (CTAB)	Carl Roth GmbH & Co. KG, Karlsruhe, Germany
Ethylene diamine tetra acetic acid (EDTA)	Sigma-Alderich Chemie GmbH, Munich, Germany
Ethanol	GeReSO mbh, Einbeck, Germany
Ethidiumbromid	Carl Roth GmbH & Co. KG, Karlsruhe, Germany
Formaldehyde	Fluka, Buchs, Switzerland
Formamid	SERVA Electrophoresis GmbH, Heidelberg, Germany
Gelrite	Duchfa Biochemie B.V., Haarlem, The Netherlands
β-mercaptoethanol	Carl Roth GmbH & Co. KG, Karlsruhe, Germany
3-(N-morpholino) ethane sulfonic acid (SDS)	Sigma-Alderich Chemie GmbH, Munich, Germany
Inositol	Duchfa Biochemie B.V., Haarlem, The Netherlands
Isopropanol	Carl Roth GmbH & Co. KG, Karlsruhe, Germany
Polyvinylpolypyrrolidone (PVPP)	Sigma, Steinheim, Germany
Roti®-Histol	Carl Roth GmbH & Co. KG, Karlsruhe, Germany
Rotiplast	Carl Roth GmbH & Co. KG, Karlsruhe, Germany
Sodium dodecyl-sulfate (SDS)	Carl Roth GmbH & Co. KG, Karlsruhe, Germany
Sodium chlorid	KMF optiChem, Lohmar, Germany

RNase-free water was obtained from Ambion Inc. (Austin, Texas, USA) and ddH_2O was produced using Satorius arium® 611VF (Sartorius, Göttingen, Germany).

2.2 Cultivation of *Populus euphratica* and *Populus* x *canescens*

Populus euphratica OLIVIER (Clone B2 from "Ein Avdat Region", obtained from Prof. A. Altman, University of Jerusalem) and *Populus* x *canescens* (Clone INRA717 1-B4) were both cloned by *in-vitro* micropropagation as described by Leplé *et al.* (1992) (Fig. 2.1).

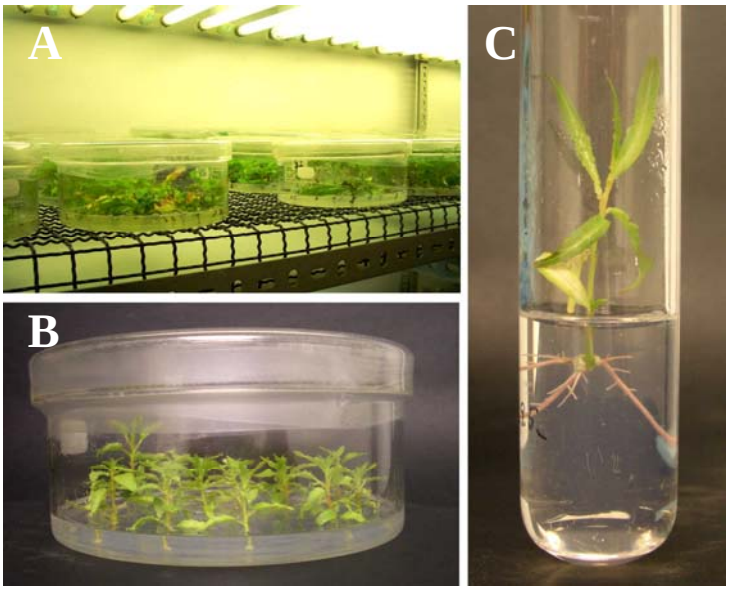

Fig. 2.1: Cultivation of *P. euphratica* and *P.* x *canescens*. Cultivation of *P.* x *canescens* (A, B) plants in plates containing rooting media under sterile conditions. (C) *P. euphratica* plant in a tube containing rooting media.

The plates were closed under sterile conditions and sealed with Parafilm M® (Pechiney Plastic Packaging, Chicago, Illinois, USA). Plants were cultivated under long day conditions (16 h light, 100 µmol PAR m^{-2} s^{-1}, 20 °C) (light: Osram L 18W/640 cool white, Osram, Munich, Germany).

2.2.1 Rooting medium

For *in-vitro* micropropagation of both species rooting medium was prepared to stimulate the development of roots. 100 ml of Macro nutrients stock solution, 1 ml of each Micro nutrients respectively Vitamins stock solution and additional nutrients (except the Gelrite) are combined and filled with ddH_2O up to 1000 ml. The pH was adjusted to 5.8. Gelrite was added and the mixture was autoclaved (20 min, 121 °C). The media was filled into the plates under sterile conditions. Cooled and sterile plates were sealed with Parafilm M®.
Small cuttings of 1 - 2 cm length with a single node were placed in plates (Fig. 2.1 A, B) or tubes (Fig. 2.1 C) both containing rooting medium (modified after Murashige and Skoog 1962) (Tab. 2.2).

Tab. 2.2: Stock solution of rooting medium for the cultivation of *P. euphratica* and *P.* x *canescens*. Concentration is noted as mixed in the stock solution. Stock solutions were prepared using ddH_2O.

Macro nutrients		
KNO_3	247.0	mM
$(NH_4)H_2PO_4$	26.1	mM
$MgSO_4$ x 7 H_2O	16.2	mM
$CaCl_2$ x 2 H_2O	13.6	mM
Micro nutrients		
$MnSO_4$ x H_2O	5.9	mM
H_3BO_3	4.9	mM
$ZnSO_4$ x 7 H_2O	1.0	mM
KJ	0.5	mM
Na_2MoO_4 x 2 H_2O	0.1	mM
$CoCl_2$ x 6 H_2O	0.1	mM
$CuSO_4$ x 5 H_2O	0.1	mM
Vitamins		
Nicotinic acid	4.061	mM
Pyridoxine-HCl	2.400	mM
Thiamine-HCl	0.297	mM
Additional nutrients		
$C_{10}H_{12}FeN_2NaO_8$	5.0	ml l^{-1}
Inosit	5.0	ml l^{-1}
Glycin	1.0	ml l^{-1}
Sucrose	25.0	g l^{-1}
Gelrite	2.8	g l^{-1}

Three to 4 weeks after the cultivation in rooting media, *P. euphratica* and *P.* x *canescens* (height: 5 – 7 cm) were transferred into hydroponic solution (1 l pot) of Long-Ashton medium (Hewitt and Smith 1975) (Tab. 2.3).

For the preparation of Long-Ashton medium, three stock solutions were used: Macro nutrients, Micro nutrients and Iron (see Tab. 2.3). Two ml of each stock solution were mixed and filled up to 1 l with ddH_2O. The pH was 5.5.

Plants were cultivated for 2 to 4 weeks under long day conditions (16 h light, 100 µmol PAR m^{-2} s^{-1}, 20 °C) in aerated media. The hydroponic solution was changed weekly.

At the start of the experiments the heights of *P. euphratica* and *P.* x *canescens* ranged from 10 to 28 cm and the fresh mass of the plants from 0.8 to 4.7 g.

Tab. 2.3: Stock solutions for Long-Ashton medium for hydroponic cultivation of *P. euphratica* and *P.* x *canescens*.

Macro nutrients		
$Ca(NO_3)_2$ x 4 H_2O	450	mM
KH_2PO_4	300	mM
$MgSO_4$ x 7 H_2O	150	mM
KNO_3	100	mM
K_2HPO_4	15.8	mM
Micro nutrients		
H_3BO_3	5.0	mM
Na_2MoO_4 x 2 H_2O	3.5	mM
$MnSO_4$ x 4 H_2O	1.0	mM
$ZnSO_4$ x 7 H_2O	0.1	mM
$CuSO_4$ x 5 H_2O	0.064	mM
$CoSO_4$ x 7 H_2O	0.020	mM
Iron		
$C_{10}H_{12}FeN_2NaO_8$	5.0	mM

2.3 Basic experimental set-up

In all experiments, plants were grown in one of two available climate chambers (Typ Z-1800 / 10-45DU-PI, Weiss Technik, Lindenstruth, Germany; or Ernst Schütt jun. Laborgerätebau, Göttingen, Germany).

The day temperature for all experiments was 22 - 25 °C and the air humidity was in the range of 40 – 70 %. All plants were kept under long day conditions (16 h light, 80 - 100 µmol PAR m^{-2} s^{-1}) (light bulb: Osram HQI-T 250W/D and Osram L 18W/640 both Osram, Munich, Germany). Aerated hydroponics containing Long-Ashton media (Tab. 2.3) were used for all experiments. For all experiments, each plant was placed in a 1 l pot, if not described otherwise.

In general, *P. euphratica* and *P.* x *canescens* were adapted to high NaCl concentration by increasing the concentration in weekly steps of 0 mM, 25 mM, 50 mM, 100 mM to 150 mM for *P. euphratica* and of 0 mM, 25 mM, 50 mM and 75 mM NaCl for *P.* x *canescens* (Fig. 2.2). Deviations from this scheme have been noted in the results section as appropriate. Control plants were maintained in aerated hydroponic solution for the same time in absence of added NaCl.

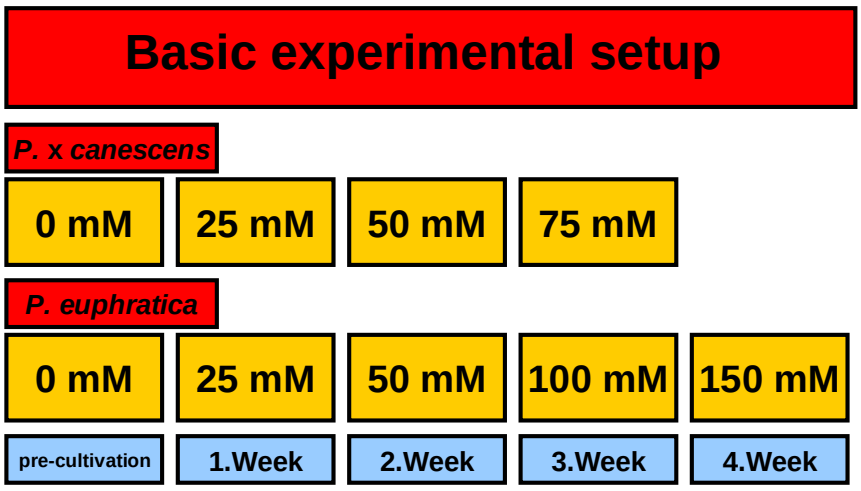

Fig. 2.2: Basic experimental setup for all experiments. The plants were adapted to high NaCl concentration by increasing the weekly external NaCl concentration, starting with 25 mM to final concentrations of 150 mM NaCl for *P. euphratica* and 75 mM NaCl for *P.* x *canescens*, respectively.

2.3.1 Short term $^{22}Na^{+}$ uptake experiments

Two independent experiments were carried out, in which the uptake of $^{22}Na^{+}$ in one single root tip for short exposure times of 1 min – 30 min and 30 min – 8 hours were measured. Five – 8-week-old *P. euphratica* were adapted to 150 mM NaCl as described under 2.3. Afterwards, plants were exposed to their final NaCl concentration for three weeks.

Single root tips attached to the plant were placed each in one Falcon tube (Sarstedt, Nümbrecht, Germany) containing 150 mM NaCl with additional 57 – 76 KBq $^{22}Na^{+}$. The remaining roots were removed from hydroponics container and placed in 1 l pots containing 150 mM NaCl (NaCl adapted) or 0 mM NaCl (control), respectively. After the exposure, the labelled root tip was immediately cut from the main root and washed for 30 s in non-labelled 150 mM NaCl solution to remove surface-bound radioactive label. Digital picture (Minolta Dimage 7, Konica Minolta Holdings Inc., Tokyo, Japan) of the root tip were taken for measurements of the surface area of the root tip (see 2.4.2). Afterwards, the harvested plant tissue was dried at 60 °C for 72 h and measurements of the radioactive tracer (see 2.6.5) were performed.

2.3.2 Split root experiments

For split root experiments, 5 – 8 weeks old *P. euphratica* and *P.* x *canescens* were adapted to high NaCl concentrations for four weeks (*P. euphratica*) or three weeks (*P.* x *canescens*) as described under 2.3.

NaCl adapted plants were placed into the split root system (Fig. 2.3), that was designed for this experiment. The split root system (25 cm x 11 cm area x 8.3 cm height) is divided in three separated chambers (Fig. 2.3 C). The two outer chambers contain nutrient solution, whereas the small, middle chamber (2 x 10 x 8.3 cm) remained empty to avoid transfer of liquid from outer chamber to the other.

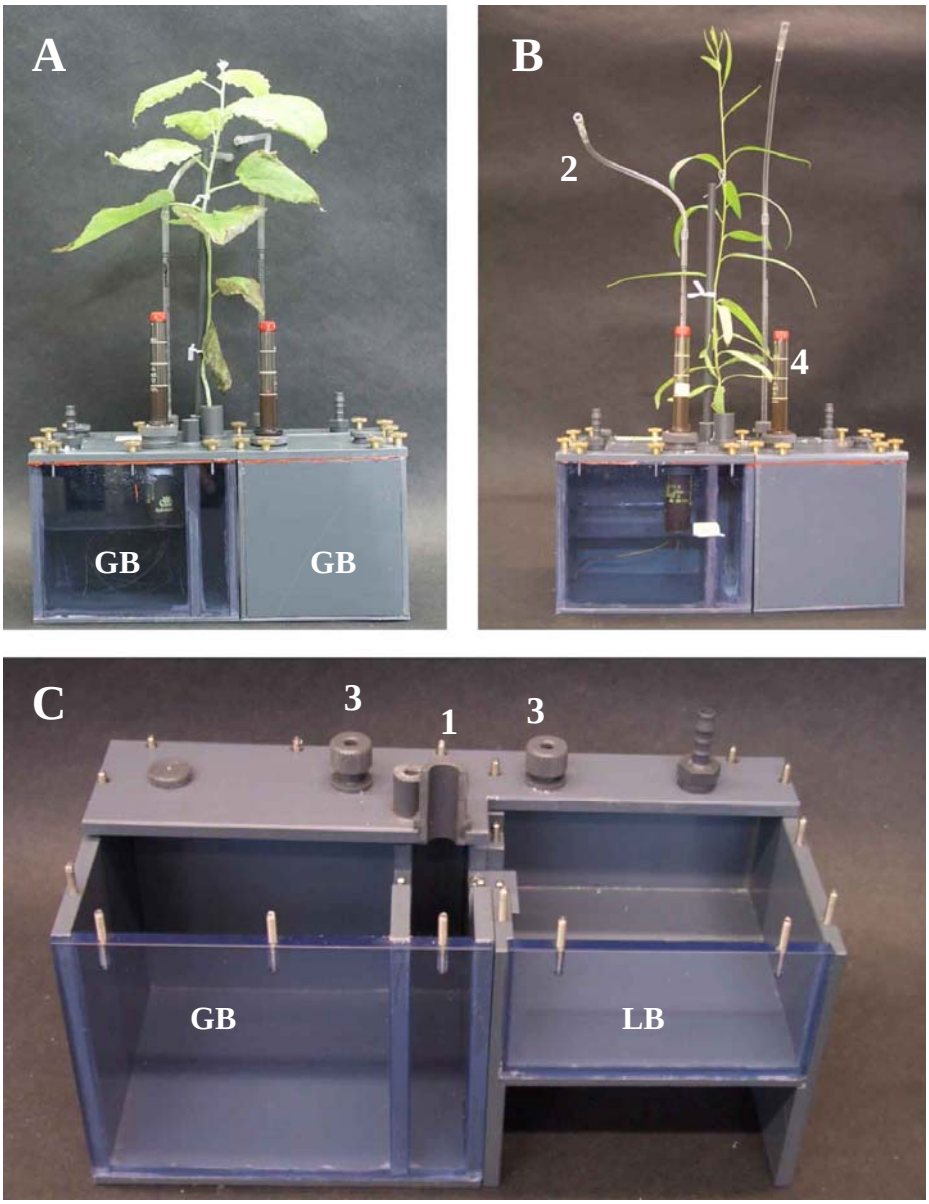

Fig. 2.3: Split root system. (A) *P.* x *canescens* and (B) *P. euphratica* in split root boxes containing hydroponics. (C) Empty split root box. LB is the removable labelling box, which was used to minimize the volume of radioactive labelled solution. It was replaced after labelling by GB, a growth box without a transparent front side (A, B). Plants were placed in the middle opening (1). Roots were aerated using an aeration system (2) through separated holes (3). The loss of water was measured using water level indicator (4).

The growth box (10 x 10 x 8.3 cm) (Fig. 2.3 C, GB) contained 600 ml hydroponics and the smaller chamber LB (10 x 10 x 3.4 cm) 200 ml hydroponics. The small chamber was only used for radioactive labelling and replaced by a growth box afterwards (10 x 10 x 8.3 cm). The split root system was covered by lids to prevent the evaporation of hydroponic solution. The loss of nutrient solution was controlled using the water level indicator (Fig. 2.3 A, B). Roots were aerated using compressed-air through aeration bars (Fig. 2.3 B). The front side of the boxes were transparent to control the growth of the roots (Fig. 2.3 C).

The plants were placed in the middle opening of the lid and the roots were separated from each other, placing one single root into the small, labelling chamber and the main root part into the growth chamber (see Fig. 2.3 C). Radioactive labelling was conducted for 7 days during the week before the final NaCl concentration for each poplar species was reached (*P. euphratica*: 100 mM NaCl; *P.* x *canescens*: 50 mM NaCl). *P. euphratica* were exposed to 185.3 KBq of $^{22}Na^{+}$ and *P.* x *canescens* were exposed to 108.4 KBq of $^{22}Na^{+}$ in the hydroponics. After radioactive labelling, the hydroponic solution was removed and radioactive labelled root tips were washed with non-labelled hydroponic solution, containing the same NaCl concentration, to remove surface-bound radioactive label. The nutrient solution was renewed and the NaCl concentration increased to final concentrations of 150 mM NaCl or 75 mM NaCl for *P. euphratica* or *P.* x *canescens*, respectively. After a chase period of 21 (*P. euphratica*) or 14 days (*P.* x *canescens*), respectively, plants were harvested. The plant shoots were divided into three part (top, middle, bottom) and the roots were divided into two parts (labelled root and non-labelled root). Plant tissue was weighed, immediately dried at 60 °C for 72 h and measurements of the radioactive tracer (see 2.6.5) were performed.

2.3.3 Leaf feeding experiments

For leaf feeding, one single leaf in the middle of the stem of each plant was fixed with modelling clay (“Nakiplast”, Pelikan, Hannover, Germany) onto a Petri dish. Afterwards, the surface of the leaf was rubbed with silicium carbid (“Carborund”, ESK-SIC GmbH, Frechen, Germany) for at least 10 s. Thereby, the surface of the leaf was injured and leaf uptake of $^{22}Na^{+}$ enabled.

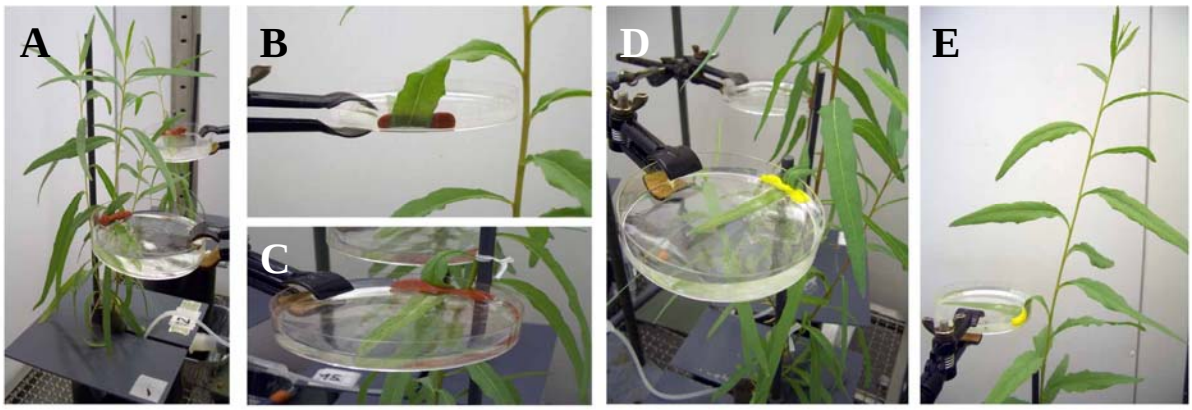

Fig. 2.4: Leaf feeding of *P. euphratica*. Leaves were placed in a Petri dish and fixed with modelling clay (B,C and D). Leaves were exposed to 35 ml $^{22}Na^{+}$ labelled nutrient solution containing 150 mM NaCl or 75 mM NaCl for *P. euphratica* or *P.* x *canescens*, respectively, for 24 h.

35 ml of the nutrient solution with 150 mM NaCl, labelled with radioactive $^{22}Na^{+}$ (7.16 - 28.28 KBq per plant) was added into the Petri dish and the set-up was incubated for 24 h. After the incubation, the Na-fed leaf was harvested and washed with ddH_2O for 5 sec to remove radioactive solution from the surface. The whole plant was harvested after further case period of 48 h. The plants shoots were divided into three parts (top, middle, bottom).
To determine $^{22}Na^{+}$ phloem content, the middle shoot part was taken and treated as described under 2.6.3 for the collection of phloem sap. Plant tissue was weighed and dried at 60 °C for 72 h. Afterwards, radioactive tracer were performed as described under 2.6.5.

2.3.4 Experiments with $^{45}Ca^{2+}$

P. euphratica and *P.* x *canescens* were adapted to final concentrations of 150 mM NaCl with weekly increasing NaCl concentrations starting with 25 mM NaCl (25 mM, 50 mM, 100 mM and 150 mM NaCl). In the first experiment, both poplar species were labelled with 526.2 KBq $^{45}Ca^{2+}$ in the hydroponics during the last three days before the start of NaCl treatment.
In the second experiment, *P. euphratica* plants were adapted to 100 mM NaCl as described above and plants were labelled during the last three days of the exposure to 100 mM NaCl with 520 KBq $^{45}Ca^{2+}$. Afterwards the plants were exposed to 150 mM NaCl for a chase period of 1 week. For both experiments, plant roots were washed after radioactive labelling with non-radioactive nutrient solution containing the respective NaCl concentration for 30 s and placed in new 1 l pots, to avoid radioactive contamination.

2.3.5 Ca^{2+} deficiency experiments

P. euphratica and *P.* x *canescens* were exposed to two different Ca^{2+} concentrations (0 mM and 1 mM) in combination with three different NaCl treatments (0 mM, 25 mM and 150 mM NaCl).
Modified Long-Ashton media with 1.8 mM KNO_3 replacing $Ca(NO_3)$ in the hydroponic solution were used for treatments without Ca^{2+}. For all other treatment, supplementary 1 mM $CaCl_2$ was added.

2.4 Growth parameters and morphology

2.4.1 Plant growth and biomass

The height of the plants shoots, the stem diameter increment and the root length increment of each plant were measured weekly. The stem diameter was measured at a marked position 2 – 4 cm above the transition area to the roots. The fresh mass accumulation of the whole plant (biomass) was determined at the start and at the end of each experiment. The daily growth rate of each parameter was calculated using the following equation.

$$\text{Growth rate} = (M_{End} - M_{Start}) / \text{days of treatment}$$

Equation 2.1: M_{Start} = Measurement at the start of treatment; M_{End} = Measurement at the end of treatment.

It was assumed that growth could be approximated by a linear relationship in the small time intervals between measurements.
Fresh mass of plant tissue was measured immediately after harvest. Dry mass was determined after 72 h at 60 °C in a drying oven (UL-40, Memmert, Schwabach, Germany).

2.4.2 Measurements of the root morphology of *P. euphratica*

P. euphratica (5 – 8 weeks old) were exposed to weekly increasing NaCl concentrations to a final concentration of 150 mM NaCl (25 mM, 75 mM and 150 mM NaCl). Afterwards, plants were exposed to their final concentration of 150 mM NaCl for additional 20 days and plants were harvested afterwards.
For measurement of the surface area, the total root length, the number of tips and the average diameter of roots, *P. euphratica* roots were placed on a dark background. Digital pictures (Minolta Dimage 7, Konica Minolta Holdings Inc., Tokyo, Japan) of the roots were taken and inverted by Adobe Photoshop software Version 8.0.1 (Adobe System, San Jose, California, USA). Inverted digital root pictures were processed using WinRHIZO software Version 3.10b (Regent Instruments Inc., Quebec, Canada). The software analysed the roots, lengths of all roots and diameter of the roots and uses this information to calculate of the surface area, the total root length, the number of root tips and the average root diameter.

2.5 Anatomical analysis

P. euphratica were exposed to weekly increasing NaCl concentration as described under 2.3. Afterwards, plants were exposed to their final concentration of 150 mM NaCl for 24 days. Harvested root tips of *P. euphratica* were placed in FAE solution (Tab. 2.4) and stored at RT. Root tips were placed in solutions with ascending ethanol (EtOH) concentrations of 70 %, 80 %, 90 %, and 96 % ethanol to dehydrate the plant tissue. Depending on the thickness of the sample, the incubation time for each step of the dehydration was 30 min to 1 h. Subsequently, the EtOH solution was exchanged, with a solution of 48 % ethanol / 50 % inositol (1 h at RT), followed by 100 % inositol (1 h at RT).

Tab. 2.4: FAE solution.

Ethanol (70 %)	90	%
Acetic acid (100 %)	5	%
Formaldehyde (37 %)	5	%

Afterwards inositol was exchanged by Roti®-Histol transferring the roots from 75 % inositol / 25 % Roti®-Histol to 50 % inositol / 50 % Roti®-Histol and finally to 25 % inositol / 75 % Roti®-Histol. Depending on the thickness of the sample, incubation times were 30 min to 1 h (at RT) for all fixation steps.

After the fixation, the samples were incubated three times for 12 h with 100 % Roti®-Histol.

A saturated solution of Roti®-Histol and Rotiplast was prepared at room temperature. The samples were incubated in this mixture for 2 h at least. Afterwards, the root tips were placed in a heated (40 °C) saturated solution of Roti®-Histol / Rotiplast for 2 h at least.

Rotiplast was melted and the samples were incubated in pure Rotiplast for 12 h at 63 °C. The solution was discarded and this step was repeated once. Afterwards, the samples cooled over night.

The embedded samples were sliced in 15µm thick cross section with a microtome (Reichert-Jung, Heidelberg, Germany). The cross sections were treated with Roti®-Histol, isopropanol and finally 96 % ethanol for 1 min each and dried afterwards. For staining all specimen sections were immersed in toluidine blue (Tab. 2.5) solution for 3 min at RT. Coloured cuttings were placed on gelatine-coated glass slides for microscopic analysis.

The specimen were viewed under a light microscope (Axioskop, Zeiss, Jena, Germany) using magnifications of 2.5x, 10x and 20x. Pictures were taken using a digital camera (Nikon

CoolPix 990, Mikon Corporation, Tokyo, Japan). Measurements of the root diameter, the cortex width, the number of cell layers in the cortex and the total area of the root were done using ImageJ software (Vers. 1.37, URL: http://rsb.info.nih.gov/ij).

Tab. 2.5: Toluidine blue staining solution. The pH was adjusted to 4.4.

Toluidine blue	0.05	%
Sodium acetate buffer	0.1	M

2.6 Physiological measurements

2.6.1 Chlorophyll fluorescence

P. euphratica were adapted to high NaCl concentration as described under 2.5. Afterwards, adapted and non-adapted plants were transferred into hydroponic solution without NaCl for 48 h ("desalinization"). Subsequently, adapted and non-adapted *P. euphratica* were NaCl shocked for 48 h. Chlorophyll fluorescence measurements were performed using the MINI-PAM chlorophyll fluorometer system (PAM, Walz, Effeltrich, Germany) during NaCl shock treatment. With the PAM, the performance of the photosystem II (PS II) can be investigated (Rascher *et al.* 2000). These measurements can be used as an indicator for plant stress (Maxwell and Johnson 2000).

The light source (halogen lamp, 8 V / 20 W blue enriched, max. 6000 µmol quanta m^{-2} s^{-1} PAR) of the PAM is placed and the lamp transmitted light with $\lambda < 710$ nm. This light produced a chlorophyll fluorescence emission by the PS II. This emitted signal is detected by the PAM and measured. For the signal detection, a PIN-photodiode protected long-pass filter ($\lambda > 710$ nm) is used.

Since all measurements were performed under light conditions, the maximal fluorescence yield in the light ($F_{m'}$) and the steady state fluorescence (F_0) were recorded. These parameters were used to calculate the quantum yield of the PS II (Maxwell and Johnson 2000) after the following equation:

$$\Phi_{II} = (F_m' - F_0) / F_m'$$

Equation 2.2: with Φ_{II} = quantum yield of PS II; F_m' = maximum fluorescence; F_0 = steady state fluorescence.

2.6.2 Electrolyte conductivity

Conductivity measurements were carried out with a conductivity meter (LF315, WTW, Weilheim, Germany). *P. euphratica* were treated with high NaCl concentrations as described under 2.6.1. Root tips were harvested after the adaptation period, after the desalinization treatment and after the exposure to NaCl shock conditions.
P. euphratica root tips were washed for at least 3 sec with ddH_2O to remove nutrient solution. Samples were immediately weighed and placed in Falcon tubes (Sarstedt, Numbrecht, Germany) containing ddH_2O. The Falcon tubes were autoclaved (121 °C, 20 min) to destroy the root tissue and to release all electrolytes into the solution. The conductivity (EC) was measured at room temperature in the solution and calculated after the following equation:

$$EC\ [\mu S * mg^{-1}] = conductivity * FM^{-1}$$

Equation 2.3: Calculation of electrolyte conductivity (EC). FM = Fresh mass of root tip

2.6.3 Collection of phloem sap

Phloem sap of *P. euphratica* and *P.* x *canescens* were collected for the leaf feeding experiment (2.3.3). Phloem sap was collected using a modification of the EDTA technique by King and Zeevart (1974), according to Schneider *et al.* (1996). Shoots of *P. euphratica* and *P.* x *canescens* plants were divided into three parts of the same length and leaves were removed from the stem. The bark was incised vertically (approximately 1 - 2 mm deep) by a razorblade cut. The bark was peeled off along this cut and separated from the wood. The bark piece was washed with ddH_2O and immediately transferred into vials containing 15 ml of exudation buffer (Tab. 2.6). Bark pieces were removed from the exudation solution after 24 h of incubation, washed with ddH_2O and dried at 60 °C for 72 h.

Tab. 2.6: Exudation buffer. The pH was adjusted to pH 7.0.

EDTA	10	mM
Chloramphenicol	0.015	mM

To quantify $^{22}Na^{+}$ in the phloem sap, exudation solution samples were measured via LSC (see 2.6.5). Dried bark pieces were measured using γ-counter measurement (see 2.6.5).

2.6.4 Element analysis

ICP optical emission spectroscopy was performed (using an ICP-spectrometer (Spectroflame; Spectro Analytical Instruments) at the Institute of Forest Soil Science and Forest Nutrition (University of Göttingen, Germany)) to determine element concentration in plant tissue. Dry plant samples (roots, barks, leaves) were weighed and ashed in 2 ml 65 % HNO_3 at 160 °C for 12 h according to Heinrichs *et al.* (1986). The supernatant was filtered over filter paper (Rundfilter 589/1, Schleicher & Schnell MicroScience GmbH, Dassel, Germany) and filled up with ddH_2O to 25 ml.

2.6.5 Measurements of radioactive tracers

The radioactive isotopes $^{22}Na^{+}$ and $^{45}Ca^{2+}$ were quantified using three different detection techniques, depending on the kind of decay, the physical condition of the sample (liquid or solid) or the kind of analysis (measurement of distribution by imaging or quantative measurement).
For the quantification of radioactivity of both isotopes, the following equation was used to calculate the radioactive decay:

$$\text{Radioactive decay [Bq]} = \text{CPM} / (\eta \times 60)$$

Equation 2.4: Calculation of radioactive decay. CPM = counts per minute; η = effectiveness of measurement

Liquid scintillation counting

Liquid scintillation counting is a method for the detection and the quantification of radioactivity in liquid samples. Different kinds of decay, like λ- and β-particles of γ-ray emitting radionuclides can be detected.

A scintillation cocktail (Lumasafe Plus©, Lumac LSC B.V., Groningen, The Netherlands) was mixed with the liquid radioactive sample. The emitted energy of the radioactive decay is transmitted to scintillation molecules within the scintillation cocktail. These molecules transform the energy into photons which emit fluorescence light that is collected by two photomultipliers and transformed into an electrical impulse.

1 ml of phloem sap samples, 2 µl of xylem sap samples and 1 ml of nutrient solution samples were transferred into scintillation vials to measure the radiation of the radioactive isotopes. Each sample was mixed with 10 ml of the scintillation cocktail. The vials were placed into the liquid scintillation analyzer (Tri-Carb 2800 TR, PerkinElmer Life Sciences, Wellesley, Massachusetts, USA) and cooled for at least 4 h to minimize the background luminescence of each sample.

γ-counter measurement

A γ-counter is a technique to quantify the radioactivity in solid samples. γ-rays are produced after an λ- or β-decay of radionuclides. γ-rays are electromagnetic radiation with shorter wavelengths than light. In a γ-counter the radiation is absorbed in a crystal (here: thallium-activated sodium iodide) and the energy creates a fluorescence light. This light is collected and transformed into an electrical impulse in a photomultipliers (see also: LSC).

For measurements of the isotopes, the radioactive plant material was harvested and dried for at least 72 h at 60 °C in a drying chamber. Dried samples were weighed and placed into scintillation vial. γ-ray decay was measured using a γ-counter (Automatic gamma counter 1480 Wizard 3´´, Wallace, Turku, Finland).

Autoradiography

Autoradiography was used to determine the distribution of the radioactive decay within a plant.

The radioactive sample is placed on a plate, covered with Europium ions (Eu^{2+}). High energy decay (β-decay, γ-rays) stimulates Eu^{2+} ions. Thereafter, the irradiated plate is scanned with a He-Na-laser (633nm) and the ions are destimulated and decay into their ground state. In this process, the originating photons are measured by a photomultiplier. The intensity of the

measured photons is proportional to the radiation of the radioactive sample and an image of the radioactive distribution in the sample is produced.

For autoradiography images, plants were harvested and dried for at least 24 h at 60°C in a drying chamber. Samples were placed on paperboards and covered with a thin transparent film (Toppits, Melitta, Minden, Germany) to avoid contamination and to minimize the shielding of radioactive decay. Samples were exposed on phosphorus imaging plates (BAS-III, Fuji Photo Film (Europe) Co., Ltd., Düsseldorf, Germany) between 10 min and 1 h depending on the amount of the radioactive decay.

Phosphorus imaging plates were read out by a phosphor imager (FLA-5100, Fuji Photo Film (Europe) Co., Ltd., Düsseldorf, Germany) using an image analysing software (AIDA Image Data Analyzer software, Version 4.10.; Raytest Isotopenmeßgeräte GmbH, Straubenhardt, Germany).

2.6.6 Calculation of wood and bark area in poplar

The wood area (A_{wood}) and the bark area (A_{bark}) of both poplar species was used for the calculation of the Na^+ xylem and Na^+ phloem transport rates (see 3.6.2 and 3.2.3).

Plants of each poplar species were taken, having a similar height and age like plants used for the experiments. The stem diameter of each plant was measured at the same stem height, as done during the experiments. Afterwards, the bark was separated from the wood (as described under 2.6.3) and the diameter of the wood was measured. Since the ratio between the diameter of the stem (d_{stem}) and the diameter of the wood (d_{wood}) was constant for each poplar species, a factor (F) for the ratio of the stem and wood diameter was calculated for each poplar species (F_{Peup} = 1.565; F_{Pcan} = 1.3095). Using the following equation, the diameter of the wood for each poplar was calculated, based on measurement of the stem diameter:

$$d_{wood}\,[\mathrm{cm}] = d_{stem} * F^{-1}$$

Equation 2.5: Calculation of the wood diameter (d_{wood}). d_{stem} = diameter of the stem; F = Factor for ratio of the stem and wood diameter

The stem (A_{stem}) and the wood area (A_{wood}) were calculated, using the following equation:

$$A_{stem/wood}\ [cm^2] = (d_{stem/wood} * 0.5)^2 * \pi$$

Equation 2.6: Calculation of the stem (A_{stem}) and the wood area (A_{wood}). $d_{stem/wood}$ = diameter of the stem/wood

Since the stem area is a sum of the xylem cross sectional area and the bark area, the bark area (A_{bark}) was calculated by subtraction of the wood area from the stem area.

$$A_{bark}\ [cm^2] = A_{stem} - A_{wood}$$

Equation 2.7: Calculation of the bark area (A_{bark}). A_{stem} = stem area; A_{wood} = wood area

2.6.7 Calculation of the Na^+ exclusion rate for both poplar species under salinity

Plants exclude most of the Na^+ that is in the hydroponic solution. This mechanism is called salt exclusion (Munns 2005). According to Munns (2005) Na^+ exclusion is the ratio of the concentration of Na^+ in the xylem sap ($c_{Na+\ xylem}$) and the concentration of Na^+ in the nutrient solution ($c_{Na+\ sol.}$). Na^+ exclusion was calculated for both poplar species during the labelling phase of the split root experiment (see 2.3.2).

First, the total amount of Na^+ taken up by the plant ($n_{Na+\ plant}$) was calculated. *P. euphratica* plants were exposed to 100 mM NaCl and *P.* x *canescens* were exposed to 50 mM NaCl (see 2.3.2). The amount of radioactivity taken up by the plant ($Bq_{taken\ up}$) was calculated as the sum of $^{22}Na^+$ incorporated at harvest and the amount of $^{22}Na^+$ released. Because the ratio of radioactivity taken up/total radioactivity in the nutrient solution is the same as the ratio of Na^+ taken up/total Na^+ in the hydroponic solution, the amount of Na^+ ($n_{Na+\ plant}$) that was taken up by each plant during this week was calculated using the following equation:

$$n_{Na+\ Plant}\ [mmol] = c_{Na+\ sol.} * V_{sol.} * (Bq_{taken\ up} * Bq_{total}^{-1})$$

Equation 2.8: Amount of Na^+ taken up by each plant ($n_{Na+\ Plant}$). $c_{Na+\ sol.}$ = concentration of Na^+ in the hydroponic solution (mmol/L), $V_{sol.}$ = volume of the hydroponic solution (L), $Bq_{taken\ up}$ = Amount of $^{22}Na^+$ taken up by the plant during one week, Bq_{total} = Amount of $^{22}Na^+$ in the hydroponic solution

For calculation of the concentration of Na^+ in the xylem sap, it was assumed that the amount of Na^+ that was taken up by the plant and present in roots had not been transported via the shoot. This was also assumed for the labelled Na^+ present in the unlabelled root compartment, since this fraction could have been transported in radial direction through the roots. The resulting xylem sap concentrations of Na^+ are therefore at the lower end representing conservative estimates.
To calculate the portion of Na^+ transported to the shoot, the distribution of incorporated $^{22}Na^+$ at the end of the split root experiment was measured for both poplar species (see Tab. 3.12 and Tab. 3.17). Using these data, the proportion of Na^+ in the shoot of the *P. euphratica* (81.56 %) and *P.* x *canescens* (95.65 %). was calculated (P_{Peup} = 0.8156 and P_{Pcan} = 0.9565). This portion was used to determine the amount of Na^+ transported via the xylem.

Using equation 2.8 for the amount of Na^+ taken up by the plant ($n_{Na+\ Plant}$) and the *P* factor, the amount of Na^+ transported via the xylem ($n_{Na+\ xylem}$) was calculated:

$$n_{Na+\ xylem}\ [mmol] = n_{Na+\ Plant} * P$$

Equation 2.9: Amount of Na^+ transported via the xylem ($n_{Na+\ xylem}$). $n_{Na+\ Plant}$ = amount of Na^+ taken up by the plant, *P* = portion of Na^+ in the shoot of the plant.

The volume of the xylem sap (V_{xylem}) was assumed to be as high as the amount of transpired water used by each plant during one week of measurement. To calculate the concentration of Na^+ in the xylem sap ($c_{Na+\ xylem}$), the following equation was used:

$$c_{Na+\ xylem}\ [mmol/ml] = n_{Na+\ xylem} * V_{xylem}^{-1}$$

Equation 2.10: Calculation of Na^+ xylem sap concentration [$c_{Na+\ xylem}$]. $n_{Na+\ xylem}$ = amount of Na^+ transported via the xylem, V_{xylem} = volume of xylem sap.

The concentration of Na^+ in the nutrient solution ($c_{Na+\ sol.}$) was 100 mM NaCl for *P. euphratica* and 50 mM NaCl for *P.* x *canescens*. Na^+ exclusion was calculated using the following equation:

$$Na^+ \text{ exclusion } [\%] = 100 - (c_{Na+\ xylem} * c_{Na+\ sol.}{}^{-1})$$

Equation 2.11: Calculation of Na^+ exclusion. $c_{Na+\ xylem}$ = concentration of Na^+ in the xylem, $c_{Na+\ sol.}$ = concentration of Na^+ in the nutrient solution.

2.7 Molecular analysis

2.7.1 Isolation of RNA

Harvested plant material for RNA isolation was immediately frozen in liquid nitrogen and stored at -80 °C. By using a wing head mill (MM 2000, Retsch, Haan, Germany) and constant cooling with liquid nitrogen, plant material was ground to fine powder.

RNA was isolated as described by Chang *et al.* (1993). For this, 150 mg of the ground plant material was placed into an Eppendorf tube. 0.75 ml of heated (65 °C) CTAB buffer (Tab. 2.7) and 15 µl β-mercaptoethanol were mixed with the plant powder. The mixture was shaken by hand several times while being incubated for 15 min at 65 °C. Afterwards, the samples were incubated for 15 min at RT and repeatedly shaken by hand.

0.75 ml of chloroform / isoamyl alcohol 24:1 were added, mixed and incubated for 15 min at RT. After centrifugation (Centrifuge 5417 R, Rotor FA 45-30-11, Eppendorf AG, Hamburg, Germany) at 14000 rpm for 15 min at RT, the supernatant was carefully removed and transferred in to a new Eppendorf tube. Thereby, proteins, polysaccharides and other compounds were removed from the solution. The washing procedure was repeated once.

Tab. 2.7: CTAB buffer for RNA isolation. RNase-free water was used for preparing the CTAB buffer. The pH was adjusted to 8.0. The buffer was autoclaved (20 min, 121 °C) except the Tris-HCl, which was added afterwards.

CTAB	2	%
PVPP	2	%
NaCl	2	M
EDTA	25	mM
Tris-HCl	100	mM

200 µl of 10 M LiCl (4 °C) was added to the supernatant and the samples were stored over night on ice at 4 °C (not longer than 18 h) to precipitate the RNA.

Afterwards, the solution was centrifuged (Centrifuge 5417 R, Rotor FA 45-30-11, Eppendorf AG, Hamburg, Germany) at 14000 rpm for 20 min at 4 °C. The supernatant was discarded and the pellet, containing the precipitated RNA, was suspended in 400 µl of heated (65 °C) SSTE buffer (Tab. 2.8). The mixture was heated for 10 min at 42 °C in a heater (HBT 130, Haep Labor Consult, Bovenden, Germany) and repeatedly mixed to dissolve the RNA.

Tab 2.8: SSTE buffer for RNA isolation. RNase-free water was used for preparing the SSTE buffer. The pH was adjusted to 8.0. Tris-HCl was added after autoclaving (20 min, 121 °C) the buffer.

SDS	0.5	%
NaCl	1	M
EDTA	1	mM
Tris-HCl	10	mM

400 µl of the chloroform / isoamyl alcohol 24 : 1 solution were added to the suspension, mixed, and centrifuged at 14000 rpm (Centrifuge 5417 R, Rotor FA 45-30-11, Eppendorf AG, Hamburg, Germany) for 5 min at RT. The supernatant was transferred into a new Eppendorf tube and the extraction with 400 µl of the chloroform / isoamyl alcohol 24 : 1 solution was repeated. 800 µl of ice-cold 96 % ethanol were added to the solution for 1 h at -80 °C to precipitate RNA.

Afterwards, the solution was centrifuged at 14000 rpm (Centrifuge 5417 R, Rotor FA 45-30-11, Eppendorf AG, Hamburg, Germany) for 20 min at 4 °C. The supernatant was discarded and the pellet was washed with 500 µl of 70 % ethanol, and afterwards with 80 µl of 70 %

ethanol. For each washing step, the pellet and the ethanol were centrifuged at 14000 rpm for 10 min at RT and the supernatant was carefully discarded.
Afterwards, the pellet was dried for 10 min at RT and subsequently resuspended in 20 µl RNase-free water at 42 °C for 10 min and repeatedly shaken to dissolve the RNA. RNA solution was stored at -80 °C.

2.7.2 Gel electrophoresis

Agarose gel electrophoresis was used to control the quality of the isolated RNA. 1 µl of each RNA sample was mixed with 2.5 µl of 2 x RNA loading buffer (Tab. 2.9) and filled up to 5 µl with RNase-free H_2O.

Tab. 2.9: 2 x RNA loading buffer

Formamide	660	µl
37 % Formaldehyde	80	µl
10 % Bromphenol blue	10	µl
Ethidium bromide	10	µl
10 x MEA	100	µl
RNA-free H_2O	140	µl

The samples were heated in a cycler (Mastercycler, Eppendorf, Hamburg, Germany) at 70 °C for 10 min. Afterwards, the samples were cooled on ice and filled into the pockets of the agarose gel which had been prepared as summarized in Tab. 2.11. The gel was placed into the electrophoresis unit (Bio-Rad Basic, Bio-Rad laboratories, Munich, Germany). The electrophoresis was performed at an amperage of 50 mA for 1 h.

Tab.2.10: 10 x MEA buffer for RNA gel electrophoresis. The solution was autoclaved (20 min, 121 °C) and stored at 4 °C.

MOPS	0.2	M
Sodium acetat	50	mM
EDTA	10	mM

After the electrophoresis run, the gel was observed using a laser at λ = 473 nm (FLA-5100, Fuji Photo Film (Europe) Co., Ltd., Düsseldorf, Germany) and Image Reader Version 3.0 (Fuji Photo Film (Europe) Co., Ltd., Düsseldorf, Germany) software to control, whether RNA isolation was successful.

Tab.2.11: Agarose gel composition for RNA isolation. The agarose, the running buffer and the H_2O were heated in a microwave (650 W, 5 min) to solve the agarose. Afterwards, the formaldehyde is added and the warm solution was filled into the mould for the gel.

Agarose	1.44	g
10 x RNA running buffer	12	ml
RNA-free H_20	84	ml
37 % Formaldehyde	24	ml

2.7.3 DNA digestion

Because the RNA has to be free of DNA before the start of the cDNA synthesis, the DNA-free Kit "Turbo DNA-free^{TM}"(Ambion Inc., Austin, Texas, USA) was used according to the manufacture's instruction. RNA samples prepared as described under 2.7.1 were subjected to this procedure.

To determine the RNA concentration after the DNA digestion, photometric measurement (BioPhotometer, Eppendorf AG, Hamburg, Germany) at λ = 260 nm were performed. For the photometric measurements, 2 µl of RNA samples were diluted using 78 µl ddH_2O and were placed in quartz curvettes. The calculation of the RNA concentration (c_{RNA}) was performed after the following equation:

$$c_{RNA} = OD_{260} * V * F$$

Equation 2.12: Calculation of the RNA concentration. c = concentration of the RNA; OD_{260} = optical density of the sample at 260 nm; V = dilution factor; F = 50 µg * ml^{-1} (concentration of DNA at OD_{260} = 1)

2.7.4 cDNA synthesis

5 µg of RNA were used for the cDNA synthesis. The "RevertAid^{TM} First Strand cDNA Synthesis Kit" (MBI Fermentas, St.-Leon, Germany) was used for the synthesis. Oligo$(dT)_{17}$ (MBI Fermentas, St.-Leon, Germany) was used as the primer, RiboLock^{TM} Ribonuclease Inhibitor (MBI Fermentas, St.-Leon, Germany) as the RNase inhibitor and M-MuLV Reverse Transcriptase (MBI Fermentas, St.-Leon, Germany) for the synthesis of cDNA. The cDNA synthesis was performed according to the manufacture's instruction in a cycler (Matercycler, Eppendorf, Hamburg, Germany).

2.7.5 Primer design for qRT-PCR

The sequences of the primer for qRT-PCR of *P.* x *canescens* (PtHKT1fwd and PtHKT1rev1) were kindly provided by Dr. Peter Ache (Julius–von–Sachs Institut für Biowissenschaften, University of Würzburg, Germany) and have been shown in Tab. 2.12.

For the design of the primer for qRT-PCR of *P. euphratica*, the NCBI data base (URL: www.ncbi.nlm.nih.gov) was used to determine the coding sequence of HKT1 in *Arabidopsis* (At 4g10310). A blast search at the EST data base resulted in a *P. tremula* EST (gi: 23994131). The EST was blasted at the JGI home page (URL: genome.jgi-psf.org/Poptr1/Poptr1.home.html) resulting in a highly matching alignment.

The coding sequence of AtHKT1, *P. tremula* EST and the sequences of both primers (PtHKT1fwd and PtHKT1rev1) were aligned using GeneDoc software (Version 2.6.002; URL: www.psc.edu/biomed/genedoc). Since PtHKT1fwd was identical to AtHKT1 and the EST sequence but PtHKT1rev showed no correlation, the primer PtHKT1rev2 (Tab. 2.12) was designed for qRT-PCR of *P. euphratica* in combination with PtHKT1fwd.

Oligo Analyzer software (Version 1.1.2; URL: http://molbiol-tools.ca/molecular_biology_freeware.htm) was used for primer design of PtHKT1rev2. Self-annealing of primer, primer dimerization, the formation of loops, the GC content and the 3`-end GC content and the annealing temperature (T_M) were analyzed by this software. The primers were obtained from MWG Biotech (Ebersberg, Germany).

Tab. 2.12: Primers used for qRT-PCR of HKT1;1.

PtHKT1fwd	5´- TCTCATTTGCGTCTCAG -3´
PtHKT1rev	5´- CCATGTTACTCCACCTT -3´
PtHKT1rev2	5´- GAACATTACTAAGATGAGG -3´

2.7.6 qRT-PCR

The use of a reverse transcription followed by a polymerase chain reaction (PCR) is a technique to analyse the amount of specific mRNA. For relative quantification, the expression of the target gene is referred to the expression of a non regulated “housekeeping” gene (Pfaffl 2001).

A fluorescence dye (SYBR® Green) is specifically binding to double stranded DNA and fluorescence light is emitted. Because the PCR product is the only double stranded DNA, the emitted light is corresponding to the quantity of the PCR product. During each cycle of the PCR, the emitted fluorescence light is measured and plotted in relation to the number of cycles. A defined fluorescence value indicates the transition of the lag phase to the exponential rise. The number of cycles required to reach this value (C_t) is recorded (Fig. 2.5). By comparing the C_t values of two samples, differences in the transcript level of the gene of interest can be calculated (Pfaffl 2004).

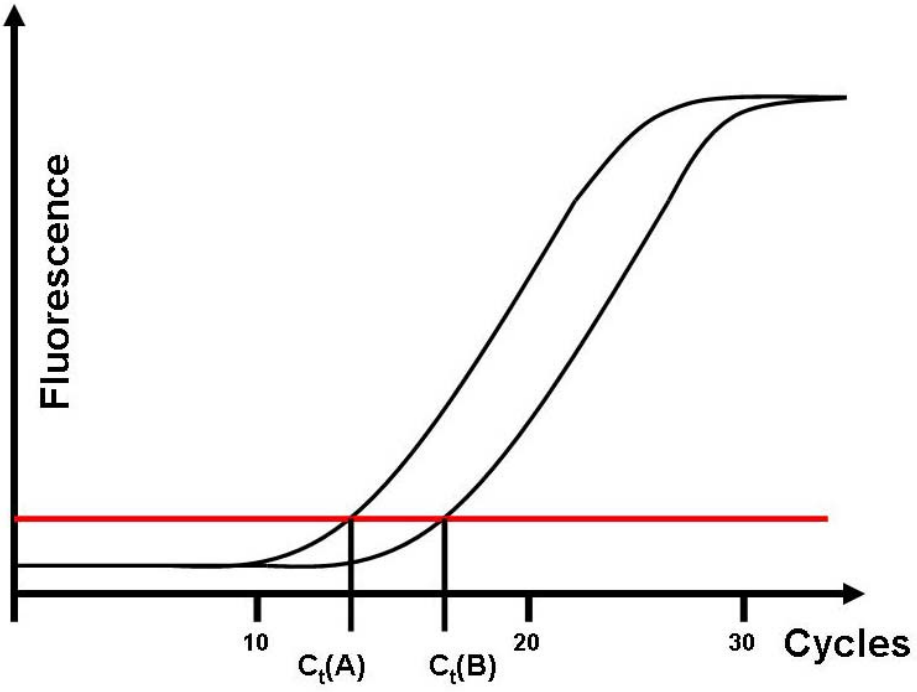

Fig. 2.5: Result of a qRT-PCR experiment (modified after Becker-Follmann & Baas 2004).

For the qRT-PCR, 2 µl of synthesized cDNA (see 2.7.4) is used. For each sample, three technical repetitions were preceded. The samples were mixed as described in Tab. 2.13. and were placed in sample plates (PCR 96er Well, Greiner bio-one, Frickenhausen, Germany),

closed with a foil and centrifuged at 1600 rpm for 3 min (Centrifuge 5810 R, Rotor A-4-62, Eppendorf AG, Hamburg, Germany).

Tab. 2.13: Schema of the qRT-PCR. RNase-free H_2O was used for the PCR. The volume of each PCR sample was 25 µl

cDNA	2	µl
2 x Abgene-Master Mix	12.5	µl
3´ Primer 10 µM	0.4	µl
5´ Primer 10 µM	0.4	µl
H_2O	9.7	µl

The qRT-PCR was performed in a PCR-Cycler (iCycler, Bio-Rad, Munich, Germany) and the emitted fluorescence was measured and calculated using the MyiQ Single Color Real-Time PCR Detection System (Bio-Rad, Munich, Germany) in combination with the MyiQ software Version 1.0 (Bio-Rad, Munich, Germany). The PCR was programmed as described in Tab. 2.14.

Tab. 2.14: PCR program scheme.

Cycle	Repeat	Describtion	°C	min
1	1 x	Denaturation	95 °C	15:00
2	45 x	Denaturation	95 °C	0:10
		Annealing	50 °C	0:30
		Extension	72 °C	1:00
3	110 x	Melting curve	40 °C + 0.5°C	0:10
			95 °C	1:00
4	1 x	Cool down	4 °C	hold

The expression of the HKT1;1 gene was calculated after the following equations (Pfaffl 2001):

$$\Delta CP = (\text{Average } Ct_C - \text{Average } Ct_T)$$

Equation 2.13: Calculation of the expression differences within a sample. ΔCP = relative expression difference; Ct_C = Ct value of the control; Ct_T = Ct value of the sample

$$\text{Ratio} = \frac{(E_{TG})^{\Delta CP_{TG}}}{(E_{RG})^{\Delta CP_{RG}}}$$

Equation 2.14: Calculation of the relative expression ratio of the target gene. E_{TG} = expression of the target gene; E_{RG} = expression of the reference (housekeeping) gene; Δ CP_{TG} = relative expression difference of the target gene; Δ CP_{RG} = relative expression difference of the reference (housekeeping) gene

As a reference (housekeeping) actin cDNA fragments were used (Langer *et al.* 2002). The primers (Tab. 2.15) were provided by Dr. Peter Ache (Julius–von–Sachs Institut für Biowissenschaften, University of Würzburg, Germany).

Tab. 2.15: Primers for housekeeping gene ACT.

PtACTfwd	5´- GGTGATGGTGTGTCT -3´
PtACTrev	5´- ACTGAGCACAATGTTAC -3´

The calculation of the relative expression was performed using REST-384 beta software Version 2 (August 2006) (http:rest-2005.gene-quantification.info) (Pfaffl *et al.* 2002).

2.8 Statistics

Data were analysed using the Student's t-test with SAS (Version 9.1) software (SAS Institute Inc., Cary, North Carolina, USA). Significant differences at $P \leq 0.05$ were indicated by *, $P \leq 0.01$ were indicated by ** and $P \leq$ were marked by ***. For analysis of significant differences between several treatments, statistical analysis were performed with SAS software using analysis of variance (ANOVA), followed by a multiple range test. Significant differences for $P \leq 0.05$ were marked by different letters.

3 Results

3.1 Influence of salinity on the performance of *P. euphratica* and *P.* x *canescens*

3.1.1 Influence of salt stress on biomass and growth of *P. euphratica* and *P.* x *canescens*

To compare the performance of *P. euphratica* and *P.* x *canescens* under high salinity, both species were exposed to weekly increasing NaCl concentration starting at 25 mM to final concentrations of 150 mM NaCl (25 mM, 50 mM, 100 mM and 150 mM NaCl). Growth parameters were measured weekly and the growth rate was calculated as described under 2.4.1.

The growth of the shoots of *P.* x *canescens* exposed to NaCl was decreased for all treatments and showed significant differences for the exposure to 100 mM NaCl (Fig. 3.1 A). In contrast to *P.* x *canescens,* a significant reduction in the growth rate of the shoots of *P. euphratica* was not detected for plants exposed to any of the NaCl treatments (Fig. 3.1 B).

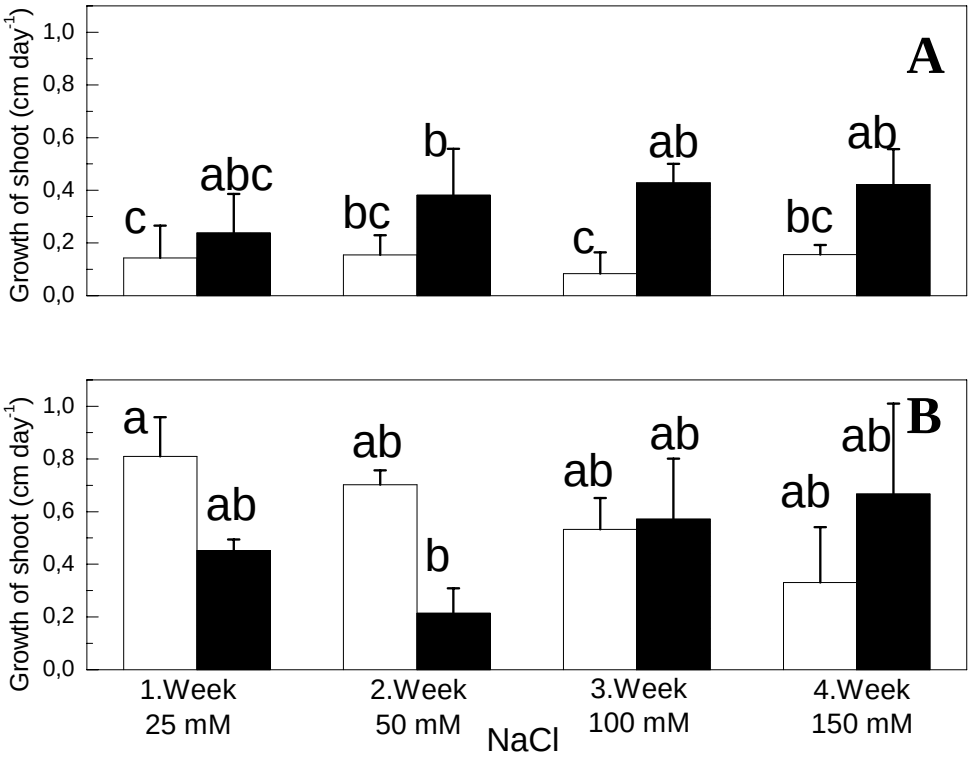

Fig. 3.1: Growth rate of the shoot height of control (black bars) and NaCl treated (white bars) *P.* x *canescens* (A) and *P. euphratica* (B). Plants were exposed to weekly increasing NaCl concentration to final concentrations of 150 mM (25 mM, 50 mM, 100 mM and 150 mM). Plant shoot growth was measured after 7 days of exposure to the indicated NaCl concentration. The shoot growth was calculated as described under 2.4.1. Data represent means ± SD; n = 3 – 4. Different letters indicate significant differences at $P \leq 0.05$.

To analyse the influence of NaCl on the growth and biomass production of *P. euphratica* and *P.* x *canescens* in greater detail, both poplar species were exposed to 25 mM NaCl, a non-injurious concentration, or 150 mM NaCl, a damaging concentration. Furthermore, *P. euphratica* was exposed to high KCl concentration (150 mM) to analyse the plants performance under ionic stress. Using the same adaptation regime to high salinity, plants were exposed to final concentrations of 150 mM of NaCl and KCl, respectively (25 mM, 50 mM, 100 mM and 150 mM). The time of exposure to NaCl and KCl was the same for all treatments. Plants were harvested 34 days after starting the treatment.

A 2-fold decrease in biomass of *P. euphratica* was observed for plants exposed to high salt treatments compared to control plants (Tab. 3.1). This decrease was mainly due to diminished leaf and stem production, whereas root biomass was less affected (Tab. 3.1). *P. euphratica* plants exposed to 25 mM NaCl showed biomass accumulation similar to that of controls and an increase in root biomass (Tab. 3.1). Since root biomass was not affected by high salt, the root / shoot ratio increased in plants exposed to 150 mM of NaCl or KCl, respectively, compared to that of controls (Tab. 3.1). Additionally, for high salt stress treatments, the DM / FM ratio of both leaves and roots increased (Tab. 3.1).

Table 3.1: The influence of salinity on biomass and morphology of *P. euphratica*. Plants were exposed to weekly increasing NaCl and KCl concentration, starting at 25 mM to final concentrations of 150 mM (25 mM, 75 mM, 150 mM). The time of exposure to salt for all treatments (25 mM NaCl, 150 mM NaCl and 150 mM KCl) was the same. The plants were harvested after 34 days of treatment. Data represent means ± SD; n = 4 - 5 plants. Significant differences at $P \leq 0.05$ are marked by different letters.

Parameter		**0 mM NaCl**	**25 mM NaCl**	**150 mM NaCl**	**150 mM KCl**
Whole plant	[g FM]	15.55 ± 3.46 ^{b}	16.82 ± 1.93 ^{b}	8.36 ± 1.85 ^{a}	8.83 ± 2.68 ^{a}
Leaves	[g FM]	8.71 ± 1.80 ^{b}	8.68 ± 0.53 ^{b}	3.77 ± 0.91 ^{a}	4.88 ± 1.94 ^{a}
Stem	[g FM]	4.54 ± 1.10 ^{b}	4.19 ± 0.70 ^{b}	1.69 ± 0.54 ^{a}	1.45 ± 0.33 ^{a}
Root	[g FM]	2.30 ± 0.77 ^{a}	3.94 ± 1.15 ^{b}	2.90 ± 0.62 ^{ab}	2.51 ± 0.63 ^{a}
DM / FM ratio (leaves)		0.15 ± 0.00 ^{a}	0.15 ± 0.01 ^{a}	0.21 ± 0.01 ^{b}	0.20 ± 0.03 ^{b}
DM / FM ratio (roots)		0.18 ± 0.03 ^{b}	0.15 ± 0.02 ^{b}	0.16 ± 0.02 ^{b}	0.10 ± 0.01 ^{a}
root / shoot ratio		0.17 ± 0.04 ^{a}	0.30 ± 0.07 ^{ab}	0.55 ± 0.13 ^{b}	0.43 ± 0.15 ^{b}

The daily biomass accumulation (see 2.4.1) of *P. euphratica* treated with high salt concentrations (150 mM NaCl, 150 mM KCl) was half of that found in controls and in 25 mM NaCl treated plants (Tab. 3.2). This result indicates, that *P. euphratica* respond to a longer time of exposure to 150 mM NaCl with a decrease in plant growth (Tab. 3.2).

The growth of the stem diameter was also only half of those found in controls (Tab 3.2). High salt concentrations of either NaCl or KCl resulted in significant reduction of *P. euphratica* biomass accumulation rates and resulted in decreases in the plants' stem diameter. The exposure to 150 mM NaCl and 25 mM NaCl had no effect on the growth of the root length, indicating a maintenance of root growth under NaCl treatment (Tab. 3.2). The leaves of *P.*

euphratica exposed to NaCl displayed no necrosis or other symptoms of leaf damage (Fig. 3.2)

Table 3.2: The influence of salt stress on the biomass production and growth rates of *P. euphratica*. The plants were exposed to three different NaCl concentrations: 0 mM (control), 25 mM, 150 mM NaCl or 150 mM KCl (without NaCl). Plants were adapted to their final concentration of 150 mM NaCl with weekly increasing NaCl concentration (25, 75 and 150 mM NaCl) and harvested 34 days after the start of treatment. Plants of the 25 mM treatment were exposed to 25 mM NaCl for 34 days. Data were calculated as described under 2.4.1. Data represent means ± SD; n = 5 plants. Significant differences at $P \leq 0.05$ are marked by different letters.

Parameter		**0 mM NaCl**	**25 mM NaCl**	**150 mM NaCl**	**150 mM KCl**
Biomass (whole plant)	[g FM/day]	0.352 ± 0.08 ^{b}	0.386 ± 0.04 ^{b}	0.136 ± 0.04 ^{a}	0.153 ± 0.06 ^{a}
Growth of stem diameter	[cm/day]	0.068 ± 0.010 ^{b}	0.060 ± 0.006 ^{b}	0.028 ± 0.009 ^{a}	0.030 ± 0.030 ^{a}
Growth of root length	[cm/day]	0.994 ± 0.400 ^{ab}	1.550 ± 0.460 ^{b}	0.647± 0.329 ^{a}	0.415 ± 0.190 ^{a}

Fig. 3.2: *P. euphratica* leaves treated without (A) and with (B) 150 mM NaCl. Leaves of plants exposed to high NaCl concentration displayed no leaf damage.

In contrast to *P. euphratica* (Fig. 3.2), *P.* x *canescens* displayed leaf damage and necrosis a few days after the start of exposure to 75 mM NaCl. As leaves became completely brownish and dry (Fig. 3.3), plants were harvested after 18 days of NaCl treatment, respectively 3 days after the start of the exposure to 150 mM NaCl.

Fig. 3.3: Damage on *P.* x *canescens* leaves treated with 150 mM NaCl. (A) Leaves developed brownish necrosis, starting at the leaf edge. (B) Black spots and leaf crinklings also developed under high NaCl concentration, indicating leaf structure damages. (C) Leaves display yellowish necrosis shortly after starting NaCl treatment. (D) Leaf of a control plant.

Biomass and morphology of *P.* x *canescens* were not affected by exposure to 25 mM NaCl compared with control conditions (Tab. 3.3). *P.* x *canescens* exposed to high NaCl concentration showed a decreased biomass accumulation and higher root and leaf DM-to-FM ratios, and root / shoot ratio, than those of controls or than those of plants exposed to 25 mM NaCl (Tab. 3.3).

Table 3.3: The influence of NaCl on biomass and morphology of *P.* x *canescens*. Plants were adapted with weekly increasing NaCl concentration starting at 25 mM to final concentrations of 150 mM NaCl (25 mM, 75 mM, and 150 mM). (*) Plants exposed to 150 mM NaCl were harvested after 18 days, due to displayed symptoms of leaf damage (see Fig. 3.3). All other plants were harvested after 34 days of treatment. Data represent means ± SD; n = 5 plants.

Parameter		**0 mM NaCl**	**25 mM NaCl**	**150 mM NaCl (*)**
Whole plant	[g FM]	36.64 ± 16.09 ^{b}	33.11 ± 12.27 ^{b}	7.31 ± 3.91 ^{a}
Leaves	[g FM]	15.91 ± 5.55 ^{b}	13.51 ± 3.16 ^{b}	1.36 ± 0.93 ^{a}
Stem	[g FM]	6.90 ± 3.20 ^{b}	5.60 ± 2.21 ^{b}	0.93 ± 0.51 ^{a}
Root	[g FM]	13.83 ± 7.54 ^{b}	14.00 ± 7.57 ^{b}	5.02 ± 2.58 ^{a}
DM / FM ratio (leaves)		0.18 ± 0.02 ^{a}	0.19 ± 0.02 ^{a}	0.54 ± 0.22 ^{b}
DM / FM ratio (roots)		0.11 ± 0.01 ^{b}	0.09 ± 0.01 ^{b}	0.07 ± 0.01 ^{a}
root / shoot ratio		0.19 ± 0.02 ^{a}	0.19 ± 0.02 ^{a}	2.32 ± 0.50 ^{b}

High NaCl treatment of *P.* x *canescens* resulted in a 4-fold decrease in biomass accumulation per day (Tab. 3.4).

The growth of the stem diameter was about 3- to 4-fold decreased in *P.* x *canescens* stressed with high NaCl concentrations and resulted in a 4-fold loss of biomass of the whole plant (Tab. 3.4) compared to that of controls and plants treated with 25 mM NaCl. In contrast to controls and plants exposed to low NaCl treatment, the root growth of plants treated with high NaCl concentration was less retarded than that of the shoots.

Table 3.4: The influence of NaCl on the biomass and growth of *P.* x *canescens*. The plants were treated with three different NaCl concentrations: 0 mM (control), 25 mM and 150 mM NaCl. Plants were adapted to their final concentration of 150 mM NaCl with weekly increasing NaCl concentration (25, 75 and150 mM NaCl). (*) Plants treated with 150 mM NaCl were harvested after 18 days of starting the NaCl treatment, because of plant death. The other plants were harvested 34 days after the start of treatment. Data were calculated as described under 2.4.1. Data represent means ± SD; n = 5 plants. Significant differences at $P \leq 0.05$ are marked by different letters.

Parameter		**0 mM NaCl**	**25 mM NaCl**	**150 mM NaCl (*)**
Biomass (whole plant)	[gFM/day]	0.966 ± 0.40 ^{b}	0.848 ± 0.28 ^{b}	0.230 ± 0.18 ^{a}
Growth of stem diameter	[cm/day]	0.087 ± 0.016 ^{b}	0.082 ± 0.014 ^{b}	0.036 ± 0.018 ^{a}
Growth of root length	[cm/day]	1.097 ± 0.449 ^{a}	0.856 ± 0.299 ^{a}	0.542 ± 0.224 ^{a}

Since *P. euphratica* had no leaf injuries and only a 2-fold decrease in plant growth compared with severe leaf injuries and a 4-fold decrease in *P.* x *canescens*, these results underline the documented fact that *P. euphratica* is more NaCl tolerant than *P.* x *canescens* (Sixto *et al.* 2005) and establish the basic culture and stress conditions for subsequent analysis.

3.2 Influence of salinity on Na^+ and Ca^{2+} tissue concentrations of *P. euphratica* and *P.* x *canescens*

The ability of plants to maintain Ca^{2+} transport under salinity had been proposed as an index of salt tolerance in plants (see 1.4, LaHaye and Epstein 1971). Since Na^+/Ca^{2+} interaction are crucial for the NaCl tolerance of plants, the influence of salinity on Na^+ and Ca^{2+} tissue concentration was investigated in both poplar species. Furthermore, the distribution of Ca^{2+} in *P. euphratica* and *P.* x *canescens* in response to salinity was analysed, to find out whether differences in Ca^{2+} transport existed between both poplar species and whether Ca^{2+} recirculation in poplar takes place.

3.2.1 Influence of salinity on the Ca^{2+} and Na^+ leaf concentration of poplar

Element analysis were carried out (see 2.6.4) to determine Na^+ and Ca^{2+} concentrations in leaves of both poplar species. Plants were exposed to weekly increasing NaCl in the hydroponic solution to final concentrations of 150 mM NaCl. Plants were harvested weekly after acclimation to 25 mM, 50 mM, 100 mM and 150 mM NaCl, respectively. A distinction between old leaves, that had been developed before the start of the NaCl treatment and new leaves, which were developed during the NaCl treatment was made.

The Na^+ concentration in new leaves of *P.* x *canescens* and of *P. euphratica* exposed to 150 mM NaCl increased 20-fold and 10-fold, respectively compared to controls (Fig. 3.4 A). The Na^+ concentration in new leaves of *P.* x *canescens* was higher for all NaCl concentrations compared to corresponding treatments of *P. euphratica*. The Na^+ concentration in new leaves of *P. euphratica* exposed to 150 mM NaCl was similar to that found in new leaves of *P.* x *canescens* exposed to lower NaCl concentrations in the range of 50 – 100 mM. This indicates

an accelerated transport of Na^+ into new leaves of *P.* x *canescens* compared to those of *P. euphratica* (Fig. 3.4 A).

The Na^+ concentration of old leaves of *P.* x *canescens* exposed to 150 mM NaCl was 25-fold and that of *P. euphratica* was 16-fold increased compared to controls (Fig. 3.4 B). For *P.* x *canescens* the Na^+ concentration did not increase furthermore after the exposure to NaCl concentrations higher than 50 mM, indicating that the Na^+ concentration in old leaves had reached a plateau.

These results point out, that both poplar species show differences regarding their Na^+ distribution between the leaves. *P.* x *canescens* accumulated Na^+ more intensively in new, developing leaves in contrast to *P. euphratica*, in which the accumulation of Na^+ was similar in old and new leaves and usually lower than in *P.* x *canescens* (Fig. 3.4 A,B).

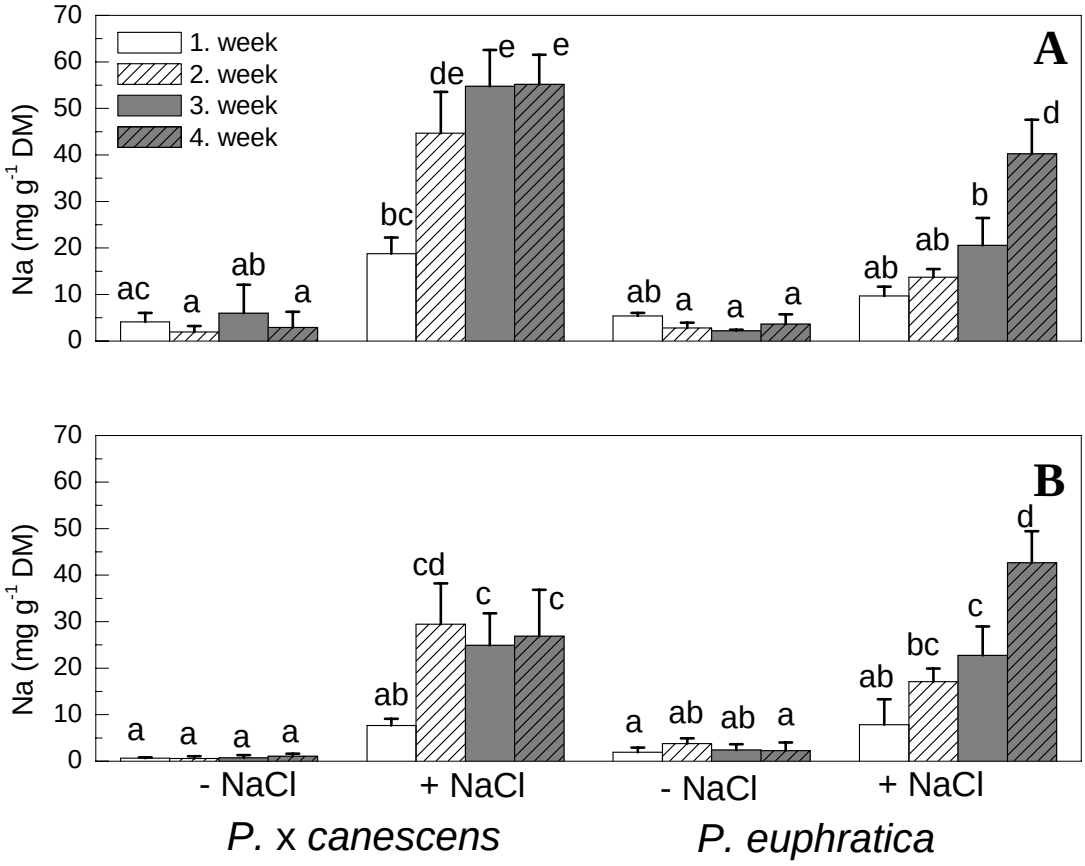

Fig. 3.4: Sodium concentrations in new leaves (A) and old leaves (B) of *P.* x *canescens* and *P. euphratica* exposed to NaCl. Plants were treated with 25 mM (white, open) in the first week, 50 mM (white, hatched) in the second week, 100 mM (grey) in the third week and 150 mM (grey, hatched) in the fourth week. Old leaves (B) are those, which were developed before the start of the NaCl treatment and new leaves (A) were developed during the exposure to NaCl. Plants were harvested weekly. Data represent means ± SD; n = 3 - 4. Significant differences at $P \leq 0.05$ are marked by different letters.

The concentration of Ca^{2+} in new leaves of both poplar species exposed to 150 mM NaCl was lower than that of controls, resulting in a 3-fold decreased Ca^{2+} concentration in new leaves of *P.* x *canescens* and a 2-fold decreased Ca^{2+} concentration in new leaves of *P. euphratica* (Fig. 3.5 A).

The Ca^{2+} concentration in old leaves of both poplar species exposed to NaCl decreased compared to controls, whereas in *P. euphratica* exposed to NaCl the Ca^{2+} concentration was significant lower compared to NaCl treated *P.* x *canescens* (Fig. 3.5 B).

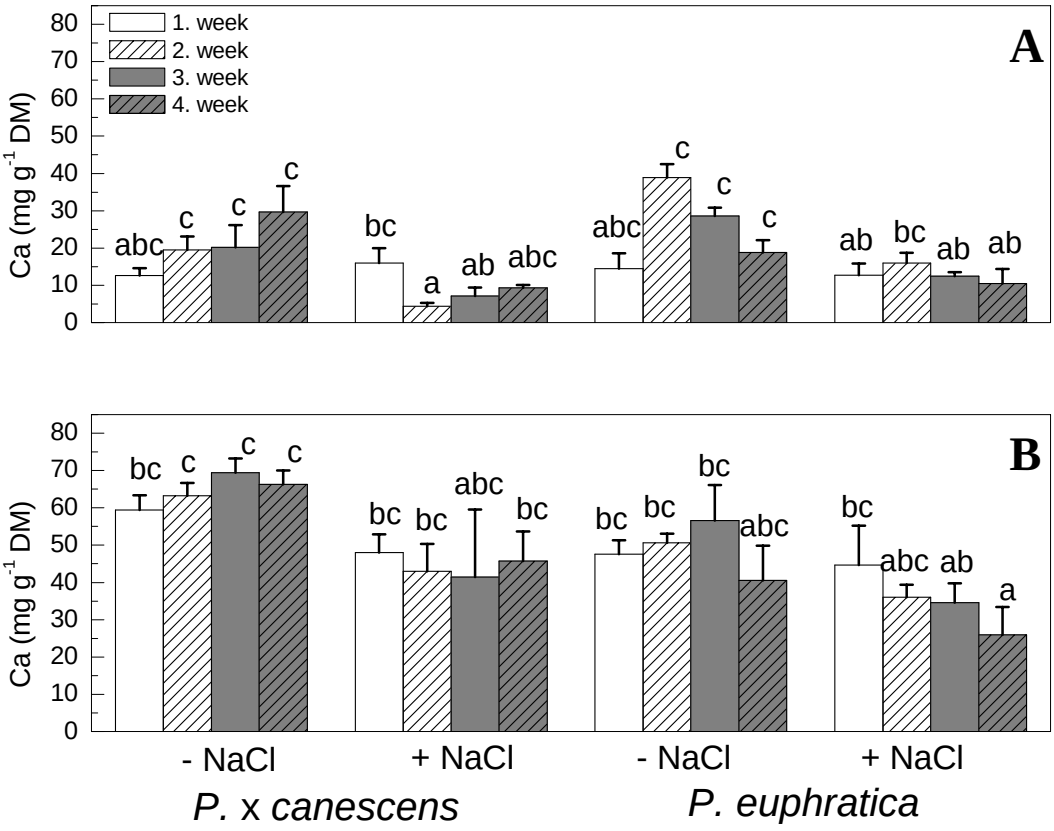

Fig. 3.5: Calcium concentrations in new leaves (A) and old leaves (B) of *P.* x *canescens* and *P. euphratica* exposed to weekly increasing NaCl concentrations. Plants were treated with 25 mM (white) in the first week, 50 mM (white, hatched) in the second week, 100 mM (grey) in the third week and 150 mM (grey, hatched) in the fourth week. Old leaves (B) were developed before the start of the NaCl treatment and new leaves (A) were developed during the exposure to NaCl. Plants were harvested weekly. Data represent means ± SD; n = 3 - 4. Significant differences at $P \leq 0.05$ are marked by different letters.

3.2.2 Influence of salinity on the Ca^{2+} and Na^{+} root concentrations of poplar

Because roots are the primary site of ion uptake, Ca^{2+} and Na^{+} concentrations were determined in the roots of *P. euphratica* and *P.* x *canescens* after acclimation to 25 mM and 150 mM NaCl, respectively.

Both poplar species exposed to NaCl showed an increased Na^{+} root concentration compared to control plants (Fig. 3.6 A). Furthermore, the Na^{+} concentration in the roots of both poplar species exposed to 25 mM and 150 mM NaCl was similar (Fig. 3.6 A). The Na^{+} concentration was 6-fold enhanced for *P.* x *canescens* and was 5-fold increased for *P. euphratica* exposed to 150 mM NaCl (Fig. 3.6 A).

For both poplar species, the Ca^{2+} content in root did not differ for plants exposed to 25 mM NaCl or 150 mM NaCl (Fig. 3.6 B).

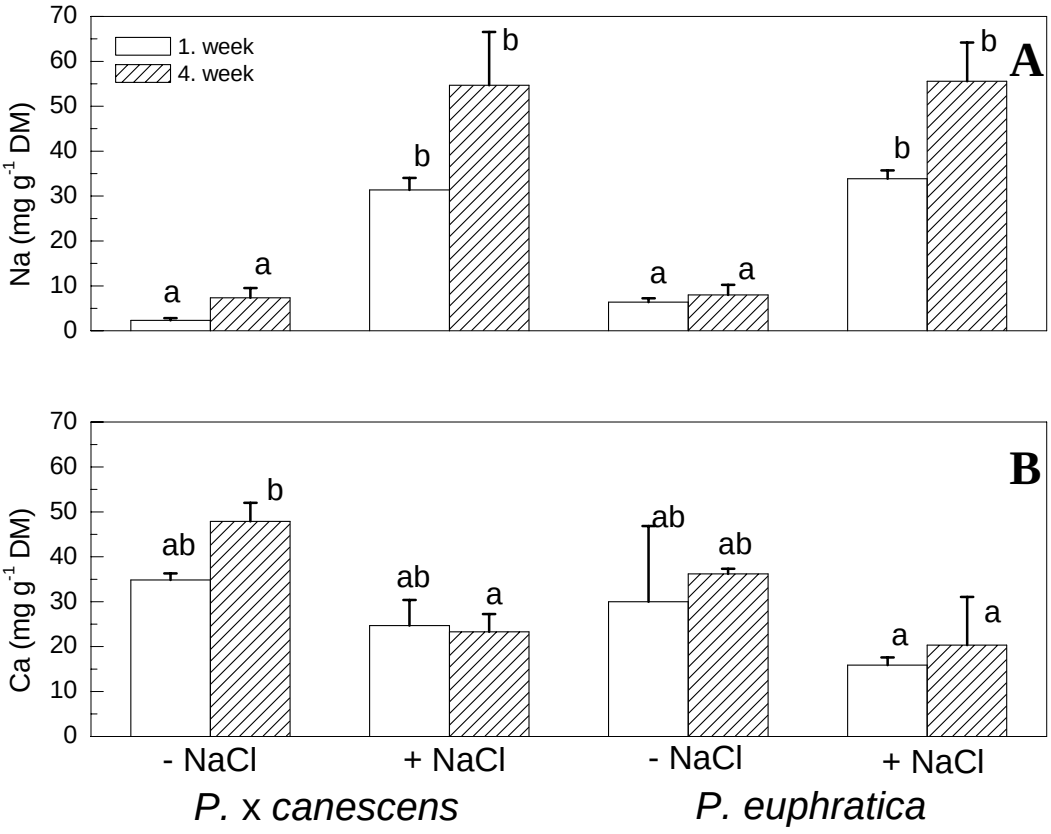

Fig. 3.6: Sodium (A) and calcium concentrations (B) in roots of *P.* x *canescens* and *P. euphratica* treated with NaCl. Plants were exposed to weekly increasing NaCl concentrations starting with 25 mM NaCl (white) to final concentrations of 150 mM NaCl (25 mM, 50 mM, 100 mM and 150 mM NaCl) (hatched). Data represent means ± SD; n = 3 - 4. Significant differences at $P \leq 0.05$ are marked by different letters.

3.2.3 Influence of Ca^{2+} deficiency on the performance of *P. euphratica* and *P.* x *canescens* under high salinity

Since excess Na^{+} can displace Ca^{2+} (see 1.4), it has been suggested that symptoms of NaCl stress may appear because of lacking Ca^{2+}. To investigate the influence of Ca^{2+} deficiency on the performance of *P. euphratica* and *P.* x *canescens*, plants were exposed to different NaCl concentrations with and without addition of Ca^{2+} in the hydroponic solution (Tab. 3.5).
The lack of Ca^{2+} in the nutrient solution led to plant death for both poplar species exposed to NaCl after 28 days of treatment (Tab. 3.5).

Table 3.5: Influence of Ca^{2+} deficiency on *P. euphratica* and *P.* x *canescens* under three different NaCl concentrations (see 2.3.6). The survival rate of plants (in %) is indicated after 14 / 28 days of NaCl treatment. n = 5 plants.

Survival rate of *P. euphratica* (in %) after (14/28) days of treatment			
	0 mM NaCl	25 mM NaCl	150 mM NaCl
0 mM Ca^{2+}	80 / 40	0 / 0	0 / 0
1 mM Ca^{2+}	100 / 100	100 / 100	100 / 100
Survival rate of *P.* x *canescens* (in %) after (14/28) days of treatment			
0 mM Ca^{2+}	100 / 0	100 / 0	0 / 0
1 mM Ca^{2+}	100 / 100	100 / 100	80 / 0

Furthermore, the lack of Ca^{2+} resulted in leaf wilting (Fig. 3.7 C,D) under salinity within two weeks of NaCl treatment for *P. euphratica*. This is in contrast to Ca^{2+} deficiency treatment without NaCl (Fig. 3.7 B) and controls (Fig. 3.7 A). In the absence of Ca^{2+}, the roots became dark, resulting in rotting of root tissue (Fig. 3.7 E).
These data indicate, that the plants performance under salinity is not attributable to root rotting due to the lack of Ca^{2+}, but is due to NaCl.

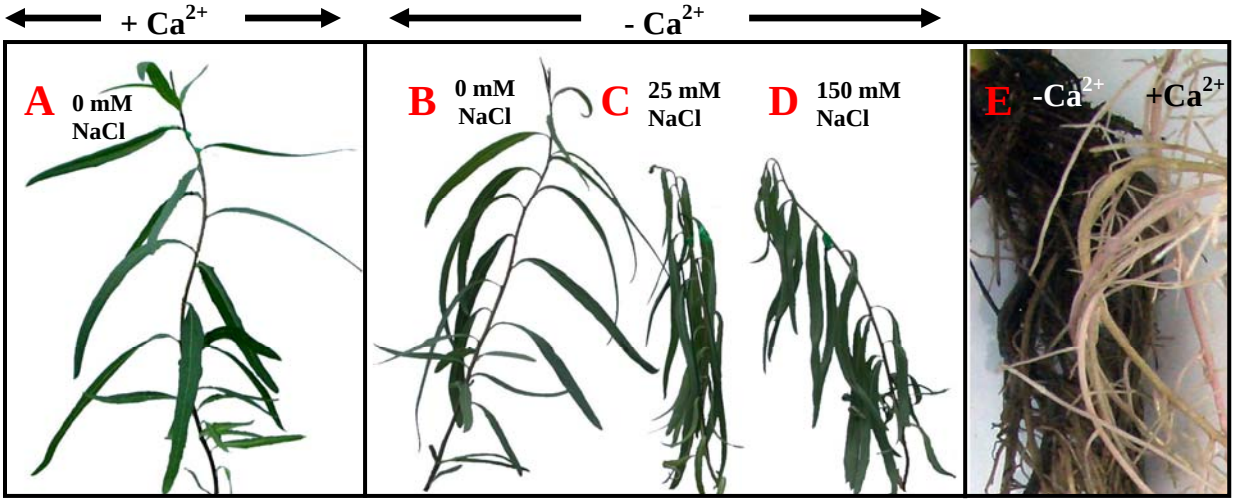

Fig. 3.7: Influence of Ca^{2+} deficiency on the performance *P. euphratica* under different NaCl concentrations. *P. euphratica* plants after 7 days without Ca^{2+} and 0 mM NaCl (B), 25 mM NaCl (C) and 150 mM NaCl (D) in the nutrient solution and controls (A). The plants of both NaCl treatments showed leaf wilting in contrast to the control (C,D). (E) Roots of *P. euphratica* plants after 7 days without (left) and with (right) Ca^{2+} in the nutrient solution.

3.2.4 Uptake of Ca^{2+} in *P. euphratica* and *P.* x *canescens* under the influence of salt

The results of the element analysis of leaves and roots showed a decrease in the concentration of Ca^{2+} in leaves of *P. euphratica* and *P.* x *canescens* under salinity (see Fig. 3.4, 3.5). To investigate, whether both polar species differ in Ca^{2+} distribution, *P. euphratica* and *P.* x *canescens* were labelled with $^{45}Ca^{2+}$ for three days and afterwards exposed to weekly increasing NaCl concentration to final concentrations of 150 mM (25 mM, 50 mM, 100 mM and 150 mM NaCl).

Both species accumulated the same whole-plant amount of $^{45}Ca^{2+}$, indicating a similar uptake of Ca^{2+} in both poplar species (Tab. 3.6).

Tab. 3.6: Uptake of $^{45}Ca^{2+}$ (in percent of the total amount) and total $^{45}Ca^{2+}$ uptake (in KBq) of *P. euphratica* and *P.* x *canescens*. Plants were exposed to 520 KBq of $^{45}Ca^{2+}$ in the external solution for three days. Data represent means ± SD; n = 29 plants.

	P. euphratica	***P.* x *canescens***
Uptake of $^{45}Ca^{2+}$ (in percent of total amount)	14.5 ± 0.06	14.0 ± 0.09
Total uptake [KBq]	76.03 ± 29.94	73.86 ± 46.60

Furthermore, $^{45}Ca^{2+}$ uptake in relation to the plants biomass was similar in for both poplar species (Fig. 3.8). This result indicates a similar accumulation of Ca^{2+} under control conditions for both poplar species.

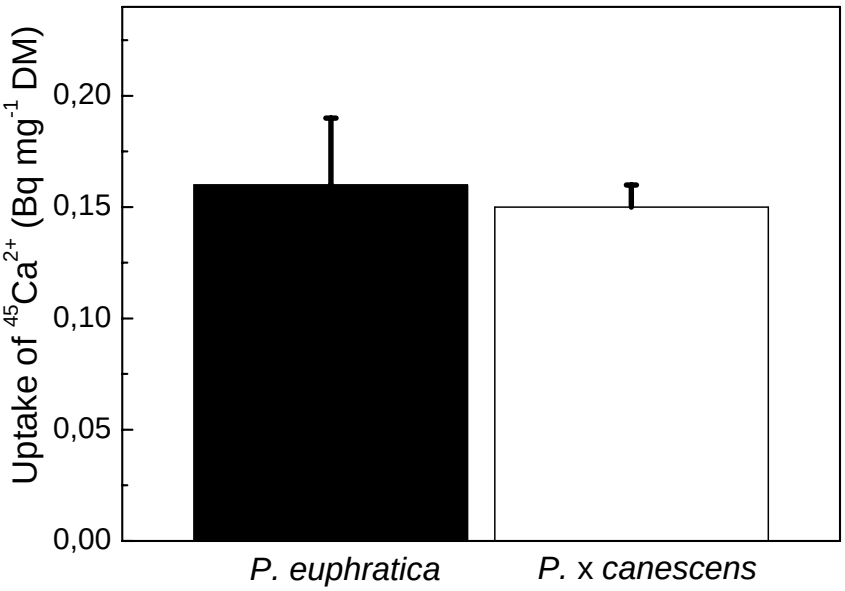

Fig. 3.8: Mean uptake of $^{45}Ca^{2+}$ in relation to dry mass of whole plants of *P. euphratica* and *P.* x *canescens*, respectively. Plants were exposed in the nutrient solution to $^{45}Ca^{2+}$ for three days and harvested afterwards. Data represent means ± SD; n = 3.

After three days of exposure, poplar species were harvested to investigate the distribution of $^{45}Ca^{2+}$. Autoradiography was used to image the pattern of $^{45}Ca^{2+}$ distribution (Fig. 3.9). Both species accumulated $^{45}Ca^{2+}$ mainly in old, bigger leaves (Fig. 3.9 A,C). For both plant species, a huge amount of $^{45}Ca^{2+}$ was located in the stem, indicating an ongoing transport of Ca^{2+}. Leaves of *P.* x *canescens* displayed a higher amount of $^{45}Ca^{2+}$ in leaf veins compared to the mesophyll.

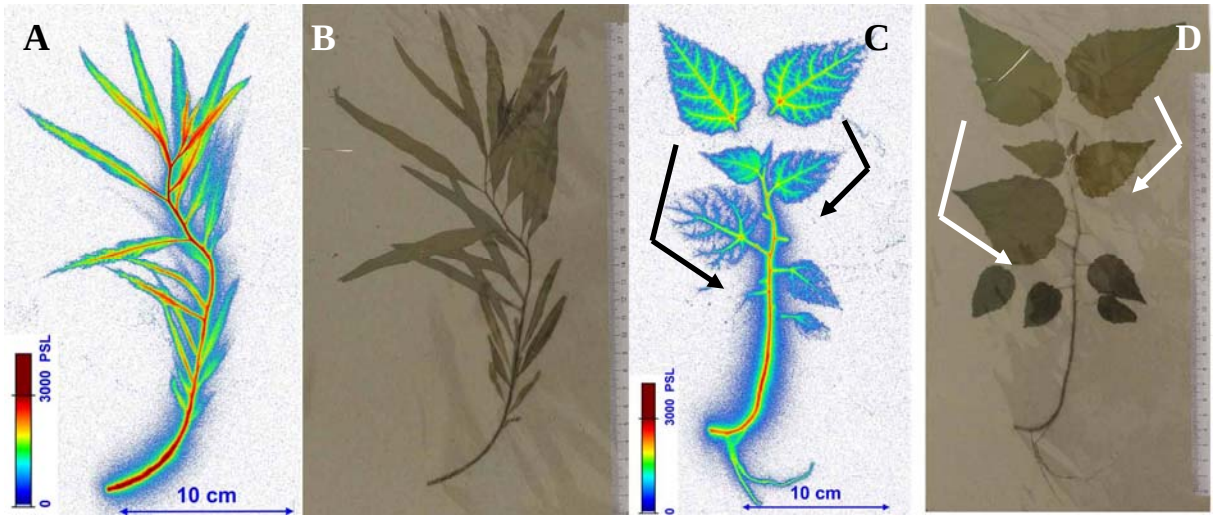

Fig. 3.9: Representative autoradiograms and photographs of *P. euphratica* (A,B) and *P.* x *canescens* (C,D) shoots after 3 days of labelling with $^{45}Ca^{2+}$ in the hydroponic solution. The colours from blue, over green to red indicate an increasing amount of incorporated $^{45}Ca^{2+}$. Arrows indicate position of detached leaves.

To find out, whether there are differences in the distribution of Ca^{2+} under NaCl exposure and whether there is a retranslocation of Ca^{2+} in both poplar species under salinity, the labelled *P. euphratica* and *P.* x *canescens* were harvested after two weeks of weekly increasing NaCl concentrations (25 mM, 50 mM) and used to image the $^{45}Ca^{2+}$ distribution in both species.

Autoradiograms showed no differences in the pattern of $^{45}Ca^{2+}$ shoot accumulation for *P.* x *canescens* between controls (Fig. 3.10 A,B) and NaCl exposed plants (Fig. 3.10 C,D). For both treatments, the $^{45}Ca^{2+}$ distribution was highest in old leaves and lowest in the newest, top leaves (Fig. 3.10 A,C).

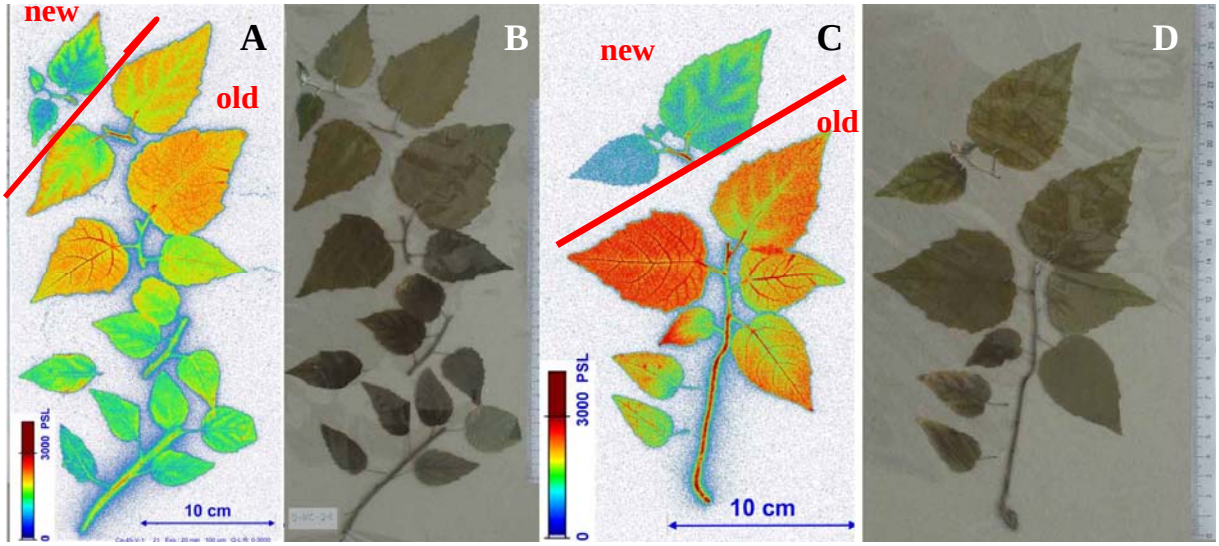

Fig. 3.10: Representative autoradiograms and photographs of *P.* x *canescens* shoot after two weeks of treatment without (A,B) and with NaCl (C,D) treatment. The plants were labelled with $^{45}Ca^{2+}$ for three days. Afterwards, plants were exposed to weekly increasing NaCl concentrations, starting at 25 mM NaCl to final concentrations of 50 mM NaCl. The red line separates new and old leaves. Old leaves were developed before the start of the NaCl treatment and new leaves were developed during the exposure to NaCl. The colours from blue, over green to red indicate an increasing amount of incorporated $^{45}Ca^{2+}$.

In *P. euphratica*, differences in the distribution of Ca^{2+} between both treatments were observed. Only a small amount of $^{45}Ca^{2+}$ was accumulated in new leaves of NaCl treated plants (Fig. 3.11 C,D). In contrast, $^{45}Ca^{2+}$ was accumulated in new leaves of control plants (Fig. 3.11 A,B).

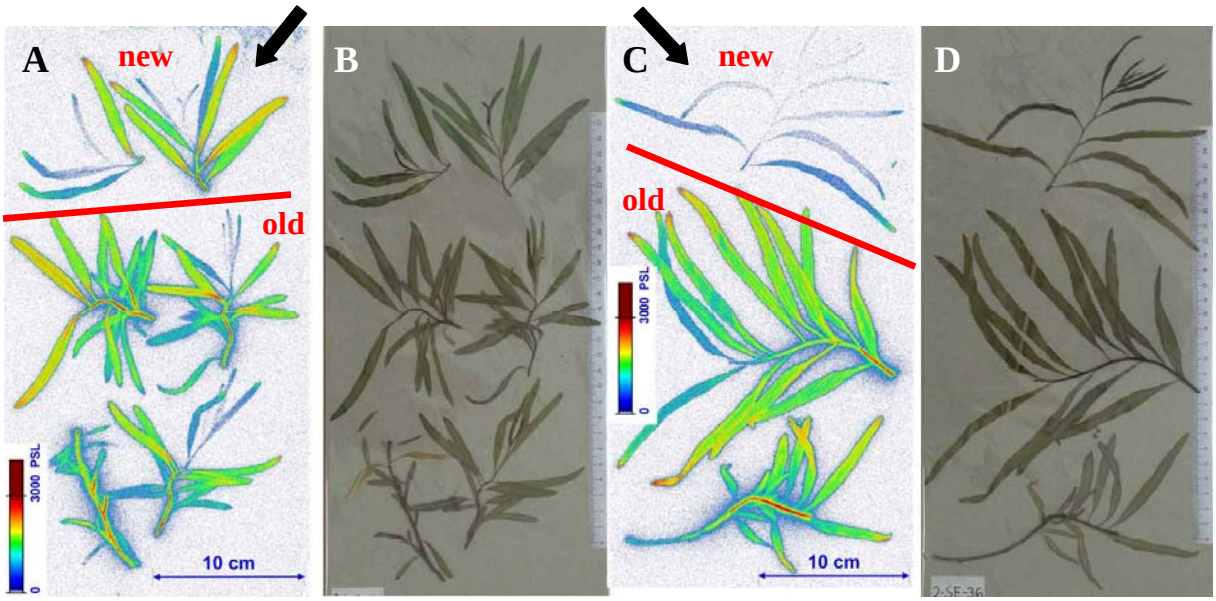

Fig. 3.11: Representative autoradiograms and photographs of *P.* euphratica shoot after two weeks of treatment without (A,B) and with NaCl (C,D) treatment. The plants were labelled with $^{45}Ca^{2+}$ for three days. Afterwards, plants were exposed to weekly increasing NaCl concentrations, starting at 25 mM NaCl to final concentrations of 50 mM NaCl. The red line separates new and old leaves. Old leaves were developed before the start of the NaCl treatment and new leaves were developed during the exposure to NaCl. The colours from blue, over green to red indicate an increasing amount of incorporated $^{45}Ca^{2+}$. Arrows indicate differences in the distribution of $^{45}Ca^{2+}$ in new leaves between both treatments.

Autoradiograms indicate that the NaCl tolerant *P. euphratica* and sensitive *P.* x *canescens* were distinguishable in their Ca^{2+} distribution under NaCl treatment. *P. euphratica* exposed to NaCl did not accumulate Ca^{2+} in new, developing leaves, in contrast to *P.* x *canescens*. This seemed to contrast previous element analysis, which showed that Ca^{2+} accumulation was stronger in new leaves of *P. euphratica* than in those of *P.* x *canescens* (Fig. 3.5).

To investigate this apparently contrasting result, Ca^{2+} distribution in *P. euphratica* was investigated during NaCl treatment (see 2.3.5). Plants were adapted to final concentrations of 100 mM NaCl with weekly increasing NaCl concentration (see 2.3). *P. euphratica* were labelled with $^{45}Ca^{2+}$ for the last three days during exposure to 100 mM NaCl. Afterwards, a subset of plants were harvested whereas the others were exposed to 150 mM NaCl for two weeks and harvested weekly.

The mean uptake of $^{45}Ca^{2+}$ was measured in *P. euphratica* exposed to 100 mM NaCl. The uptake of $^{45}Ca^{2+}$ in control plants was 2-fold higher compared to NaCl treated *P. euphratica*. This indicates a suppression of Ca^{2+} uptake under salinity (Fig. 3.12).

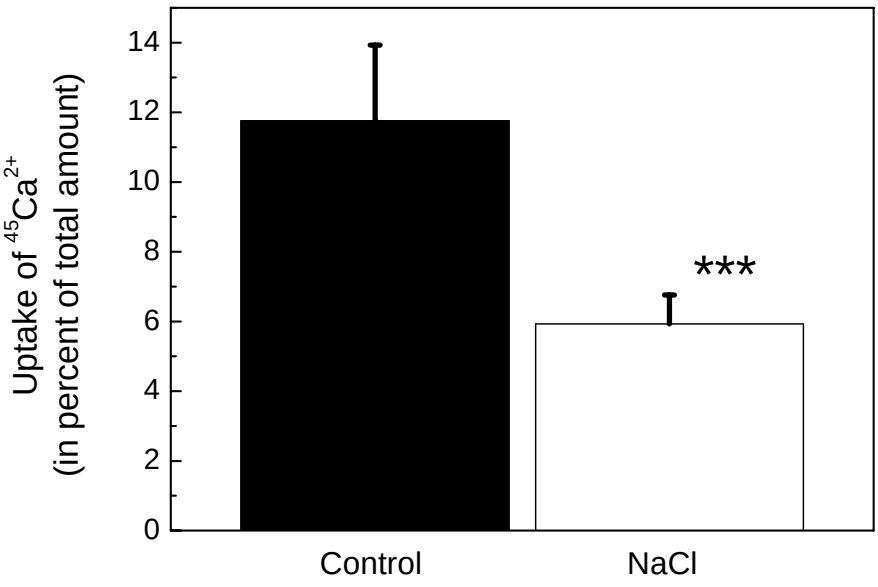

Fig. 3.12: Mean uptake of $^{45}Ca^{2+}$ (in percent of the total amount) in control (black bar) and NaCl treated (white bar) *P. euphratica*. Plants were exposed to weekly increasing concentration of NaCl starting with 25 mM, to final concentrations of 150 mM NaCl (see 2.3). Plants were placed in 1 l pots for radioactive labelling with 520 KBq $^{45}Ca^{2+}$ during the last three days of the exposure to 100 mM NaCl. Afterwards, plants were exposed to 150 mM NaCl for a chase period of two weeks. Plants were harvested after the radioactive labelling and after each week of exposure to 150 mM NaCl. n = 12. Data represent means ± SD. *** indicates significant differences at $P \leq 0.001$.

Autoradiograms were carried out to image the distribution of $^{45}Ca^{2+}$ in *P. euphratica* under salinity. Plants were harvested immediately after their labelling with $^{45}Ca^{2+}$ for three days during exposure to 100 mM NaCl.

Autoradiograms of the controls (Fig 3.13 A,B) showed a high accumulation of $^{45}Ca^{2+}$ at the top leaves of the plant, indicating preferential allocation of $^{45}Ca^{2+}$ to the youngest, newly developed leaves. The accumulation of $^{45}Ca^{2+}$ in NaCl treated *P. euphratica* (Fig. 3.13 C,D) showed an enhanced accumulation of $^{45}Ca^{2+}$ in the top leaves, but displayed no $^{45}Ca^{2+}$ in old leaves.

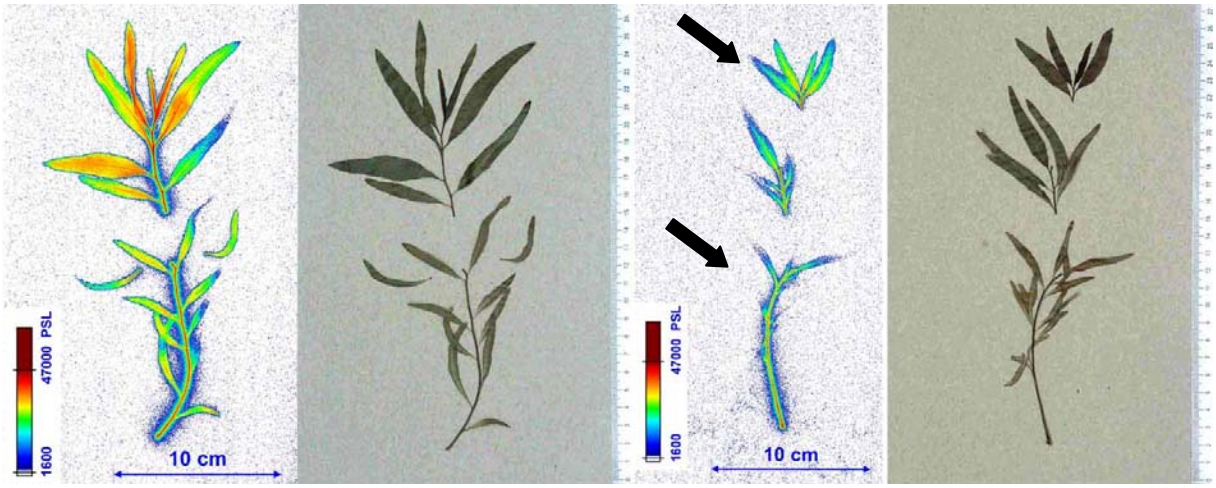

Fig. 3.13: Representative autoradiograms and photographs of *P. euphratica* shoot after 3 days of additional $^{45}Ca^{2+}$ in control (A,B) and NaCl treatment (C,D). Plants were exposed to weekly increasing concentrations of NaCl starting with 25 mM to final concentrations of 150 mM NaCl (see 2.3). The radioactive labelling was conducted during last three days of the exposure to 100 mM NaCl and plants were harvested afterwards. The colours from blue, over green to red indicate an increasing amount of incorporated $^{45}Ca^{2+}$. Arrow indicates the accumulation of $^{45}Ca^{2+}$ in new leaves of NaCl treated *P. euphratica*.

To investigate the distribution of the incorporated $^{45}Ca^{2+}$ in *P. euphratica* under salinity, plants were treated as described above and afterwards exposed to 150 mM NaCl for a chase period of two weeks. Afterwards, plants were harvested and autoradiograms were made.

Control plants showed an increased accumulation of $^{45}Ca^{2+}$ in old, large leaves of *P. euphratica* (Fig. 3.14 A). In contrast, NaCl treated plants showed an increased $^{45}Ca^{2+}$ accumulation in the youngest, developing leaves (Fig. 3.14 C). This distribution of $^{45}Ca^{2+}$ suggest an enhanced transport of Ca^{2+} to the newest, developing leaves in *P. euphratica* under salinity.

These results indicate differences between both poplar species in response to salinity. *P. euphratica* maintains Ca^{2+} transport to new, developing leaves under salinity (Fig. 3.14). In contrast, *P.* x *canescens* had a decreased Ca^{2+} allocation to new, developing leaves under salinity (Fig. 3.10). *P. euphratica* showed no decrease in shoot growth under these moderate conditions of salinity (Fig. 3.1 B), in contrast to *P.* x *canescens* that diminished shoot growth. These results indicate differences in the plants response to salinity in relation to their ability of maintenance Ca^{2+} shoot transport.

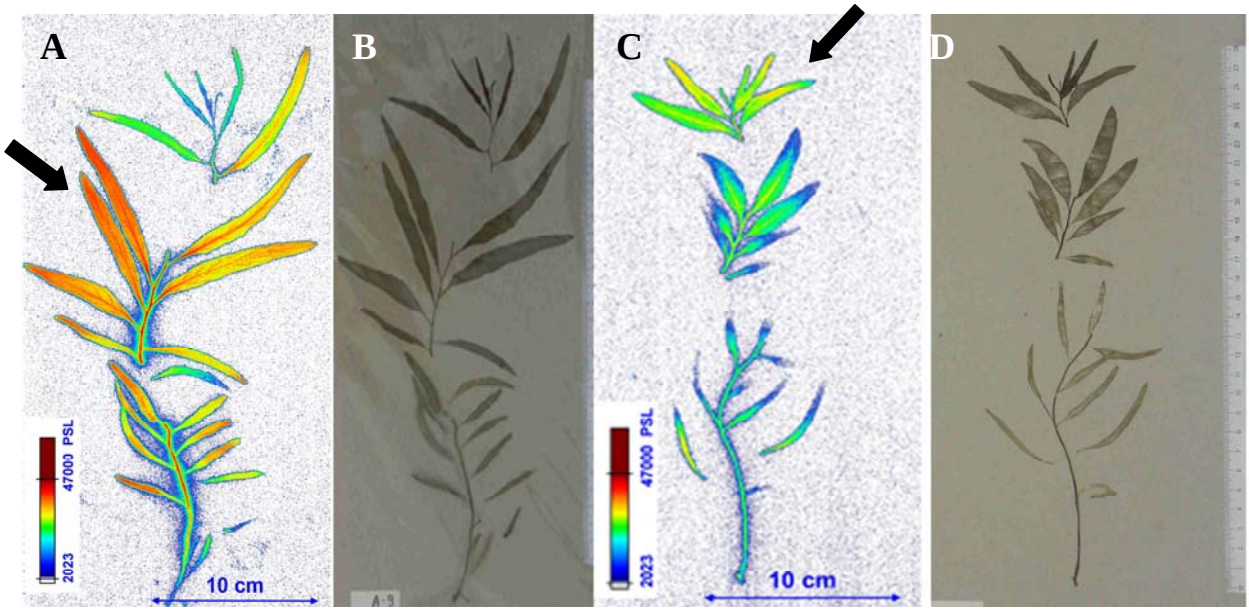

Fig. 3.14: Representative autoradiograms and photographs of control (A,B) and NaCl treated (C,D) *P. euphratica* shoots. Plants were exposed to weekly increasing concentrations of NaCl starting with 25 mM, to final concentrations of 150 mM NaCl (see 2.3). During the last three days of exposure to 100 mM NaCl, radioactive labelling with $^{45}Ca^{2+}$ was conducted. Afterwards, plants were exposed to 150 mM NaCl for two weeks. The colours from blue, over green to red indicate an increasing amount of incorporated $^{45}Ca^{2+}$. Arrows indicate the main accumulation of $^{45}Ca^{2+}$ in the shoot of *P. euphratica* for both treatments.

Element analysis were performed to determine the concentration of Na^+ and Ca^{2+} in *P. euphratica* leaves under salinity (Fig. 3.15).

The Na^+ concentration was 6-fold increased in old leaves compared to the controls (Fig. 3.15 A). In new leaves the Na^+ concentration was increased for plants exposed to 100 mM NaCl (Fig. 3.15 B), but showed no differences for the exposure to 150 mM NaCl compared to controls. This indicates that *P. euphratica* is able to protect its new leaves against Na^+ accumulation.

The Ca^{2+} concentration in old and new leaves of plants exposed to NaCl was the half of the concentration in the controls (Fig. 3.15 C,D), indicating a 2-fold decrease in Ca^{2+} uptake under salinity, but showed no significant differences for new leaves exposed to 150 mM NaCl, indicating an increased Ca^{2+} allocation to new, developing leaves.

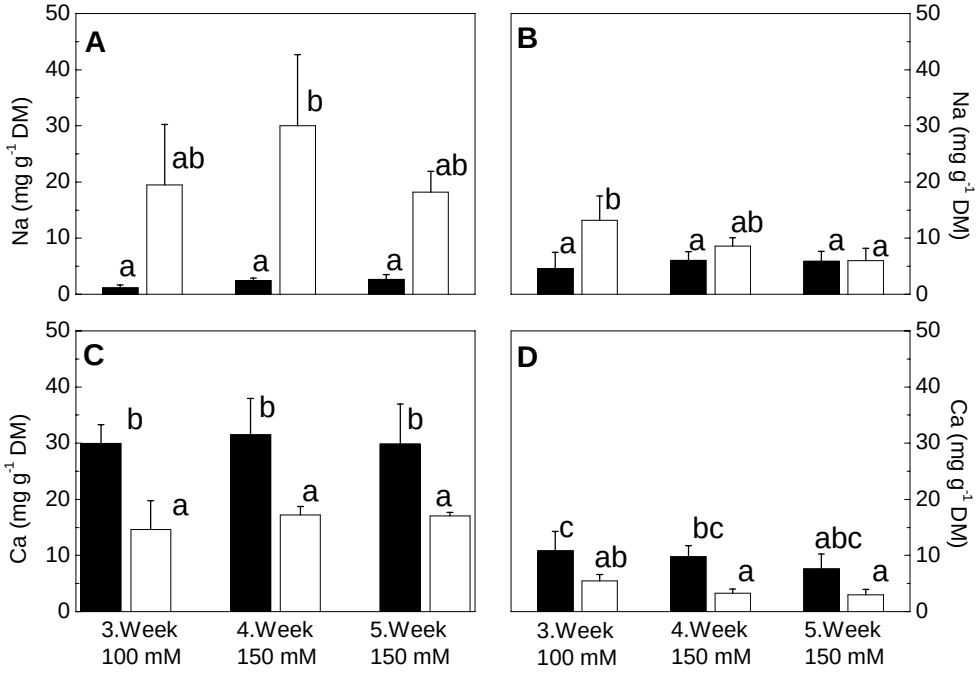

Fig. 3.15: Sodium (A,B) and calcium (C,D) concentration in old (left) and new (right) leaves of *P. euphratica* treated without (■) and with NaCl (□). Plants were exposed to weekly increasing NaCl concentrations starting with 25 mM NaCl, to final concentrations of 150 mM NaCl. Plants were exposed to the final NaCl concentration for combined two weeks. Data represent means ± SD; n = 3 - 4. Different letters indicate significant differences at $P \leq 0.05$.

To analyse the root to shoot transport of Ca^{2+} of both poplar exposed to NaCl and to analyse whether Ca^{2+} recirculation takes place, autoradiograms of the roots were analysed.

Plants were exposed to $^{45}Ca^{2+}$ for three days (Fig. 3.16). Afterwards, plants were exposed to final concentrations of 150 mM NaCl with weekly increasing NaCl concentration (see 2.3) (Fig. 3.16 C,D). A subset of plants of *P. euphratica* and *P.* x *canescen*s was harvested after the exposure to radioactive label (Fig. 3.16 A,B for *P. euphratica*; Fig. 3.16 E,F for *P.* x *canescens*). Autoradiograms of these roots showed $^{45}Ca^{2+}$ in the whole roots. Furthermore, plants of both species were harvested after a chase period of 4 weeks (Fig. 16 C,D for *P. euphratica*; Fig. 16 G,H for *P.* x *canescens*). Autoradiograms showed that $^{45}Ca^{2+}$ was present in a small part of the coarse roots located at the transition area to the stem for both plant species.

P. euphratica and *P.* x *canescens* showed no differences in Ca^{2+} distribution in roots for plants of the same treatment (Fig. 3.16).

Since radioactivity was not detected in the nutrient solution (data not shown), these data indicate that $^{45}Ca^{2+}$ was unlikely transported from the shoot to the roots of the plant, concluding that Ca^{2+} recirculation did not occur.

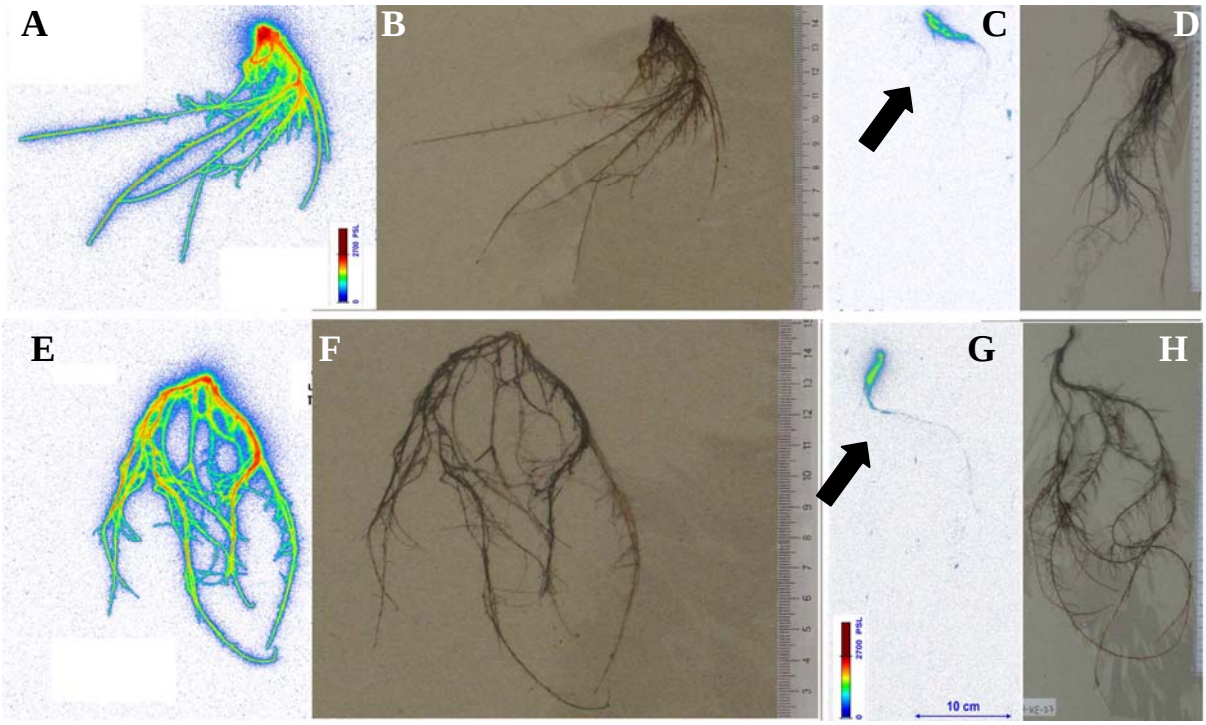

Fig. 3.16: Representative autoradiograms and photographs of *P. euphratica* (A–D) and *P.* x *canescens* (E-H) roots after 3 days of additional $^{45}Ca^{2+}$ in the nutrient solution (A,B) and after a chase period of four weeks without NaCl in the hydroponic solution (C,D). The colours from blue, over green to red indicate an increasing amount of incorporated $^{45}Ca^{2+}$. Arrow indicates the accumulation of $^{45}Ca^{2+}$ in the root.

3.3 Characterisation of root anatomy, morphology and Na^+ uptake in response to high salinity in *P. euphratica*

3.3.1 Influence of salt stress on root morphology of *P. euphratica*

Since the roots are the main site for ion uptake, the performance of roots of *P. euphratica* under salinity was investigated. *P. euphratica* plants were exposed to weekly increasing concentrations of either NaCl or KCl to final concentrations of 150 mM in steps of 25 mM, 75 mM and 150 mM, respectively. Afterwards, the plants were maintained at their final salt concentrations for 20 days. A further subset of plants was treated with 25 mM NaCl for the same exposure time. All plants were harvested after 34 days of NaCl treatment. The root morphology was analyzed as described above (2.4.2).

An increase in the surface area of the root, an increase in the total root length and a higher number of root tips were measured for 25 mM NaCl treated plants compared to control and to high salt treatments with KCl or NaCl (Tab. 3.7).

Tab. 3.7: Root morphology of *P. euphratica* exposed to NaCl or KCl. Plants were treated with weekly increasing NaCl or KCl concentrations to final concentrations of 150 mM (25 mM, 75 mM, 150 mM NaCl or KCl, respectively) and exposed to their final concentration for 20 days. Further plants were treated with 25 mM NaCl for the same time of exposure. After 34 days of treatment, plants were harvested. Analysis of whole root system were performed by using WinRHIZO analysis software (see 2.4.2). Measurements of the average diameter of root tips were carried out for randomly selected (5 – 10) root tips of one whole root system. Data represent means ± SD; n = 3 - 5 plants. Significant differences at $P \leq 0.05$ are marked by different letters.

Parameter		0 mM NaCl	25 mM NaCl	150 mM NaCl	150 mM KCl
Surface area	[cm²]	252.5 ± 53.3 ^{b}	357.9 ± 43.4 ^{c}	222.4 ± 25.4 ^{b}	94.1 ± 32.7 ^{a}
Total root length	[cm]	646.71 ± 94.27 ^{a}	1367.25 ± 193.63 ^{b}	748.00 ± 162.04 ^{a}	434.33 ± 56.20 ^{a}
Number of root tips		511.5 ± 100.9 ^{a}	942.8 ± 165.7 ^{b}	502.5± 164.9 ^{a}	432.5 ± 128.7 ^{a}
Average diameter whole root system	[cm]	0.121 ± 0.011 ^{b}	0.085 ± 0.005 ^{a}	0.089 ± 0.022 ^{a}	0.068 ± 0.013 ^{a}
Average diameter root tip	[cm]	0.11 ± 0.02 ^{ab}	0.11 ± 0.00 ^{bc}	0.14 ± 0.01 ^{c}	0.08 ± 0.01 ^{a}

The mean diameter of all roots of the whole root decreased for all NaCl treatments compared to controls (Tab. 3.7). In contrast to the whole root system, the mean diameter of root tips increased after the exposure to 150 mM NaCl compared to the controls and compared to plants exposed to 150 mM KCl. The reason for this was that high NaCl concentration induced

swelling of roots of *P. euphratica* leading to a cob-like appearance. These roots that were formed only under high NaCl, but not under high KCl, were named cob roots (Fig. 3.17). This effect is specific for *P. euphratica*, because it was not detected for *P.* x *canescens*.

Under high KCl the mean diameter of root tips and the surface area of the whole root system were decreased to controls and plants exposed to 150 mM NaCl (Tab. 3.7). These results point out, that the development of cob roots is an ion specific effect of high Na^+, because root thickening was not occurring in the presence of KCl treated roots.

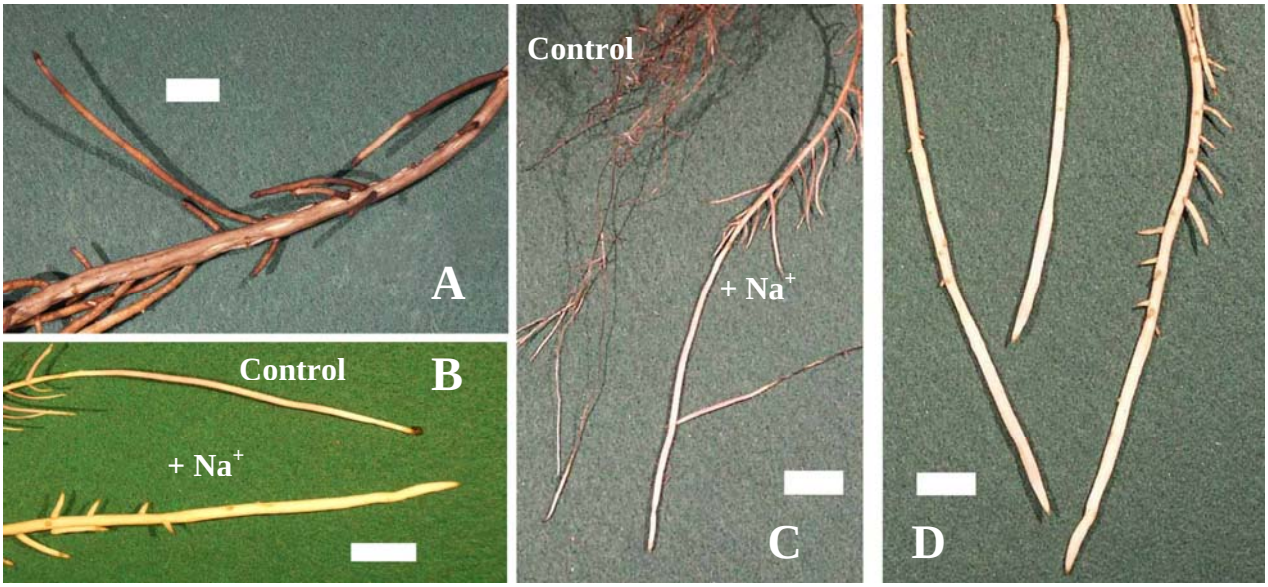

Fig. 3.17: Changes in the morphology of *P. euphratica* under high salinity. Plants were adapted to final concentrations of 150 mM NaCl (25 mM, 75 mM, 150 mM NaCl) and exposed to their final NaCl concentration for at least 20 days to initiate the development of cob roots. (A) Cob root *P. euphratica* after exposure to 150 mM NaCl. (B,C) Cob root and control. (D) Several cob root tips of one single *P. euphratica*. Bars indicate 1 cm length.

To investigate whether the surprising differences in root tip diameter were caused by swelling of cells or formation of additional cell layers, root anatomy was analysed (see 2.5).

P. euphratica were adapted to final concentrations of 150 mM NaCl (see 2.3) and exposed to this concentration for additional 3 weeks.

Cob roots displayed a 2-fold increase in root diameter compared to controls (Fig. 3.18; Tab. 3.8).

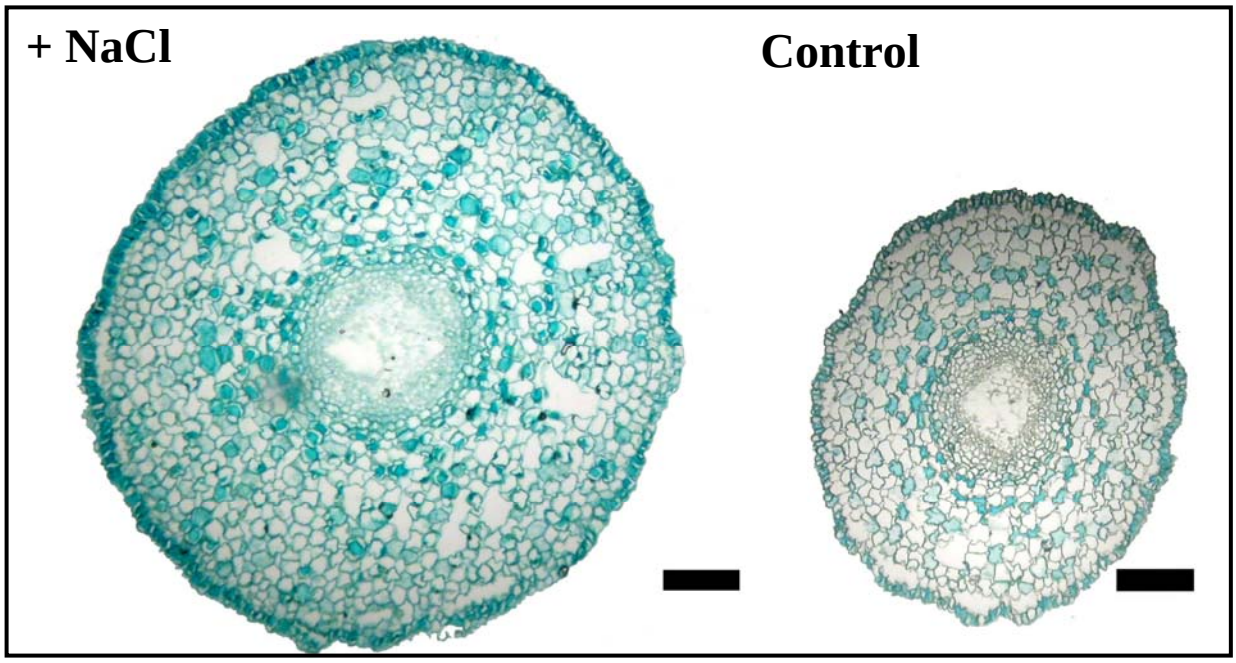

Fig 3.18: Cross section of NaCl adapted (left) and control root (right) of *P. euphratica*. Plants were adapted to final concentrations of 150 mM NaCl (see 2.3) and exposed to their final NaCl concentration for additional 3 weeks to initiate the development of cob roots. Afterwards, plants were harvested. Root tip samples were prepared as described under 2.5. The magnification was 2.5x. The black bar indicates 1 mm.

The cortex width of cob roots and the number of cell layers in the cortex both showed a 2-fold increase (Tab. 3.8). This observation indicates that the swelling of roots is caused by an increase in the number of cortex cells and not by an increase in cell volume.

The thickening of adapted roots took place only at few root tips of *P. euphratica* and not for the whole root system. This explains differences in the measurement of the average diameter of root tips (Tab. 3.7), that was carried out for few root tips of one root system, and the root diameter measurement of cross sections (Tab. 3.8), which was performed for one single root tip of the whole root system.

Tab. 3.8: Anatomical analysis of control and cob roots of *P. euphratica*. Plants were NaCl adapted to final concentrations of 150 mM (see 2.3) and exposed to their final NaCl concentration for additional 21 days to initiate the development of cob roots. Root tips of both treatments were cut and analyzed under the microscope using ImageJ software. Data indicate means (± SD) of n = 6 - 9. *** indicate significant differences at $P \leq 0.001$.

		Control	**NaCl adapted**
Root diameter	[cm]	0.40 ± 0.02	0.82 ± 0.05***
Cortex width	[cm]	0.13 ± 0.01	0.32 ± 0.02***
Cell layers in the cortex		6.21 ± 0.32	11.57 ± 0.67***
Total cross sectional of root	[cm^2]	0.13 ± 0.01	0.51 ± 0.06***
Number of cells per 1 mm^2 cortex area		26.08 ± 4.93	26.55 ± 10.13

3.4 The significance of cob roots for the NaCl tolerance of *P. euphratica*

3.4.1 Influx of Na^+ in cob roots

To find out, whether cob roots influence Na^+ influx and Na^+ accumulation in root tissue, short term (1 min - 30 min) and long term (30 min – 8 h) exposure to $^{22}Na^+$ was performed. A single attached root tip of these salt-adapted plants was placed in a separate tube, containing the radioactively labelled solution. The remaining root system was placed in a 1 l pot. Both compartments also contained 150 mM NaCl (see 2.3.1).

Before the experiment, *P. euphratica* were adapted to final concentrations of 150 mM NaCl with weekly increasing NaCl concentration (see 2.3). Plants maintained at 150 mM NaCl in the hydroponic solution for additional three weeks. For comparison, *P. euphratica* not previously adapted to high salinity were also analysed.

The uptake of $^{22}Na^+$ per g FM of the roots was enhanced in non-adapted root tips compared to NaCl adapted root tips for root tips exposed to nutrient solution containing 150 mM NaCl for 30 min to 8 h (Fig. 3.19 A). After 8 h of exposure, non-adapted root tips had a 4-fold increased $^{22}Na^+$ influx, compared to adapted root tips. This results indicates, that cob roots function in decreasing Na^+ uptake under salinity.

The accumulation of $^{22}Na^+$ per surface area in cob roots was similar to that of control roots (Fig. 3.19 C, D). This result indicates, that the root surface does not play an important role in

the Na^+ exclusion. But the higher root volume leads to a larger dilution of NaCl during short-term uptake and may provide larger storage capacities for salinity than thinner roots.

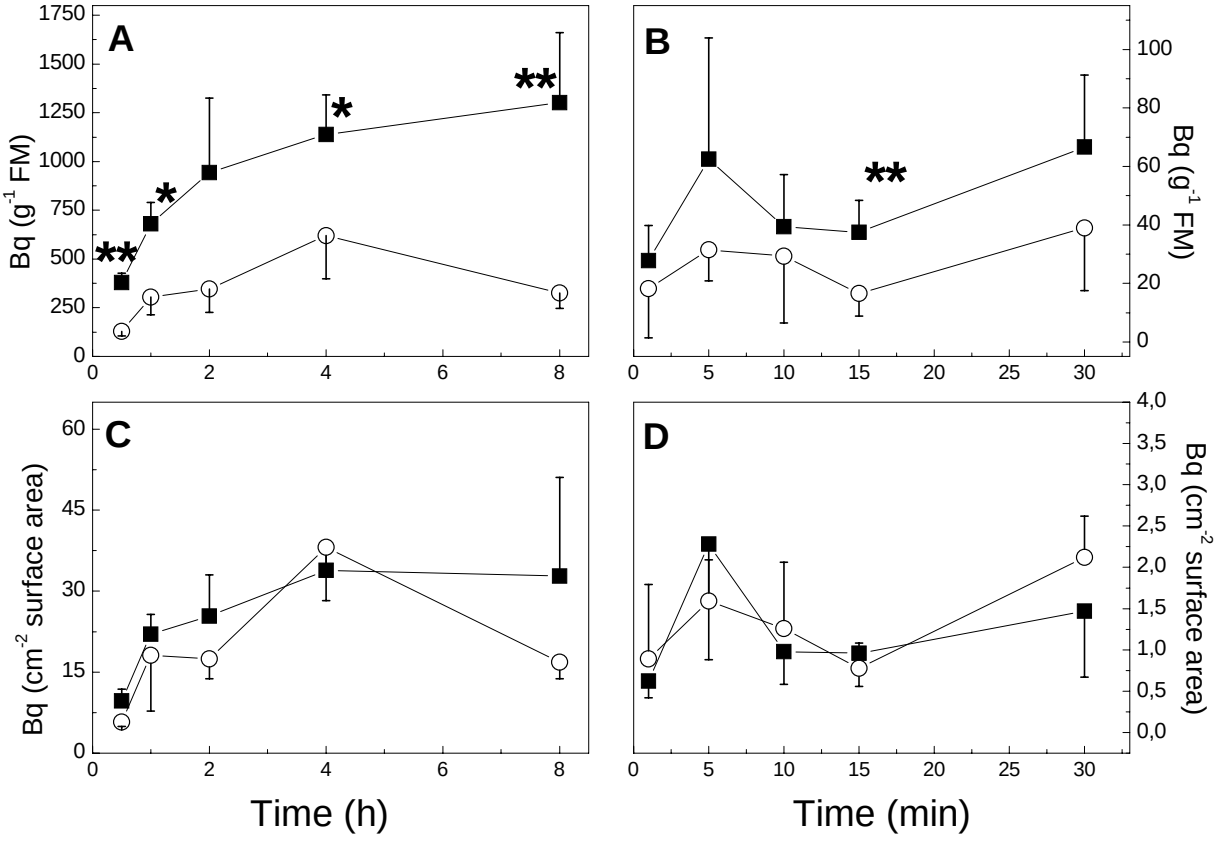

Fig. 3.19: Influx of $^{22}Na^+$ into adapted (cob roots) (○) and non-adapted (■) roots of *P. euphratica*, in relation to the FM of the roots (A,B) and the surface area of the roots (C,D). Plants were adapted to final concentrations of 150 mM NaCl with weekly increasing NaCl concentrations (see 2.3). Afterwards, plants were exposed to their final concentration for three weeks. Plants were exposed to 76 KBq (1 – 30 min) and 57 KBq (30 min – 8 h), respectively (see 2.3.1). Symbols indicate means ± SD; n = 5 plants. * indicates significant differences at $P \leq 0.05$ and ** at $P \leq 0.01$.

3.4.2 Effects of cob roots on Na^+ uptake and Na^+ distribution

To investigate whether cob roots have a function for the NaCl tolerance of *P. euphratica*, *P. euphratica* were adapted with weekly increasing NaCl concentrations in the nutrient solution to final concentrations of 150 mM NaCl (see 2.3). Afterwards, the final concentration was maintained for furthermore three weeks. Control plants were grown for the same period of time in the absence of NaCl addition. The transport studies started after cob root formation was

observed. At the end of this adaptation phase salt accumulation was monitored by electrolyte measurements. Roots of NaCl adapted plants showed higher conductivity than that of controls (Fig. 3.20).

Subsequently, all plants were placed in fresh nutrient solution in the absence of NaCl. After 48 h, excess NaCl was removed from the roots as evident from a decrease in tissue conductivity to control levels (Fig. 3.20).

Afterwards, plants without and with NaCl preadaptation were treated with 300 mM NaCl (shock treatment) labelled with additional 330 KBq $^{22}Na^{+}$. After a 24 h labelling period, plants were exposed to non-labelled hydroponic solution containing 300 mM NaCl and harvested after a chase period of 24 h. Salt shock caused a decrease in electrolyte conductivity in both treatments.

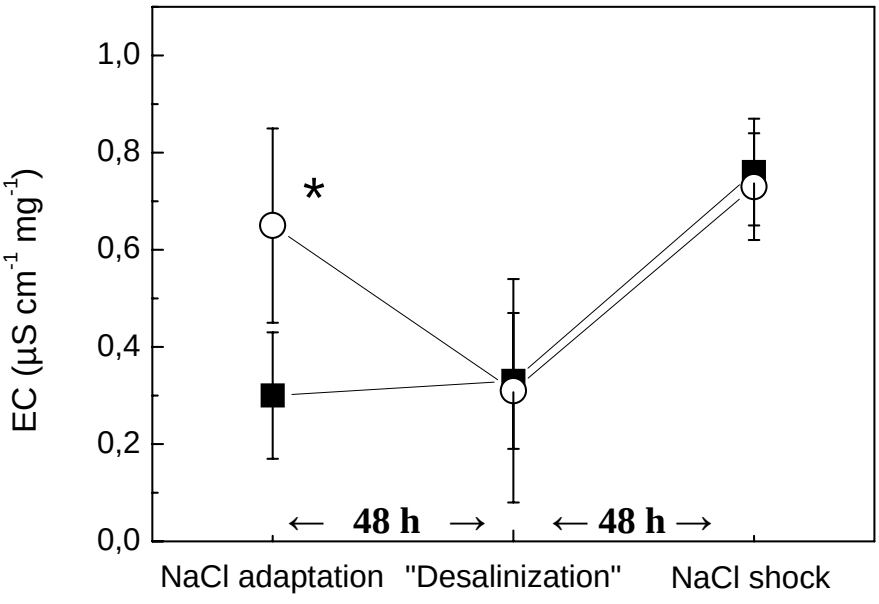

Fig. 3.20: Electrolyte conductivity (EC) of non-adapted (control) roots (■) and NaCl adapted roots (cob roots) (○) of *P. euphratica*. Plants were exposed with weekly increasing NaCl concentration to final concentrations of 150 mM NaCl (see 2.3.1). Plants were exposed to 150 mM NaCl for furthermore 3 weeks (NaCl adaptation) and afterwards placed in control nutrient solution for 2 days ("Desalinization"). Following this treatment, plants were treated with 300 mM NaCl for 2 days (NaCl shock). Measurements were conducted as described under 2.6.2. Data represent means ± SD; n = 2 – 8 samples. * indicate significant differences at $P \leq 0.05$.

The uptake of $^{22}Na^{+}$ within 24 h of NaCl shock treatment differed significantly between NaCl adapted and non-adapted plants (Tab 3.9). Since the biomass of the root system of NaCl adapted plants was similar to those of non-adapted plants, the uptake of $^{22}Na^{+}$ of non-adapted

plants was 2-fold higher compared to adapted plants. This finding indicates that the preadaptation decreased the accumulation of $^{22}Na^{+}$ under high NaCl concentration.

Tab. 3.9: Uptake of $^{22}Na^{+}$ within 24 h of NaCl shock (300 mM) in percent of the total offered $^{22}Na^{+}$ in the labelled solution. Plants were treated as described above (3.4.2). For NaCl shock treatment, hydroponic solution were labelled with additional $^{22}Na^{+}$ for 24 h. Data represent means ± SD; n = 4. ** indicates significant differences at $P \leq 0.01$.

		non-adapted	**NaCl adapted**
Uptake of $^{22}Na^{+}$ (in percent of total applied $^{22}Na^{+}$)	[%]	1.40 ± 0.32	0.61 ± 0.24**
Biomass (DM root)	[mg]	264 ± 51.6	254 ± 56.7

To investigate Na^{+} allocation after 48 h of NaCl shock treatment, plants were harvested. The shoots of the harvested plants were divided into three parts of the same stem length. The roots were divided into coarse and fine roots, depending on the thickness of the roots, and the transition area to the stem.

At the whole plant level, the accumulation of $^{22}Na^{+}$ was 2-fold higher in non-adapted plants than in adapted plants (Tab. 3.10). A decrease in $^{22}Na^{+}$ accumulation from the apex to bottom of the stem and from top to lower leaves was observed for both treatments, indicating an enhanced Na^{+} accumulation under NaCl shock in the youngest parts of the plant.

Significant differences in $^{22}Na^{+}$ accumulation between the two treatments for the roots were also detected. Non-adapted plants had a higher incorporation of $^{22}Na^{+}$ in all root parts, compared to adapted plants (Tab. 3.10).

Because the accumulation of $^{22}Na^{+}$ was about 1.5 to 3-fold higher in all parts of salt-shocked compared to adapted plants, it is assumed both treatments differ in the amount of Na^{+} taken up. The results therefore suggest that cob roots contribute to decrease the flux of Na^{+} into the shoot and thus provide an important protective mechanism.

Tab. 3.10: Total amount of $^{22}Na^{+}$ [Bq] and concentration of $^{22}Na^{+}$ [Bq / mg DW] in different tissues of *P. euphratica* after 48 h of NaCl shock. Labelled $^{22}Na^{+}$ was applied for 24 h of NaCl shock (300 mM) and a chase period of 24 in hydroponic solution containing the same NaCl concentration (300 mM NaCl). Plants were treated as described above (3.4.2) and harvested after 48 h of NaCl shock treatment. Data are means (± SD) of n = 4 plants. * indicates differences at $P \leq 0.05$, ** at $P \leq 0.01$ and *** at $P \leq 0.001$.

Leaves		**non-adapted**	**NaCl adapted**
Top	[Bq]	330.1 ± 102.9	116.9 ± 79.6*
Middle	[Bq]	294.6 ± 149.8	235.3 ± 163.7
Bottom	[Bq]	120.5 ± 44.6	82.6 ± 64.0
Stem			
Top	[Bq]	392.0 ± 45.4	152.7 ± 42.3***
Middle	[Bq]	454.2 ± 77.4	157.2 ± 31.1***
Bottom	[Bq]	430.1 ± 98.0	153.2 ± 35.2**
Roots			
Transition	[Bq]	53.3 ± 19.1	29.2 ± 11.7
Coarse root	[Bq]	42.1 ± 12.9	13.5 ± 6.6**
Fine root	[Bq]	211.1 ± 64.6	103.4 ± 47.6*

Leaves		**non-adapted**	**NaCl adapted**
Top	[Bq / mg DM]	8.42 ± 2.65	4.10 ± 1.68**
Middle	[Bq / mg DM]	3.41 ± 0.92	3.98 ± 1.70
Bottom	[Bq / mg DM]	4.77 ± 2.56	2.16 ± 1.50*
Stem			
Top	[Bq / mg DM]	7.78 ± 0.78	2.45 ± 0.66***
Middle	[Bq / mg DM]	3.85 ± 0.97	1.12 ± 0.19***
Bottom	[Bq / mg DM]	2.42 ± 0.37	0.87 ± 0.12***
Roots			
Transition	[Bq / mg DM]	1.68 ± 0.48	0.89 ± 0.24*
Coarse root	[Bq / mg DM]	1.59 ± 0.15	0.44 ± 0.14***
Fine root	[Bq / mg DM]	0.90 ± 0.31	0.50 ± 0.13*
Total plant biomass	DM [mg]	1103.1 ± 146.1	983.5 ± 194.7
Total plant uptake	[KBq]	267.6 ± 63.9	117.8 ± 54.7*
Mean concentration of $^{22}Na^{+}$ per whole plant	[Bq / mg DM]	4.02 ± 0.63	1.95 ± 0.58**

Additionally, element analysis were carried out to determine the Na^{+} concentration in the leaves and the roots of NaCl shocked plants. NaCl-adapted plants showed a 2-fold higher concentration of Na^{+} in the middle and bottom leaves than salt-shocked non-adapted plants

(Fig. 3.21). These higher concentrations in the leaves of adapted than in non-adapted plants was probably due to the long time of previous NaCl exposure. Since old leaves and new leaves of *P. euphratica* had an Na^+ concentration of 40 mg per g DM in previous experiment (see Fig. 3.4), the present concentration of 35 – 45 mg suggest that only little additional Na^+ was accumulated in the leaves during NaCl shock treatment in adapted plants. In contrast, non-adapted *P. euphratica* displayed massive Na^+ accumulation after 48 h (Fig. 3.21). Differences in the Na^+ concentrations in roots of both treatments were not observed (Fig. 3.21), suggesting that NaCl adaptations had no influence on Na^+ root concentrations but on Na^+ shoot transfer.

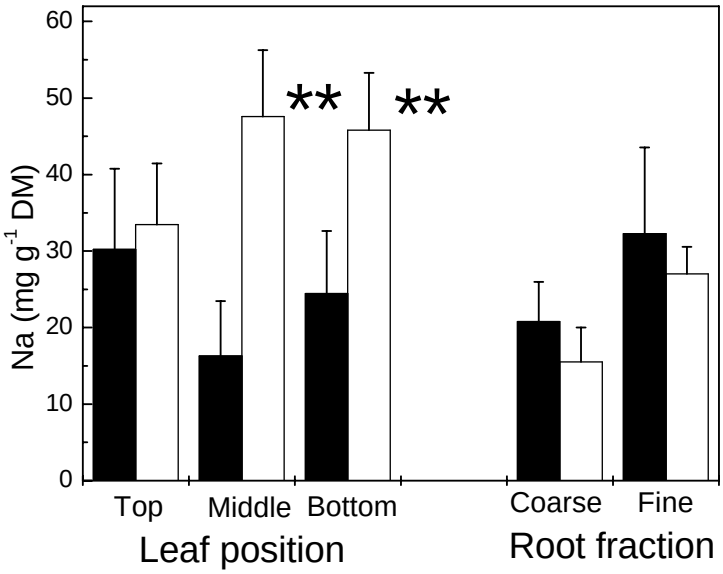

Fig. 3.21: Na^+ concentration in leaves and roots after 48 h of exposure to 300 mM NaCl for non-adapted (■) and NaCl adapted (□) *P. euphratica*. Plants were treated as described above (3.4.2). Data represent means ± SD; n = 4 plants. ** indicates significant differences at $P \leq 0.01$.

3.4.3 Effects of NaCl shock treatment on plants performance NaCl adapted *P. euphratica*

To find out, whether the increased Na^+ accumulation in non-adapted plants compared to the Na^+ concentration in leaves of NaCl adapted plants caused enhanced physiological stress, the quantum yield of PS II was determined (see 2.6.1) during 48 h of NaCl shock treatment (see 3.4.2). The quantum yield Φ decreased in NaCl shocked plants after 48 h of treatment (Fig. 3.22). Non-adapted plants displayed a higher decrease in the quantum yield of PS II after 48 h

of NaCl shock, than to adapted plants, indicating an increased NaCl resistance in NaCl adapted *P. euphratica*.

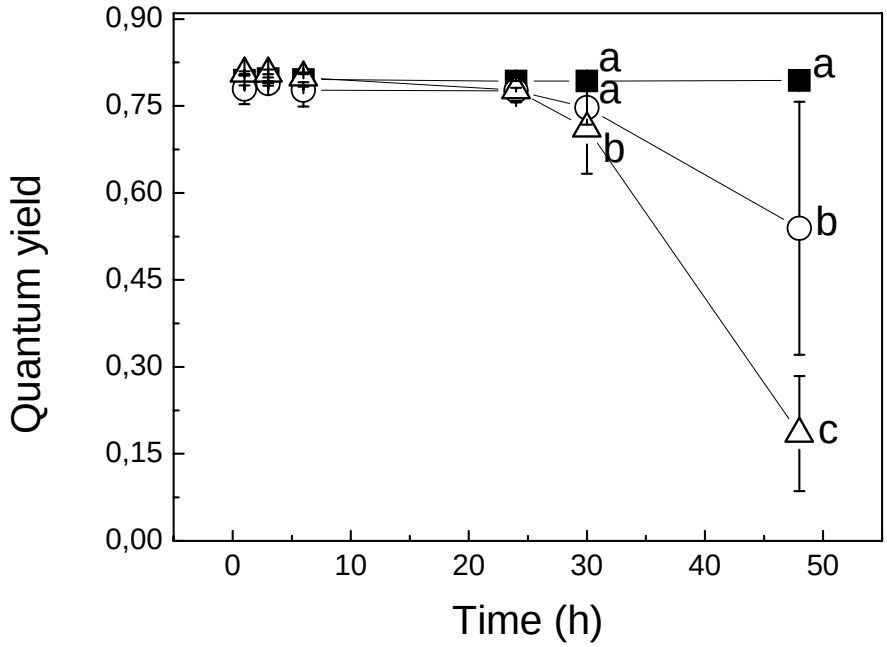

Fig. 3.22: Quantum yield of PS II of NaCl shocked adapted (○), NaCl shocked non-adapted (Δ) and non-shocked (■) plants. Plants were treated as described under 3.4.2. Measurements were performed as described under 2.6.1. Data represent means ± SD; n = 2 – 8 plants. Significant differences at $P \leq 0.05$ are marked by different letters.

These results on quantum yield of PS II of adapted and non-adapted *P. euphratica* corroborate visual observations: non-adapted plants that were NaCl shocked had curled and dried leaves (Fig 3.23 C) as symptoms of osmotic stress. NaCl adapted plants with cob roots performed better (Fig. 3.23 B), because only few leaves at the top of the plant were wilting and in contrast to non-adapted plants, no symptoms of curling or drying of leaves were observed.

These results indicate that NaCl adaptation and the development of cob roots in *P. euphratica* result in a decrease in Na^+ uptake, leading to an enhanced NaCl resistance of *P. euphratica*.

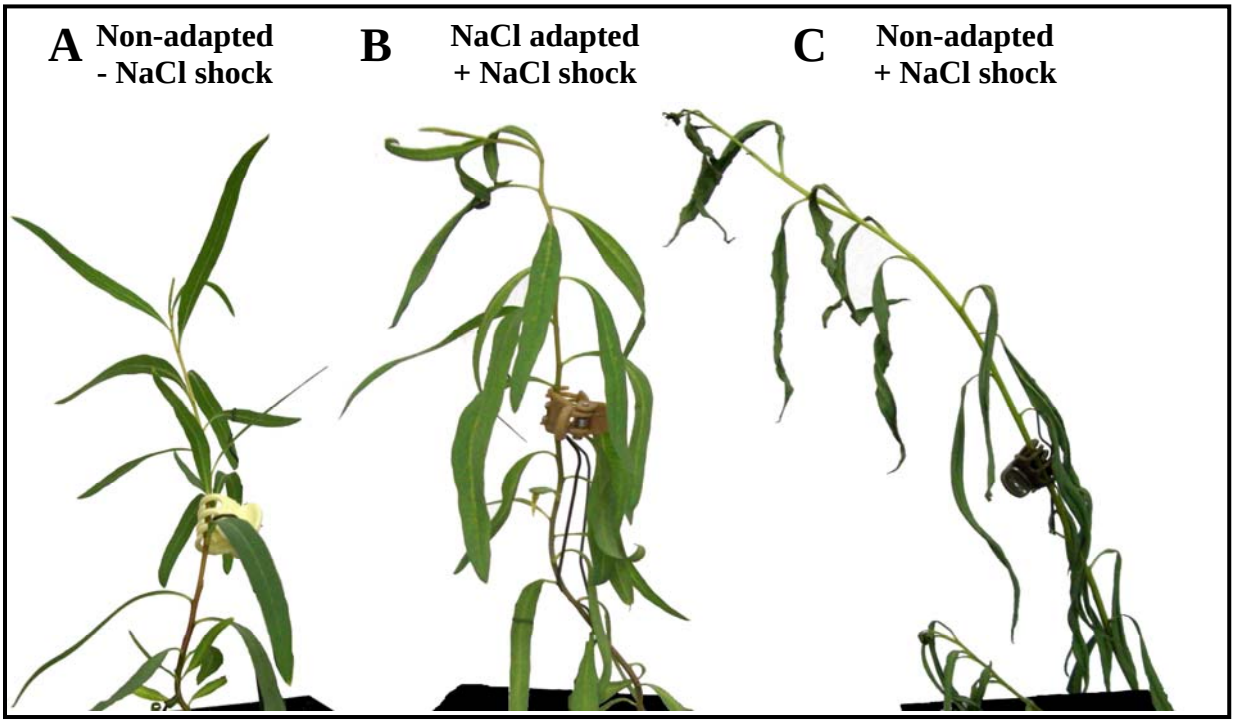

Fig. 3.23: Non-adapted (A,C) and NaCl adapted (B) *P. euphratica* after 48 h of 300 mM NaCl (B) shock treatment (C) and after 48 h of control treatment (A). Plants were adapted as described under 3.4.2.

3.5 Na^+ transport and Na^+ distribution in *P.* x *canescens* and *P. euphratica* under salinity

To analyse, whether both poplar species differ in their Na^+ transport and Na^+ distribution in response to salinity, a split root experiment and a leaf feeding experiment were carried out.
Due to the differences in Na^+ tolerance in both species, *P. euphratica* and *P.* x *canescens* were exposed to different external NaCl concentrations. Since *P.* x *canescens* displayed leaf damage in response to 150 mM NaCl (Fig. 3.3) and plants died after few days of exposure to this NaCl level, a NaCl concentration for *P.* x *canescens* of 75 mM was applied to determine Na^+ transport over a longer period of time. Therefore, Na^+ transport experiments were performed at external concentrations of 75 mM NaCl for *P.* x *canescens* and 150 mM NaCl for *P. euphratica*.

3.5.1 Na^+ transport and Na^+ distribution in *P.* x *canescens*

Split root experiment

To investigate whether Na^+ recirculation takes place in *P.* x *canescens* a split root experiment was carried out (see 2.3.2). Additionally, Na^+ xylem transport rates were calculated to find out whether both poplar species can be distinguished by different Na^+ xylem transport rates and whether Na^+ release via the roots plays a role for NaCl tolerance of *P. euphratica*.

Plants were adapted to final concentrations of 75 mM NaCl (see 2.3). Radioactive labelling was performed during the week before the final NaCl concentration was reached. Before labelling the root systems were separated and exposed to NaCl in two compartments. The label was added only to one compartment (see 2.3.2) and the plants were exposed for 7 days to 50 mM NaCl solution. After labelling, the radioactive solution was removed and NaCl concentrations were increased to 75 mM NaCl. *P.* x *canescens* were harvested 21 days after starting the radioactive labelling (see 2.3.2).

The uptake of $^{22}Na^+$ for plants exposed to 50 mM NaCl was measured for one week. *P.* x *canescens* incorporated about 7 % of the total amount of $^{22}Na^+$ (Tab. 3.11).

Tab. 3.11: Uptake of $^{22}Na^+$ (in percent of the total amount) for *P.* x *canescens* in split root experiment. Plants were exposed to weekly increasing NaCl concentrations starting with 25 mM NaCl up to a concentration of 50 mM NaCl for *P.* x *canescens*. Plants were labelled with additional $^{22}Na^+$ for one week. Afterwards, plants were exposed to their final concentration of 75 mM NaCl for 14 days and harvested. The amount of $^{22}Na^+$ that was taken up by the plant was calculated as the sum of $^{22}Na^+$ incorporated at the end of the experiment and $^{22}Na^+$ released during the experiment. Data represent means ± SD; n = 9 plants.

	P.* x *canescens
Uptake of $^{22}Na^+$ (in percent of total amount)	6.88 ± 4.29

The uptake of the tracer $^{22}Na^+$ was used to calculate the amount of total Na^+ take up from the hydroponic solution during one week assuming that the ratio of radioactivity taken up/total radioactivity in the hydroponic solution is directly proportional to the ratio of the amount of Na^+ taken up/total amount of Na^+ in the hydroponic solution (see equation 2.8).

It was assumed that only Na^+ present in the shoot of the plant was transported via the xylem. Based on this assumption, the total amount of Na^+ transported in the xylem sap of each *P.* x *canescens* was calculated (see equation 2.9). Since all losses in nutrient solution were caused

by transpiration, which is driven by water transport through the xylem, the volume of the xylem sap is the same as the loss of volume of nutrient solution (Tab. 3.12).

Using these data, the concentration of Na^+ in the xylem sap ($c_{\text{xylem sap}}$) and the Na^+ exclusion was calculated using equations 2.10 and 2.11 (see 2.6.7). The Na^+ exclusion of *P.* x *canescens* exposed to 50 mM NaCl for one week was 99.616 % which indicates that *P.* x *canescens* excluded a high fraction of external Na^+ from its shoot (Tab. 3.12).

The Na^+ exclusion (Tab. 3.12) was determined using calculated Na^+ xylem sap concentration. In contrast, uptake of $^{22}Na^+$ (Tab. 3.11) was measured using a different calculation. These differences explain the contrasting Na^+ incorporation rates.

Tab. 3.12: Exclusion of Na^+ for *P.* x *canescens* in split root experiment. Plants were exposed to weekly increasing NaCl concentrations starting with 25 mM NaCl up to a concentration of 50 mM NaCl for *P.* x *canescens*. Plants were labelled with additional $^{22}Na^+$ for one week. Afterwards, plants were exposed to their final concentration of 75 mM NaCl for 14 days and harvested.
The exclusion of Na^+ was calculated as described under 2.6.7. $c_{\text{Na+ sol.}}$ = concentration of Na^+ in the nutrient solution, $V_{\text{sol.}}$ = volume of the hydroponic solution, $\text{Bq}_{\text{taken up}}$ = amount of $^{22}Na^+$ taken up by the plant during one week, Bq_{total} = amount of $^{22}Na^+$ in the hydroponic solution, V_{xylem} = volume of the xylem sap = volume of loss of nutrient solution, $n_{\text{Na+ plant}}$ = amount of Na^+ taken up by the plant, P = portion of Na^+ in the shoot of the plant, $n_{\text{Na+ xylem}}$ = amount of Na^+ transported via the xylem, $c_{\text{Na+ xylem}}$ = concentration of Na^+ in the xylem sap.

$c_{\text{Na+ sol.}}$	[mmol/l]	50
$V_{\text{sol.}}$	[ml]	800
$\text{Bq}_{\text{taken up}}$	[KBq]	7.12 ± 3.74
Bq_{total}	[KBq]	108.4
V_{xylem}	[ml]	131 ± 8.9
$n_{\text{Na+ plant}}$	[mmol]	2.63 ± 1.38
P		0.9565
$n_{\text{Na+ xylem}}$	[mmol]	2.51 ± 1.32
$c_{\text{Na+ xylem}}$	[µmol/ml]	19.18 ± 10.31
Na^+ exclusion	[%]	99.616 ± 0.21

To find out the amount of Na^+ that was released by the plants via the roots into the hydroponic solution, the release of $^{22}Na^+$ was measured during and after radioactive labelling by analysing samples of the hydroponic solution daily (see 2.6.5 and 2.3.2).

During their exposure to 50 mM and 75 mM NaCl for 21 days, *P.* x *canescens* released about 75 % of incorporated $^{22}Na^+$ into the hydroponic solution (Fig. 3.24).

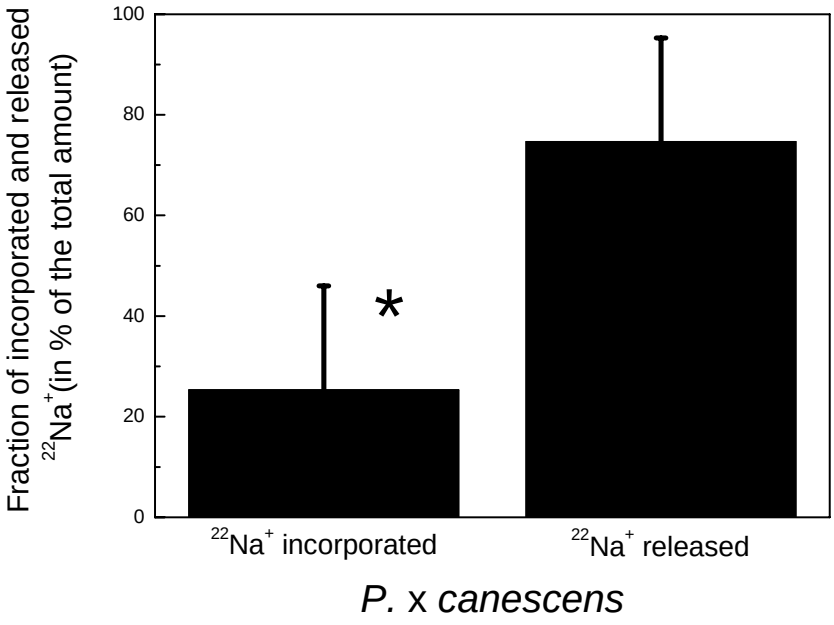

Fig. 3.24: Fraction of $^{22}Na^{+}$ incorporated in the plant and $^{22}Na^{+}$ released into the nutrient solution (in % of the total amount of $^{22}Na^{+}$ uptake) after two weeks of exposure to NaCl. Plants were adapted to final concentrations 75 mM NaCl with weekly increasing NaCl concentrations (see 2.3). Plants were labelled for one week during exposure to 50 mM NaCl. Afterwards, plants were exposed to their final concentration of 75 mM NaCl for two weeks. The amount of $^{22}Na^{+}$ released was analysed by measuring samples of the hydroponic solution daily. Data represents means ± SD; n = 8 plants. * indicates significant differences at $P \leq 0.05$.

P. x *canescens* released the highest amount of incorporated $^{22}Na^{+}$ into the non-labelled compartment of the split root box (Fig. 3.25). Since roots were separated in the split root system, this suggest transport of a large portion of Na^{+} uptake through the plant, indicating Na^{+} recirculation via the phloem.

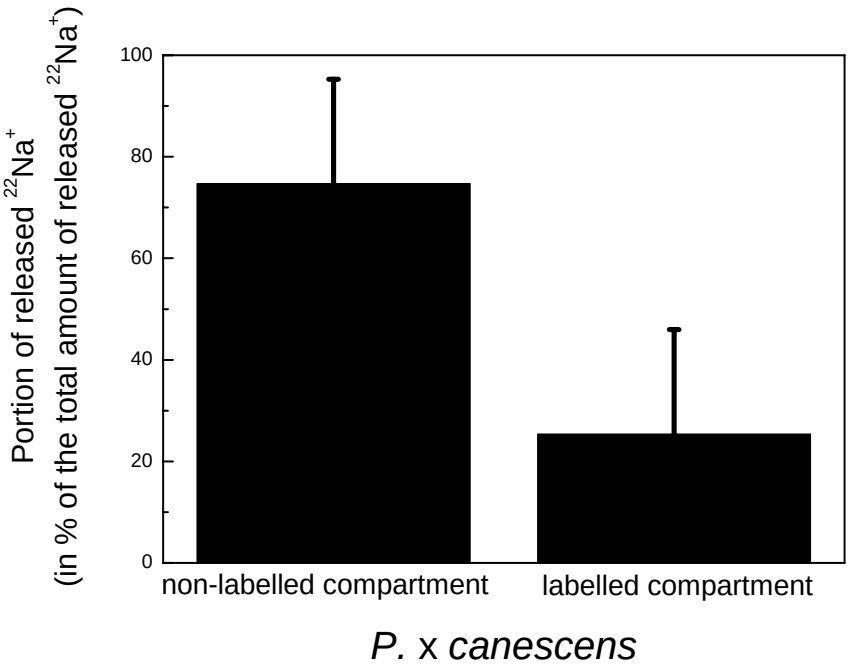

Fig. 3.25: Portion of $^{22}Na^{+}$ (in percent of the total amount of released $^{22}Na^{+}$) released by *P.* x *canescens* into the labelled and non-labelled compartment of a split root system under salinity. Plants were adapted to final concentrations of 75 mM NaCl with weekly increasing NaCl concentrations (25 mM, 50 mM and 75 mM NaCl). Plants were labelled for one week during their exposure to 50 mM NaCl. Afterwards, plants were exposed to their final concentration of 75 mM NaCl for two weeks. Data represent means ± SD; n = 8 plants.

The time course of Na^{+} release for *P.* x *canescens* was analysed (Fig. 3.26). *P.* x *canescens* released over 65 % of $^{22}Na^{+}$ uptake in the first week during the labelling phase (Fig. 3.26) and about 10 % of $^{22}Na^{+}$ uptake in the first week after the labelling period.

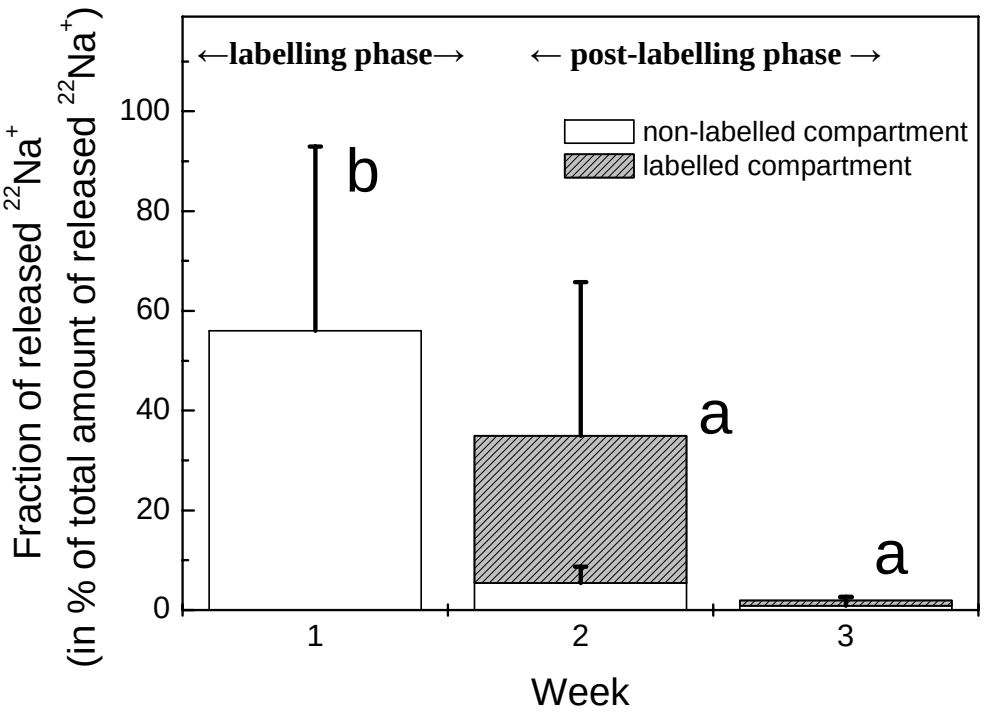

Fig. 3.26: Fraction of $^{22}Na^{+}$ (in percent of the total amount of released $^{22}Na^{+}$) released into the labelled (grey) and non-labelled (white) compartment of the split root system. Plants were adapted to final concentrations of 75 mM NaCl with weekly increasing NaCl concentrations (25 mM, 50 mM and 75 mM NaCl). Plants were labelled for one week during their exposure to 50 mM NaCl (week 1). Afterwards, plants were exposed to their final concentration of 75 mM NaCl for two weeks (week 2 – 3). The release of $^{22}Na^{+}$ via the roots was measured daily. Data represent means ± SD; n = 6 plants. Different letters indicate significant differences at $P \leq 0.05$.

At harvest the plants contained about 20 % of the fraction of Na^{+} that had been taken up during the labelling phase (Fig. 2.24).

To investigate how the remaining $^{22}Na^{+}$ was distributed within the plant imaging was performed. Autoradiograms of harvested plants showed $^{22}Na^{+}$ present in the shoot of *P.* x *canescens* (Fig. 3.27 A,C). $^{22}Na^{+}$ was mainly distributed in the leaves at the top of the shoot (Fig. 3.27 A,B) and in the stem (Fig. 3.27), but could hardly be detected in leaves of the lower shoot part (Fig. 3.27 C,D).

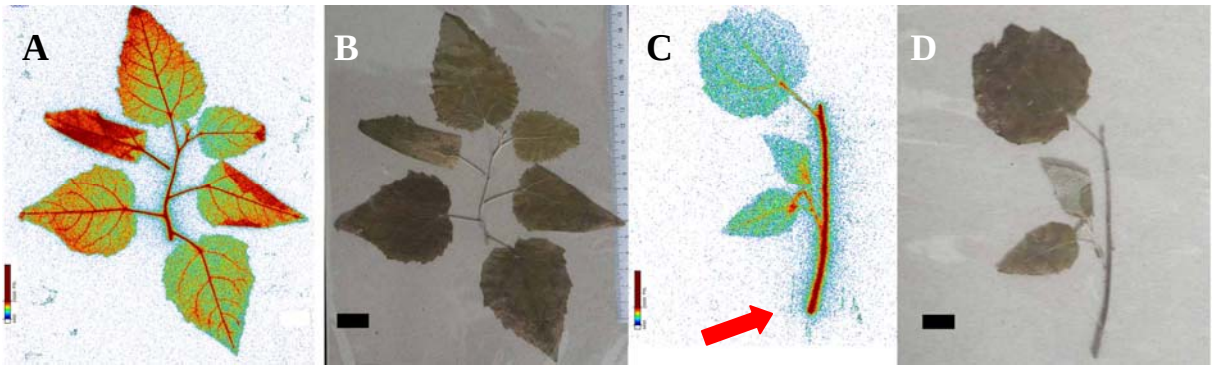

Fig. 3.27: Representative autoradiograms and photographs of *P.* x *canescens* shoot parts (top shoot part: A,B; bottom shoot part: C,D). Plants were adapted to final concentrations of 75 mM NaCl with weekly increasing NaCl concentration (25 mM, 50 mM and 75 mM NaCl). Plants were labelled with $^{22}Na^{+}$ for one week during plant exposure to 50 mM. Afterwards, plants were exposed to their final concentrations of 75 mM NaCl for two weeks. The colours from blue, over green to red indicate an increasing amount of incorporated $^{22}Na^{+}$. Arrow indicates main accumulation of $^{22}Na^{+}$.

In the non-labelled (Fig. 3.28 A,B) and labelled root fractions (Fig. 3.28 C,D), $^{22}Na^{+}$ was present in a small part of the coarse root in the transition area to the stem (Fig. 3.28 A,C), but not alongside the whole root. This indicates that only a small amount of incorporated Na^{+} was retained in roots of *P.* x *canescens* and that Na^{+} was mainly transported to the shoots under salinity. Since imaging of $^{22}Na^{+}$ showed the presence of labelled sodium in non-labelled 2 weeks after the $^{22}Na^{+}$ feeding phase, it can be assumed that Na^{+} transport from shoot to the roots via the phloem and/or lateral root transport takes place in *P.* x *canescens* exposed to NaCl.

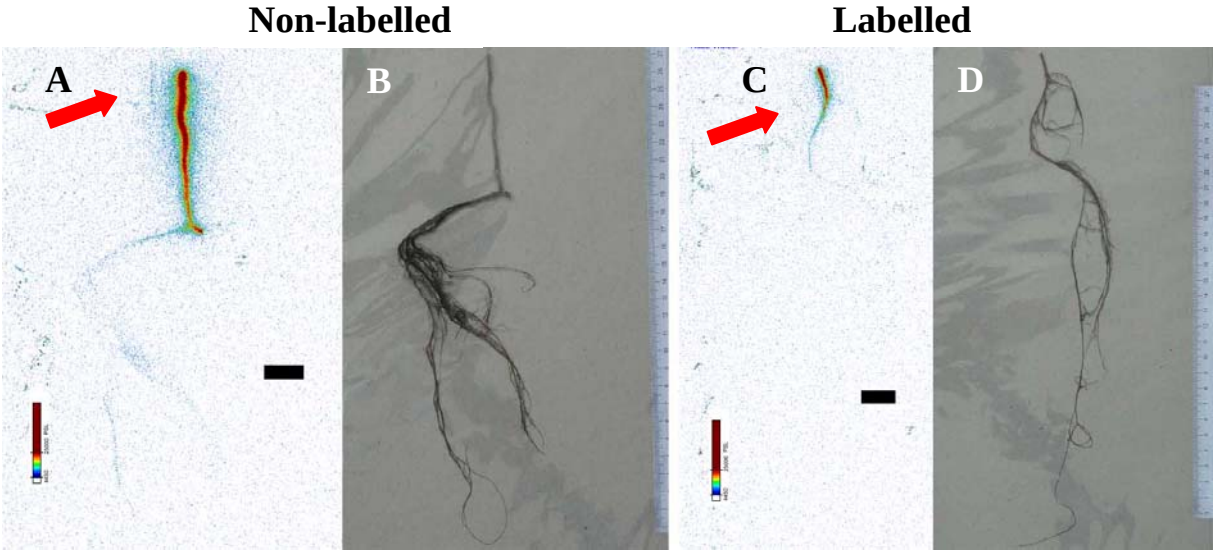

Fig. 3.28: Representative autoradiograms and photographs of *P.* x *canescens* (A-D) roots. Plants were adapted to final concentrations of 75 mM NaCl with weekly increasing NaCl concentration (25 mM, 50 mM and 75 mM NaCl). Plants were labelled for one week during its exposure to 50 mM NaCl. Afterwards, plants were exposed to their final concentrations 75 mM NaCl for two weeks. The colours from blue, over green to red indicate an increasing amount of incorporated $^{22}Na^{+}$. Black bars indicate 2 cm. Arrows indicate main accumulation of $^{22}Na^{+}$.

To quantify Na^+ allocation to different plan parts, the fraction of $^{22}Na^+$ in plant tissue of *P.* x *canescens* was measured at harvest. Nearly 80 % of total $^{22}Na^+$ still present at that time in the plant was found in the leaves but only about 5 % of $^{22}Na^+$ was still present in the roots of *P.* x *canescens* (Tab. 3.13).

Tab. 3.13: Fraction of $^{22}Na^+$ (in percent of the total amount of $^{22}Na^+$ incorporated in the plant) and fresh mass of *P.* x *canescens* at harvest. Plants were adapted to final concentrations of 75 mM NaCl with weekly increasing NaCl concentrations (25 mM, 50 mM and 75 mM NaCl). Plants were labelled for one week during its exposure to 50 mM NaCl. Afterwards, plants were exposed to their final concentration of 75 mM NaCl for two weeks. Data represent means ± SD; n = 8 plants. Different letters indicate significant differences at $P \leq 0.05$.

Plant		***P.* x *canescens***
Leaves	[%]	78.87 ± 4.01 [c]
Stem	[%]	16.78 ± 3.78 [b]
Roots	[%]	4.35 ± 2.80 [a]
Leaves	[g FM]	1.74 ± 1.19 [b]
Stem	[g FM]	0.80 ± 0.38 [a]
Roots	[g FM]	2.90 ± 1.50 [c]
Whole plant	[g FM]	5.44 ± 3.00

Leaf feeding experiment

Since the previous results pointed to a Na^+ downward directed transport via the phloem from the shoot to the root in *P.* x *canescens* under salinity (see 3.25) a leaf feeding experiment was carried out to investigate Na^+ phloem transport from shoot to roots (see 2.3.3). *P.* x *canescens* were pre-adapted to final concentrations 75 mM NaCl with weekly increasing NaCl concentrations (see 2.3). In addition, *P.* x *canescens* exposed to 25 mM NaCl and to hydroponic solutions without NaCl were also analysed.

After NaCl adaptation, the surface of a single leaf in the middle of the plant was rubbed with siliciumcarbid to injure the surface and enable sodium uptake. The treated leaf was submerged for 24 h in hydroponic solution with additional $^{22}Na^+$ in 75 mM NaCl. After 24 h, the labelling solution was removed and the treated leaf was harvested. The remaining plants were harvested after 48 h chase period (see 2.3.3).

P. x *canescens* plants exposed to 25 mM and 75 mM NaCl displayed leaf necrosis due to pre-adaptation to NaCl (Fig. 3.29).

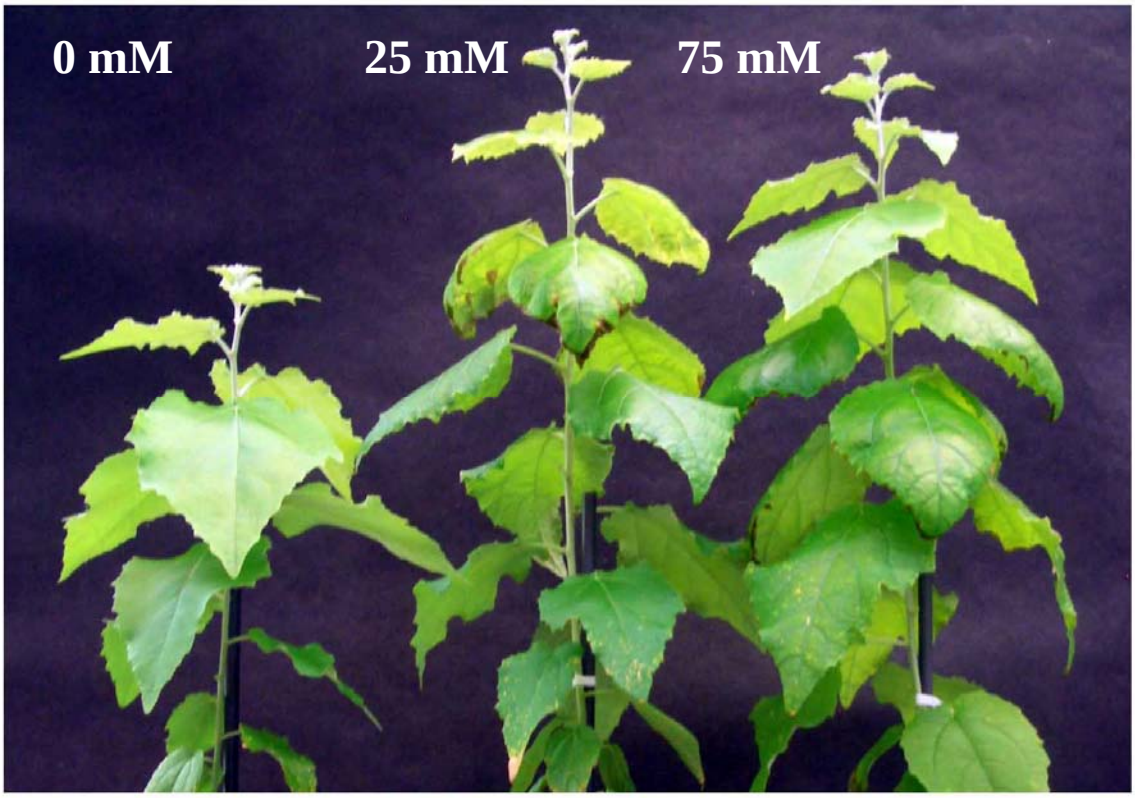

Fig. 3.29: *P.* x *canescens* after 24 h of leaf feeding (75 mM for *P.* x *canescens*) and a chase period of 48 h. Plants were adapted to their final concentrations of 75 mM NaCl with weekly increasing NaCl concentrations (25 mM, 50 mM and 75 mM NaCl). Furthermore, plants were exposed to 25 mM NaCl and to hydroponic solution without NaCl (0 mM NaCl). One single leaf in the middle of the shoot was treated for leaf feeding (see 2.3.3). The leaf was harvested after 24 h of treatment. The remaining plant was harvested after a chase period of 48 h.

After a chase period of 48 h, $^{22}Na^{+}$ was distributed within the whole plants of *P.* x *canescens* (Fig. 3.30). Since $^{22}Na^{+}$ was detected in plant tissues below the exposed leaf, Na^{+} recirculation via the phloem in *P.* x *canescens* must have taken place.

The relative $^{22}Na^{+}$ distribution pattern was not affected by the three different NaCl adaptation regimes (Fig. 3.30). The highest portion of $^{22}Na^{+}$ was present in the middle shoot part of *P.* x *canescens*.

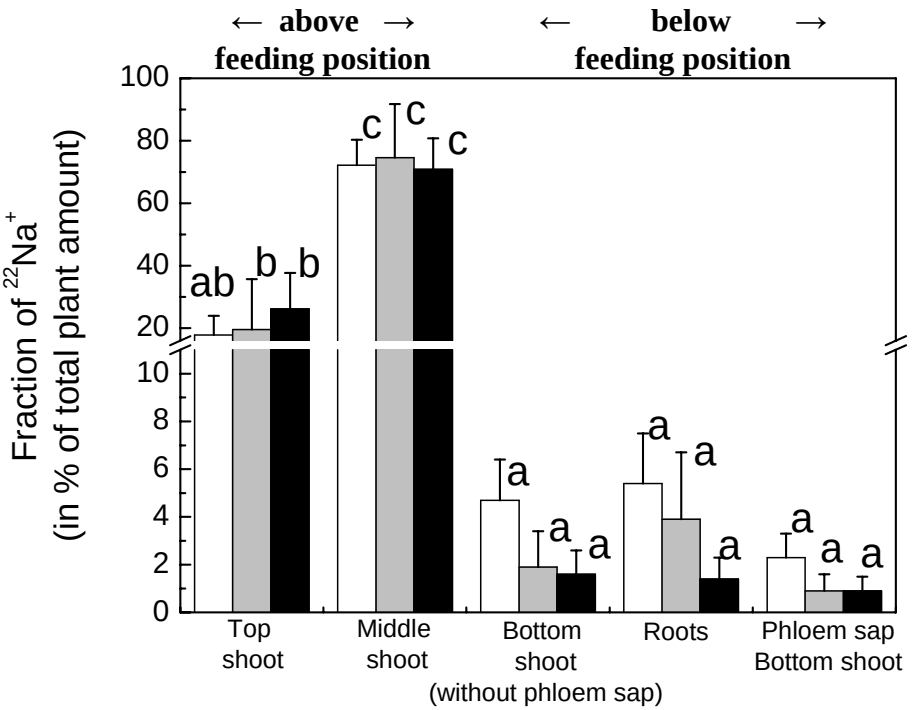

Fig. 3.30: Fraction of $^{22}Na^{+}$ (in percent of the total amount of $^{22}Na^{+}$ incorporated; without the treated leaf) in *P.* x *canescens* controls (white bars), exposed to 25 mM NaCl (grey bars) and 75 mM NaCl (black bars) after leaf feeding. Plants were adapted to final concentrations of 75 mM with weekly increasing NaCl concentrations (see 2.3). Furthermore, plants were exposed to 25 mM NaCl and to hydroponic solution without NaCl. One single leaf in the middle of the shoot was exposed to labelled 75 mM NaCl as described under 2.3.3 for 24 h. Afterwards, the leaf was harvested and the remaining plants were harvested after a chase period of 48 h. Plants were divided into three shoot parts (top, middle, bottom) and the roots. Further, phloem sap from the bottom shoot part was taken (see 2.6.3). Data represent means ± SD, n = 4 - 5 plants. Different letters indicate significant differences at $P \leq 0.05$.

After harvest, biomass and the amount of accumulated $^{22}Na^+$ were determined (see 2.6.5) in all different tissue fractions of *P.* x *canescens* to observe $^{22}Na^+$ accumulation in plant tissue (Tab. 3.14). The data indicates that the main fraction of $^{22}Na^+$ accumulation was the top and middle shoot part, indicating that the main fraction of Na^+ was upwards transported.

Tab. 3.14: Biomass and the amount of $^{22}Na^+$ incorporated for *P.* x *canescens*. Plants were adapted to their final concentrations of 75 mM NaCl with weekly increasing NaCl concentrations, as described under 2.3. Additionally, plants were exposed to 25 mM NaCl and to hydroponic solution without NaCl. One single leaf in the middle of the shoot was exposed to 14.38 KBq $^{22}Na^+$ for leaf feeding (see 2.3.3). The leaf was harvested after 24 h of treatment. The remaining plants were harvested after chase periods of 48 h. Data represent means ± SD; n = 4 – 5 plants. Different letters indicate significant differences at $P \leq 0.05$.

		Biomass		
		0 mM	**25 mM**	**75 mM**
Top shoot	[g DM]	0.64 ± 0.17^a	1.14 ± 0.41^a	1.09 ± 0.41^a
Middle shoot	[g DM]	0.74 ± 0.16^a	1.68 ± 0.53^b	1.90 ± 0.68^b
Bottom shoot	[g DM]	0.29 ± 0.10^a	0.75 ± 0.30^b	0.59 ± 0.24^{ab}
Roots	[g DM]	0.46 ± 0.11^a	1.21 ± 0.47^b	1.13 ± 0.45^{ab}
Total	[g DM]	2.13 ± 0.50^a	4.78 ± 1.53^b	4.73 ± 1.71^b
		Total $^{22}Na^+$		
Top shoot	[Bq]	261.7 ± 152.2^a	298.9 ± 306.3^a	460.6 ± 398.0^a
Middle shoot	[Bq]	1010.1 ± 425.8^a	917.6 ± 96.2^a	1064.4 ± 249.7^a
Bottom shoot	[Bq]	62.3 ± 19.0^b	25.7 ± 19.2^a	20.2 ± 10.7^a
Roots	[Bq]	75.2 ± 36.7^a	53.3 ± 37.7^a	17.5 ± 11.9^a
Total	[Bq]	1409.3 ± 586.5^a	1295.6 ± 368.6^a	1562.7 ± 616.5^a

The radioactivity in the nutrient solution was also measured, but no radioactivity was detected indicating that Na^+ taken up from the exposed leaf was not released via the roots.

Expression of HKT1;1 in *P.* x *canescens* under salinity

HKT1;1 has been proposed to be crucial for Na^+ accumulation in plants under salinity and to influence Na^+ recirculation via the phloem (Davenport *et al.* 2007; see Introduction). Since differences in the Na^+ phloem transport rate for *P.* x *canescens* exposed to different NaCl adaptation regimes were measured, HKT1;1 expression was determined to analyse whether

changes in HKT1;1 expression in *P.* x *canescens* were related to differences in Na^+ phloem transport rates.

P. x *canescens* were adapted to increasing NaCl concentrations to a final concentration of 75 mM NaCl and 25 mM NaCl as described above. Root and bark tissue were used for qRT-PCR analysis (see 2.7).

P. x *canescens* plants exposed to 75 mM NaCl showed a significant 34-fold decrease in the transcript level of HKT1;1 in bark tissue (Fig. 3.31), indicating a changed transcription of HKT1;1 of an elevated mRNA degradation under salinity. This might explain decreased transport under salinity in *P.* x *canescens*.

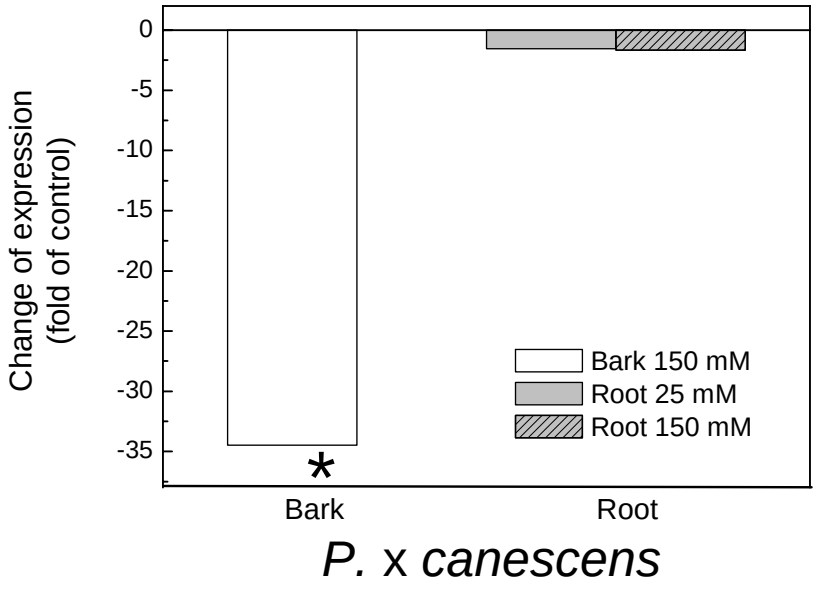

Fig. 3.31: Change in the transcript level of HKT1;1 (At 4g10310) in *P.* x *canescens* bark (white) and root (grey) tissues. Plants were adapted to their final concentration of 25 mM NaCl and 75 mM NaCl (hatched). For qRT-PCR specific primers were used (2.7.5). n = 3 plants; * indicates significant change of expression of a gene.

3.5.2 Na^+ transport and Na^+ distribution in *P. euphratica*

Split root experiment

To determine Na^+ transport in *P. euphratica* and to compare Na^+ transport in both poplar species, a split root experiment was performed (see 2.3.2) and Na^+ xylem transport rates were calculated.

P. euphratica were adapted to final concentrations of 150 mM NaCl (see 2.3). Radioactive labelling was performed during the week before the final NaCl concentration was reached. Plants were exposed to labelled 100 mM NaCl for 7 days and afterwards treated with 150 mM NaCl for 21 days. The plants were harvested 28 days after starting the labelling.

The $^{22}Na^+$ uptake of *P. euphratica* was 1.84 % of the total amount of radioactivity during one week of exposure to 100 mM NaCl (Tab. 3.15).

Tab. 3.15: Uptake of $^{22}Na^+$ (in percent of the total amount) for *P. euphratica* in split root experiment. Plants were exposed to weekly increasing NaCl concentrations starting with 25 mM NaCl up to a concentration of 100 mM NaCl. Plants were labelled with additional $^{22}Na^+$ for one week. Afterwards, plants were exposed to their final concentration of 150 mM NaCl for 21 days and harvested. The amount of $^{22}Na^+$ that was taken up by the plant was calculated as the sum of $^{22}Na^+$ incorporated at the end of the experiment and the amount of $^{22}Na^+$ released during the experiment. Data represent means ± SD; n = 8 plants.

	P. euphratica
Uptake of $^{22}Na^+$ (in percent of total amount)	1.84 ± 1.27

The Na^+ exclusion was calculated as described under 2.6.7, according to the calculation of Na^+ exclusion in *P.* x *canescens* (see 3.5.1) and using equation 2.11.

The Na^+ exclusion during one week of exposure to 100 mM NaCl was 99.95 % (Tab. 3.16). Furthermore, the Na^+ exclusion during one week of exposure to 100 mM NaCl was significantly higher (at $P \leq 0.01$) for *P. euphratica* than for *P.* x *canescens* exposed to 50 mM NaCl for one week (Tab. 3.11). These data indicates an enhanced Na^+ uptake in *P.* x *canescens* compared to *P. euphratica* under salinity.

Tab. 3.16: Exclusion of Na^+ for *P. euphratica* in split root experiment. Plants were exposed to weekly increasing NaCl concentrations starting with 25 mM NaCl up to a concentration of 100 mM NaCl for *P. euphratica*. Plants were labelled with additional $^{22}Na^+$ for one week. Afterwards, plants were exposed to their final concentrations of 75 mM NaCl for 14 days and harvested.
The exclusion of Na^+ was calculated as described under 2.6.7. $c_{Na+\ sol.}$ = concentration of Na^+ in the nutrient solution, $V_{sol.}$ = volume of the hydroponic solution, $Bq_{taken\ up}$ = amount of $^{22}Na^+$ taken up by the plant during one week, Bq_{total} = amount of $^{22}Na^+$ in the hydroponic solution, V_{xylem} = volume of the xylem sap = volume of loss of nutrient solution, $n_{Na+\ plant}$ = amount of Na^+ taken up by the plant, P = portion of Na^+ in the shoot of the plant, $n_{Na+\ xylem}$ = amount of Na^+ transported via the xylem, $c_{Na+\ xylem}$ = concentration of Na^+ in the xylem sap.

$c_{Na+\ sol.}$	[mmol/l]	100
$V_{sol.}$	[ml]	800
$Bq_{taken\ up}$	[KBq]	3.92 ± 2.80
Bq_{total}	[KBq]	185.3
V_{xylem}	[ml]	337 ± 49.5
$n_{Na+\ plant}$	[mmol]	1.69 ± 1.21
P		0.8156
$n_{Na+\ xylem}$	[mmol]	1.38 ± 0.99
$c_{Na+\ xylem}$	[µmol/ml]	4.09 ± 2.75
Na^+ exclusion	[%]	99.959 ± 0.03

The release of Na^+ under salinity was determined by measuring the radioactivity in the nutrient medium (see 2.6.5). Samples of the nutrient solution in both compartments of the split root system (see 2.3.2) were taken daily and the amount of released $^{22}Na^+$ was measured. The portion of $^{22}Na^+$ that was released by the plants via the roots into the hydroponic solution was measured during radioactive labelling and afterwards during the chase period by analysing samples of the hydroponic solution daily (see 2.3.2 and 2.6.5). *P. euphratica* released about 80 % of $^{22}Na^+$ uptake into the nutrient solution and incorporated about 20 % (Fig. 3.32). This result is similar to that found in *P.* x *canescens* that released about 75 % of incorporated $^{22}Na^+$ into the hydroponic solution (Fig. 3.24).

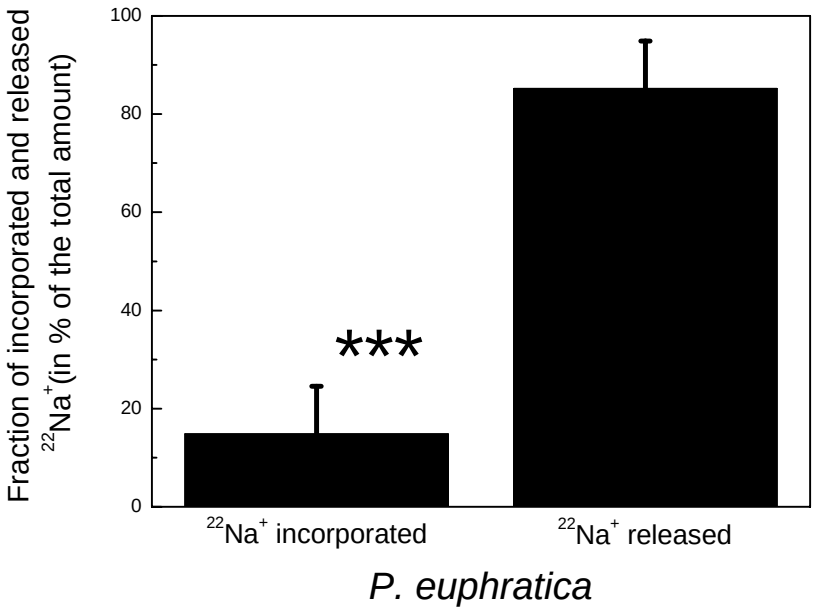

Fig. 3.32: Fraction of $^{22}Na^{+}$ incorporated in the plant and $^{22}Na^{+}$ released into the nutrient solution (in % of the total amount of $^{22}Na^{+}$ uptake) after three weeks of exposure to NaCl. Plants were adapted to final concentrations of 150 mM NaCl with weekly increasing NaCl concentrations (see 2.3). Plants were labelled for one week at 100 mM NaCl. Afterwards, plants were exposed to their final concentrations of 150 mM NaCl for three weeks. Data represents means ± SD; n = 8 plants. *** indicates significant differences at $P \leq 0.001$.

However, in contrast to *P.* x *canescens*, *P. euphratica* released about 70 % of the total amount of $^{22}Na^{+}$ taken up via the labelled root part (Fig. 3.33), indicating that a large portion of the Na^{+} uptake was not transported.

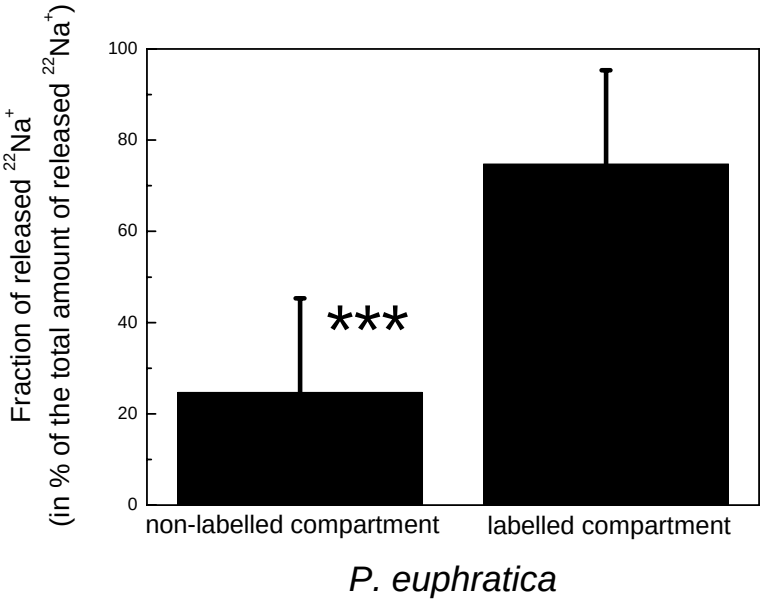

Fig. 3.33: Fraction of $^{22}Na^{+}$ (in percent of the total amount of released $^{22}Na^{+}$) released by *P. euphratica* into the labelled and non-labelled compartment of a split root system under salinity. Plants were adapted to final concentrations of 150 mM NaCl with weekly increasing NaCl concentrations (see 2.3). Plants were labelled for one week during their exposure to 100 mM NaCl. Afterwards, plants were exposed to their final concentration of 150 mM NaCl for three weeks. Data represent means ± SD; n = 8 plants. *** indicates significant differences at $P \leq 0.001$.

Na^{+} release in *P. euphratica* exposed to 150 mM NaCl was analysed in the course of time of the split root experiment (Fig. 3.34). About 75 % of Na^{+} taken up was retrieved in the second week of the experiment in the compartment in which the labelling had taken place (Fig. 3.34). This is in contrast to *P.* x *canescens* that released over 65 % of $^{22}Na^{+}$ uptake in the first week during the radioactive labelling to the non-labelled compartment (Fig. 3.26).

These results suggest, that both species differ in their strategies of Na^{+} release under high NaCl concentrations. *P.* x *canescens* released the highest portion of Na^{+} incorporated via the non-labelled root part (Fig. 3.25), in contrast to *P. euphratica* that released most of its Na^{+} incorporated via the labelled root part (Fig. 3.33). This suggests an increased Na^{+} transport in *P.* x *canescens* under salinity and that in *P. euphratica* roots Na^{+} seems to be retained and freely exchangeable.

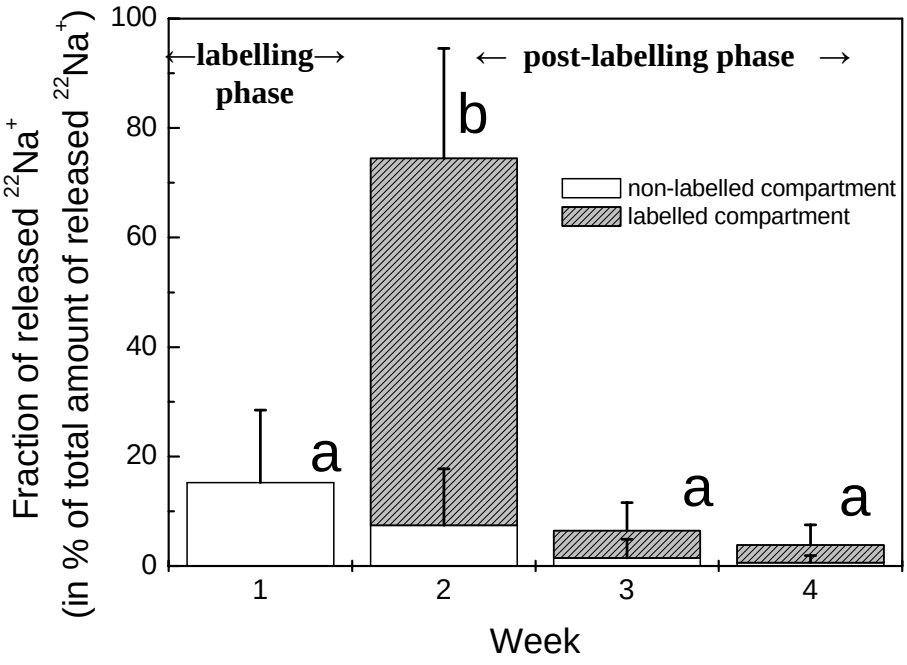

Fig. 3.34: Fraction of $^{22}Na^{+}$ (in percent of the total amount of released $^{22}Na^{+}$) released into the labelled (grey) and non-labelled (white) compartment of the split root system. Plants were adapted to final concentrations of 150 mM NaCl with weekly increasing NaCl concentrations (see 2.3). Plants were labelled for one week during their exposure to 100 mM NaCl (week 1). Afterwards, plants were exposed to their final concentration of 150 mM NaCl for three weeks. The release of $^{22}Na^{+}$ via the roots was measured daily. Data represent means ± SD; n = 8 plants. Different letters indicate significant differences at $P \leq 0.05$.

To gain information about the $^{22}Na^{+}$ distribution within the plant, autoradiography imaging was performed. The distribution of $^{22}Na^{+}$ in *P. euphratica* at harvest was imaged by taking autoradiograms (Fig. 3.35 and Fig. 3.36). $^{22}Na^{+}$ was hardly detected in the youngest leaves of *P. euphratica* (Fig. 3.35 A) that were developed during a chase period of 150 mM NaCl, in which *P. euphratica* exposed to 150 mM NaCl maintained growth (Fig. 3.1). $^{22}Na^{+}$ was mainly present in older leaves and in the stem part in the transition area to the roots (Fig. 3.35 C,D).

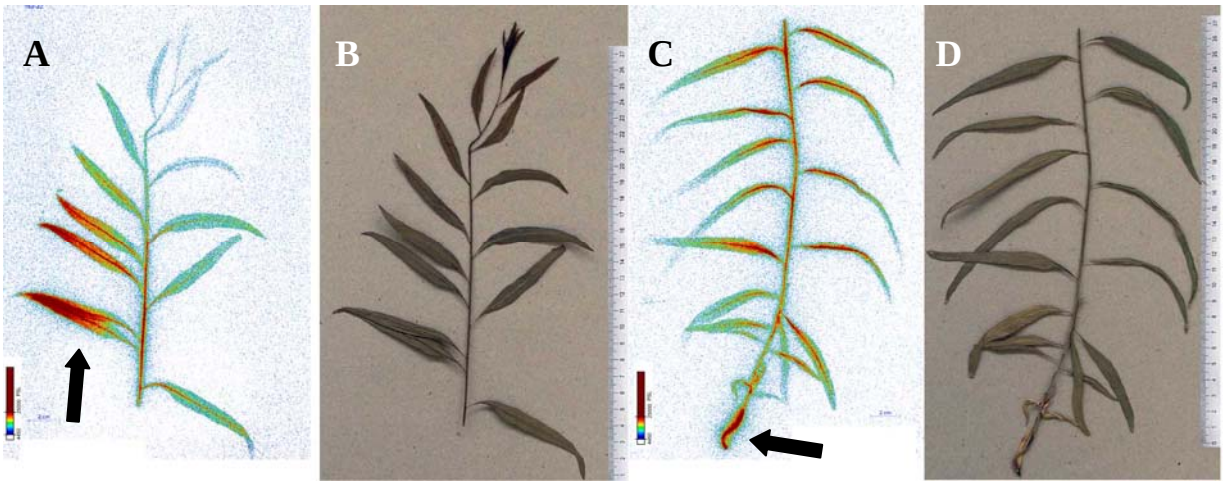

Fig 3.35: Representative autoradiograms and photographs of *P. euphratica* shoot parts (top shoot part: A,B; bottom shoot part: C,D). Plants were adapted to final concentrations of 150 mM NaCl for *P. euphratica* with weekly increasing NaCl concentration (see 2.3). Plants were labelled with $^{22}Na^{+}$ for one week during the plants exposure to 100 mM. Afterwards, plants were exposed to their final concentration of 150 mM NaCl for three weeks. The colours from blue, over green to red indicate an increasing amount of incorporated $^{22}Na^{+}$. Arrows indicate main accumulation of $^{22}Na^{+}$.

Autoradiograms of *P. euphratica* roots (Fig. 3.36) showed an incorporation of the radioisotopes alongside the main root parts for both, the non-labelled (Fig. 3.36 A,B) and labelled (Fig. 3.36 C,D) roots. In the non-labelled roots, a huge amount of $^{22}Na^{+}$ was present in the coarse roots in the transition area to the stem (Fig. 3.36 A,B). This observation suggests that lateral Na^{+} transport in the root system can take place and/or that Na^{+} transported to the shoot can be retranslocated in the phloem down into the roots. Furthermore, this indicates that *P. euphratica* roots have a greater capacity for Na^{+} accumulation, since autoradiograms of *P.* x *canescens* roots exposed to labelled NaCl in a split root system imaged $^{22}Na^{+}$ present only in a small root part at the transition area to the stem (Fig. 3.27).

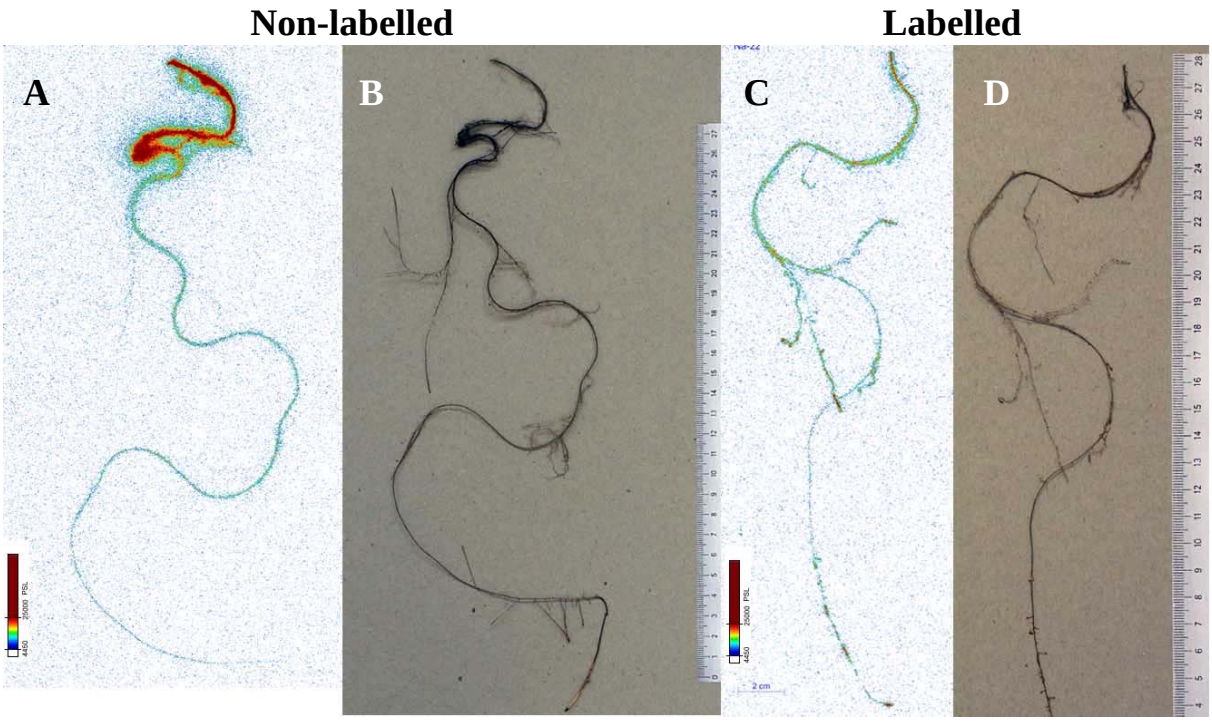

Fig. 3.36: Representative autoradiograms and photographs of *P. euphratica* (A-D) roots. Plants were adapted to final concentrations of 150 mM NaCl with weekly increasing NaCl concentration (see 2.3). Plants were labelled for one week during its exposure to 100 mM NaCl. Afterwards, plants were exposed to their final concentration of 150 mM NaCl for three weeks. The colours from blue, over green to red indicate an increasing amount of incorporated $^{22}Na^{+}$. Black bars indicate 2 cm. Arrows indicate main accumulation of $^{22}Na^{+}$.

The relative fraction of $^{22}Na^{+}$ present in plant tissue of *P. euphratica* at harvest was measured (Tab. 3.17). About 60 % of $^{22}Na^{+}$ taken up were incorporated in the leaves and about 20 % were present in the stem and in the roots each.

Nearly 80 % of $^{22}Na^{+}$ was present in leaves of *P.* x *canescens* (Tab. 3.12), whereas *P. euphratica* accumulated less (60 %) (Tab. 3.17). Furthermore, the fraction of $^{22}Na^{+}$ present in roots at harvest was 3-fold higher in *P. euphratica* than in *P.* x *canescens* (Tab. 3.12 and Tab. 3.17), indicating a higher Na^{+} accumulation capacity of the roots of *P. euphratica* compared to those of *P.* x *canescens*.

Tab. 3.17: Fraction of $^{22}Na^{+}$ (in percent of the total amount of $^{22}Na^{+}$ incorporated) and fresh mass of *P. euphratica* at harvest. Plants were adapted to final concentrations of 150 mM NaCl with weekly increasing NaCl concentrations (see 2.3). Plants were labelled for one week during its exposure to 100 mM NaCl. Afterwards, plants were exposed to their final concentration of 150 mM NaCl for three weeks. Data represent means ± SD; n = 6 - 8 plants. Different letters indicate significant differences for $P \leq 0.05$.

Whole plant		***P. euphratica***
Leaves	[%]	60.37 ± 12.22 [b]
Stem	[%]	21.18 ± 13.26 [a]
Roots	[%]	18.44 ± 6.46 [a]
Leaves	[g FM]	2.93 ± 1.40 [b]
Stem	[g FM]	1.20 ± 0.54 [a]
Roots	[g FM]	2.08 ± 0.69 [ab]
Whole plant	[g FM]	6.21 ± 2.40

Leaf feeding experiment

Since the previous results suggested a Na^{+} downward directed transport via the phloem in *P. euphratica* under salinity (Fig. 3.33) a leaf feeding experiment was carried out to determine the Na^{+} phloem transport rate (see 2.3.3). Plants were adapted to final concentrations of 150 mM NaCl (see 2.3). Furthermore, plants were exposed to 25 mM NaCl and to hydroponic solutions without NaCl for the same time of exposure.

After NaCl adaptation, leaf feeding was conducted as described under 3.5.1 for *P.* x *canescens* with the exception that the radioactive labelled solution that was used for leaf feeding contained 150 mM NaCl instead of 75 mM (see 2.3.3). The treated leaf was submerged for 24 h in the labelled nutrient solution and removed afterwards. The remaining plant was harvested after a chase period of 48 h.

P. euphratica exposed to hydroponic solution without NaCl showed wilting of the top leaves. In contrast, *P. euphratica* pre-adapted to NaCl (Fig. 3.37) did not display these symptoms.

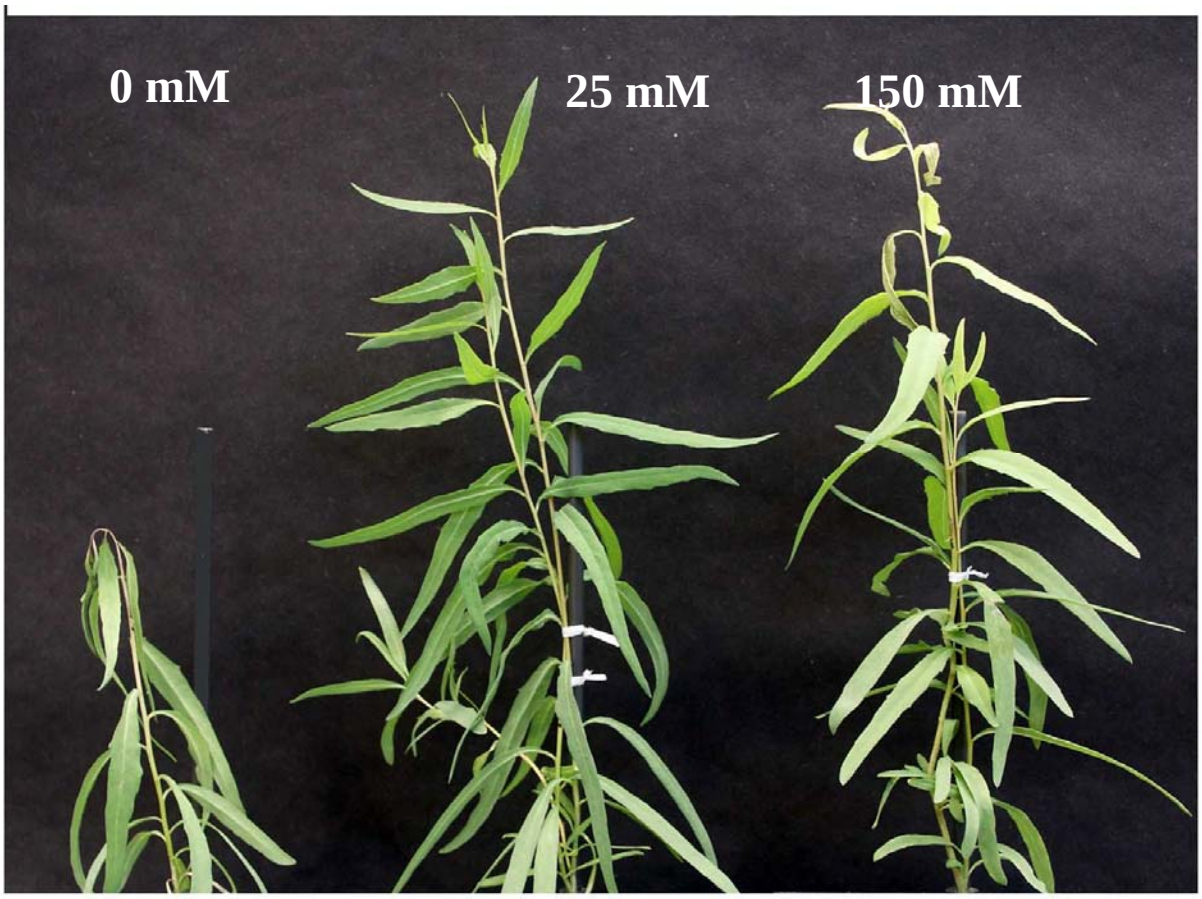

Fig. 3.37: *P. euphratica* after 24 h of leaf feeding and a chase period of 48 h. Plants were adapted to their final concentration of 150 mM NaCl with weekly increasing NaCl concentrations, as described under 2.3. Additionally, plants were exposed to 25 mM NaCl and to hydroponic solution without NaCl. One single leaf in the middle of the shoot was treated for leaf feeding (see 2.3.3). The leaf was harvested after 24 h of treatment. The remaining plants were harvested after a chase period of 48 h.

72 h after the start of leaf feeding, $^{22}Na^{+}$ was distributed within the whole plant for all NaCl regimes (Fig. 3.38). Furthermore, leaf feeding of *P. euphratica* exposed to three different external NaCl concentrations did not affect the relative Na^{+} distribution pattern (Fig. 3.38). Differences in $^{22}Na^{+}$ distribution between the three treatments in the shoot were not detected.
For *P. euphratica* exposed to 150 mM NaCl, the $^{22}Na^{+}$ accumulation into the top shoot part was 74-fold higher, compared to the bottom shoot part. In the roots, the portion of $^{22}Na^{+}$ was around 10-fold increased for controls, compared to plants exposed to 150 mM NaCl (Fig. 3.38).

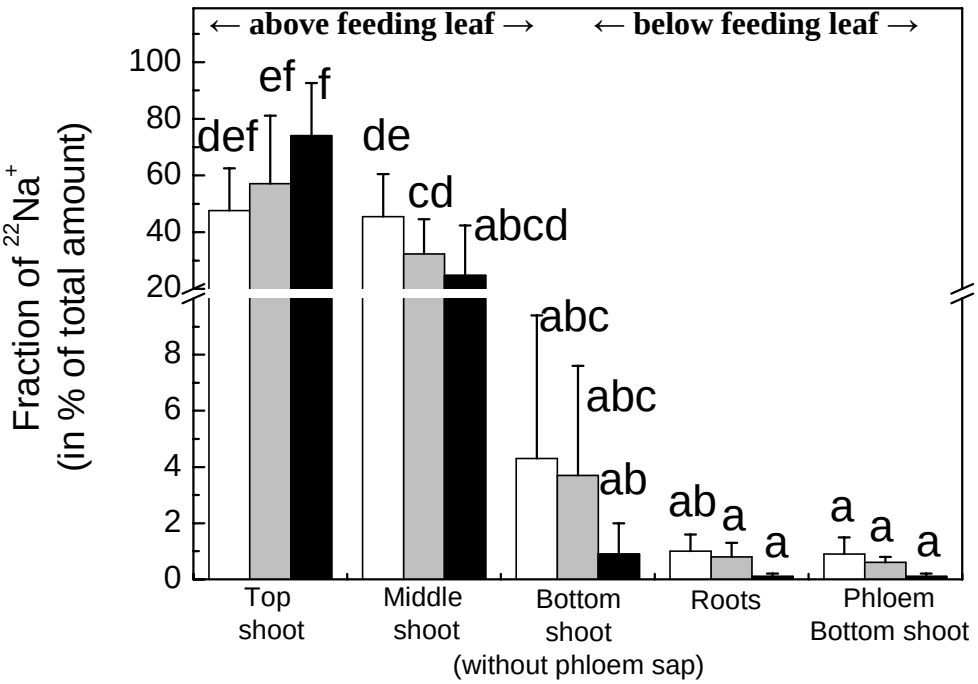

Fig. 3.38: Fraction of $^{22}Na^{+}$ (in percent of the total amount of $^{22}Na^{+}$ incorporated; without the treated leaf) in *P. euphratica* controls (white bars), exposed to 25 mM NaCl (grey bars) and 150 mM NaCl (black bars) after leaf feeding. Plants were adapted to final concentrations of 150 mM with weekly increasing NaCl concentrations (see 2.3). Furthermore, plants were exposed to 25 mM NaCl and to hydroponic solution without NaCl. One single leaf in the middle of the shoot was exposed to labelled 150 mM NaCl as described under 2.3.3 for 24 h. Afterwards, the leaf was harvested and the remaining plants were harvested after a chase period of 48 h. For harvest, plants were divided into three shoot parts (top, middle, bottom) and the roots. Further, phloem sap from the bottom shoot part was taken (see 2.6.3). Data represent means ± SD, n = 4 - 5 plants. Different letters indicate significant differences at $P \leq 0.05$.

Biomass and the amount of $^{22}Na^{+}$ incorporated in the plants were determined at harvest (Tab. 3.18). The amount of radioactivity in the top shoot part of the plants exposed to 150 mM NaCl was 2-fold increased than in plants treated with 25 mM NaCl (Tab. 3.18), indicating an increased Na^{+} transport from the leaves to the top shoot part under high NaCl treatment.

Tab. 3.18: Biomass and the amount of accumulated $^{22}Na^{+}$ for *P. euphratica*. Plants were adapted to their final concentrations of 150 mM NaCl with weekly increasing NaCl concentrations, as described under 2.3. Additionally, plants were exposed to 25 mM NaCl and to hydroponic solution without NaCl. One single leaf in the middle of the shoot was exposed to 7.2 or 28.3 KBq $^{22}Na^{+}$, respectively, for leaf feeding (see 2.3.3). The leaf was harvested after 24 h of treatment. The remaining plants were harvested after chase periods of 48 h. n = 4 -5 plants. Different letters indicate significant differences at $P \leq 0.05$.

		Biomass		
		0 mM	**25 mM**	**150 mM**
Top shoot	[g DM]	0.24 ± 0.15^{a}	0.21 ± 0.10^{a}	0.48 ± 0.15^{a}
Middle shoot	[g DM]	0.42 ± 0.29^{a}	0.36 ± 0.18^{a}	0.57 ± 0.15^{a}
Bottom shoot	[g DM]	0.33 ± 0.35^{a}	0.27 ± 0.18^{a}	0.54 ± 0.24^{a}
Roots	[g DM]	0.25 ± 0.07^{a}	0.51 ± 0.11^{b}	0.43 ± 0.17^{ab}
Total	[g DM]	1.31 ± 0.75^{a}	1.72 ± 0.29^{ab}	2.20 ± 0.50^{b}
		Total $^{22}Na^{+}$		
Top shoot	[Bq]	773.8 ± 222.3^{ab}	440.8 ± 352.9^{a}	1058.4 ± 80.8^{b}
Middle shoot	[Bq]	923.1 ± 831.9^{a}	262.5 ± 190.3^{a}	432.1 ± 346.7^{a}
Bottom shoot	[Bq]	124.7 ± 202.1^{a}	25.9 ± 26.0^{a}	17.2 ± 21.7^{a}
Roots	[Bq]	20.6 ± 19.6^{a}	6.4 ± 3.8^{a}	1.2 ± 1.4^{a}
Total	[Bq]	1864.0 ± 1207.4^{a}	764.9 ± 478.9^{a}	1509.4 ± 443.6^{a}

Expression of HKT1;1 in *P. euphratica* under salinity

Because HKT1;1 has been assumed to be important for Na^{+} recirculation in plants (Davenport *et al.* 2007), the transcript levels of HKT1;1 in root and bark tissue of *P. euphratica* were determined (see 2.7).

P. euphratica showed no significant changes in the transcript levels of HKT1;1 in roots and barks exposed to both NaCl concentrations (Fig. 3.39), indicating that the transcription of HKT1;1 is unchanged in response to salinity in *P. euphratica*.

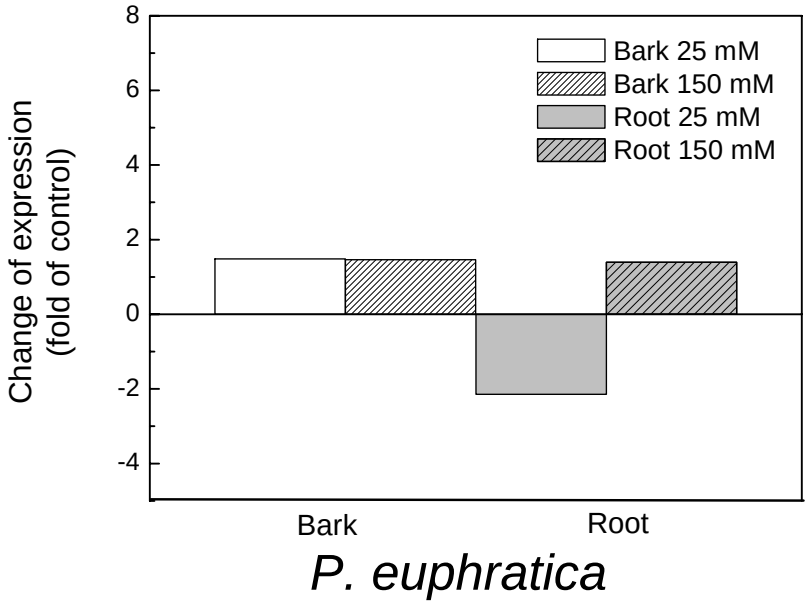

Fig. 3.39: Change in the transcript level of HKT1;1 (At 4g10310) in *P. euphratica* bark (white) and root (grey) tissues. Plants were adapted to their final concentration of 25 mM NaCl and 150 mM NaCl (grey). For qRT-PCR specific primers were used (2.7.5). n = 3 plants.

3.6 Comparing Na^+ uptake and fraction of transported Na^+ in *P. euphratica* and *P.* x *canescens* under salinity

3.6.1 Na^+ xylem transport rates in both poplar species

To calculate the total amount of Na^+ taken up by each plant ($n_{Na+\ plant}$) equation 2.8 was used. To calculate the portion of Na^+ transported to the shoot, the fraction of $^{22}Na^+$ at the end of the split root experiment was measured for both poplar species (see Tab. 3.12 and Tab. 3.16). Since the labelling was done only during one week all activity present later in the plant must have been taken up in the preceding labelling phase. Using data from Tab. 3.12 and Tab. 3.16, the following proportions (*P*) of Na^+ in the shoot of the *P. euphratica* and *P.* x *canescens* were calculated (Tab. 3.19). These portions were used to determine the amount of Na^+ transported via the xylem by the following equation:

$$n_{Na+\ xylem}\ [mmol] = n_{Na+\ Plant} * P$$

Equation 3.1: Amount of Na^+ transported via the xylem ($n_{Na+\ xylem}$). $n_{Na+\ Plant}$ = amount of Na^+ taken up by the plant; P = portion of Na^+ in the shoot of the plant.

The Na^+ xylem transport rate was calculated for *P. euphratica* exposed to 100 mM NaCl and for *P.* x *canescens* exposed to 50 mM NaCl. The plants were exposed for 1 week to these concentrations in the present of the radioactive label.

The wood area of the stem was calculated as described under 2.6.6. Since the plants used for this experiment were very young, the whole wood area was assumed to transport water and Na^+ via its xylem tissue. To calculate the Na^+ xylem transport rate ($v_{Na+\ xylem}$), the following equation was used:

$$v_{Na+\ xylem}\ [\mu mol * cm^{-2} * h^{-1}] = n_{Na+\ xylem} * A_{wood}^{-1} * t^{-1}$$

Equation 3.2: Calculation of Na^+ xylem transport rate ($v_{Na+\ xylem}$). $n_{Na+\ xylem}$ = amount of Na^+ transported via the xylem, A_{wood} = wood area (xylem cross sectional area), t = time of measurement

Although the external concentration of *P. euphratica* (100 mM NaCl) was 2-fold higher than in *P.* x *canescens* (50 mM NaCl) during the labelling phase, the Na^+ xylem transport rate per wood area and time was 2-fold decreased in *P. euphratica* (Tab. 3.19) compared to that established for *P.* x *canescens* (Tab. 3.19). This indicates an increased Na^+ shoot transport via the xylem in *P.* x *canescens* under salinity.

Tab. 3.19: Calculation of the Na^+ xylem transport rate ($v_{Na+\ xylem}$). $n_{Na+\ xylem}$ = Amount of Na^+ transported via the xylem, d_{stem} = diameter of the stem, F = ratio of the stem and the wood diameter (see 2.6.6), d_{wood} = diameter of the wood (see 2.6.6), A_{xylem} = xylem cross sectional area (see 2.6.6), $v_{Na+\ xylem}$ = Na^+ xylem transport rate. Data represent means ± SD; n = 5 plants. ** indicate significant differences at $P \leq 0.01$.

		P. euphratica	***P.* x *canescens***
Portion (P)		0.8156	0.9565
$n_{Na+\ xylem}$	[mmol]	1.38 ± 0.99	2.51 ± 1.32
d_{stem}	[cm]	2.04 ± 0.23	2.64 ± 0.33
F		1.565	1.3095
d_{wood}	[cm]	1.30 ± 0.15	2.01 ± 0.25
A_{wood}	[cm^2]	1.35 ± 0.30	3.22 ± 0.82
$v_{Na+\ xylem}$	[$\mu mol*cm^{-2}*h^{-1}$]	8.04 ± 1.81	19.17 ± 4.89**

3.6.2 Na^+ phloem transport rates in both poplar species

Downward directed transport in plants proceeds via the phloem. Therefore, radioactivity of $^{22}Na^+$ that was present in plant parts below the feeding leaf (see 3.5.1 and 3.5.2) is due to phloem transport. The amount of radioactivity (Bq_{phloem}) that was present in the roots, the bottom shoot part and in the phloem sap (see 2.6.3) below the exposed leaf was measured. Further, the total amount of radioactivity in the plant (Bq_{total}) present at harvest was measured (see Tab. 3.14 and Tab. 3.18).

Because the ratio of radioactivity transported via the phloem/total radioactivity in the plant is the same as the ratio of Na^+ transported via the phloem/total Na^+ in the plant, the amount of Na^+ transported via the phloem ($n_{Na+\ phloem}$) was calculated, using the following equation:

$$n_{Na+\ phloem}\ [mmol] = (Bq_{phloem} * Bq_{total}^{-1}) * c_{Na+} * V$$

Equation 3.3: Calculation of the amount of Na^+ ($n_{Na+\ phloem}$) transported via the phloem. Bq_{phloem} = amount of radioactivity transported via the phloem, Bq_{total} = total amount of radioactivity in the plant, c_{Na+} = concentration of Na^+ in the labelled solution, V = volume of labelled solution

Since the treated leaf of each plant was exposed to radioactivity for 24 h, followed by a chase period of 48 h, Na^+ phloem transport was measured for 72 h.

Because the plants used for this experiment were very young, the whole bark area was assumed to transport Na^+ via the phloem tissue. The diameter of the bark was determined as described above (see 2.6.6). To calculate the Na^+ phloem transport rate per bark area and time, the following equation was used:

$$v_{Na+\ phloem}\ [\mu mol * cm^{-2} * h^{-1}] = n_{Na+\ phloem} * A_{bark}^{-1} * t^{-1}$$

Equation 3.4: Calculation of Na^+ phloem transport rate ($v_{Na+\ phloem}$). $n_{Na+\ phloem}$ = amount of Na^+ transported via the phloem, A_{bark} = area of the bark (phloem cross sectional area), t = time of measurement.

Since *P. euphratica* were exposed to 2-fold increased NaCl concentrations for leaf feeding (see 2.3.3), Na^+ phloem transport rates were 2-fold enhanced in *P. euphratica* exposed to

control nutrient solution (0 mM NaCl) and high NaCl concentrations (150 mM) compared to *P.* x *canescens* exposed to control nutrient solution and high (75 mM) NaCl treatment (Tab. 3.20). This indicates similar Na^+ phloem transport rates in both poplar species. Furthermore, Na^+ phloem transport rates declined in both poplar species with increasing NaCl concentrations in the nutrient solution showing significant higher Na^+ phloem transport rates for *P.* x *canescens* without NaCl in the nutrient solution than in plants treated with NaCl (Tab. 3.20).

Tab. 3.20: Calculation of the Na^+ phloem transport rate ($v_{Na+\ phloem}$) for *P. euphratica* and *P.* x *canescens*. Bq_{phloem} = amount of radioactivity transported via the phloem, Bq_{total} = total amount of radioactivity in the plant, c_{Na+} = concentration of Na^+ in the labelled solution, *V* = volume of labelled solution, $n_{Na+\ phloem}$ = Amount of Na^+ transported via the phloem, d_{stem} = diameter of the stem, *F* = ratio of the stem and the wood diameter (see 2.6.6), d_{wood} = diameter of the wood (see 2.6.6), A_{bark} = bark area (see 2.6.6), *t* = time of measurement, $v_{Na+\ phloem}$ = Na^+ phloem transport rate. Data represent means ± SD; n = 4 - 5 plants. Different letters indicate significant differences at $P \leq 0.05$.

		P. euphratica		
		0 mM	**25 mM**	**150 mM**
Bq_{phloem}	[Bq]	187.0 ± 267.1	43.1 ± 32.3	30.3 ± 28.8
Bq_{total}	[Bq]	1840.5 ± 1219.7	735.4 ± 500.8	1506.6 ± 442.5
c_{Na+}	[mmol/ml]	0.15	0.15	0.15
V	[ml]	35	35	35
$n_{Na+\ phloem}$	[mmol]	0.052 ± 0.044	0.015 ± 0.014	0.020 ± 0.023
d_{stem}	[cm]	1.58 ± 0.29	2.32 ± 0.51	2.22 ± 0.54
F		1.565	1.565	1.565
d_{bark}	[cm]	1.01 ± 0.19	1.48 ± 0.33	1.42 ± 0.34
A_{bark}	[cm^2]	1.18 ± 0.42	2.57 ± 1.06	2.39 ± 1.02
t	[h]	72	72	72
$v_{Na+\ phloem}$	[µmol*cm^{-2}*h^{-1}]	0.672 ± 0.497 [a]	0.096 ± 0.099 [a]	0.209 ± 0.317 [a]
		P.* x *canescens		
		0 mM	**25 mM**	**75 mM**
Bq_{phloem}	[Bq]	181.9 ± 63.3	113.4 ± 66.5	70.0 ± 27.0
Bq_{total}	[Bq]	1405.7 ± 584.3	1293.4 ± 368.2	1558.8 ± 618.0
c_{Na+}	[mmol/ml]	0.075	0.075	0.075
V	[ml]	35	35	35
$n_{Na+\ phloem}$	[mmol]	0.033 ± 0.012	0.021 ± 0.012	0.013 ± 0.005
d_{stem}	[cm]	2.10 ± 0.27	2.92 ± 0.37	2.81 ± 0.46
F		1.3095	1.3095	1.3095
d_{bark}	[cm]	1.61 ± 0.21	2.23 ± 0.28	2.14 ± 0.35
A_{bark}	[cm^2]	1.47 ± 0.34	2.82 ± 0.72	2.63 ± 0.86
t	[h]	72	72	72
$v_{Na+\ phloem}$	[µmol*cm^{-2}*h^{-1}]	0.327 ± 0.111 [b]	0.177 ± 0.080 [a]	0.072 ± 0.032 [a]

3.6.3 Scheme of Na^+ uptake and Na^+ transport in both poplar species

To display differences in the Na^+ transport between both poplar species Na^+ uptake and Na^+ transport of *P. euphratica* and *P.* x *canescens* (Fig. 3.40) were compared.

The scheme distinguishes three plant organs (roots, stem, and leaves). The roots are divided into an influx root, in which Na^+ uptake by the plant takes place, and the remaining roots. The stem is divided into xylem and phloem tissue and the leaves are divided into top, middle and bottom leaves. The scheme also displays Na^+ exclusion for *P. euphratica* exposed to 100 mM NaCl and for *P.* x *canescens* exposed to 50 mM NaCl, both for one week each. Afterwards both poplar species were exposed to their final concentrations of 150 mM NaCl for *P. euphratica* and 75 mM NaCl for *P.* x *canescens* for 2 weeks.

Na^+ uptake by the influx root and Na^+ exclusion is displayed in the outer left root part of Fig. 3.40. Na^+ transport within the plant and Na^+ release via the roots is displayed by arrows with the thickness of each arrow being related to the relative fraction of transported Na^+.

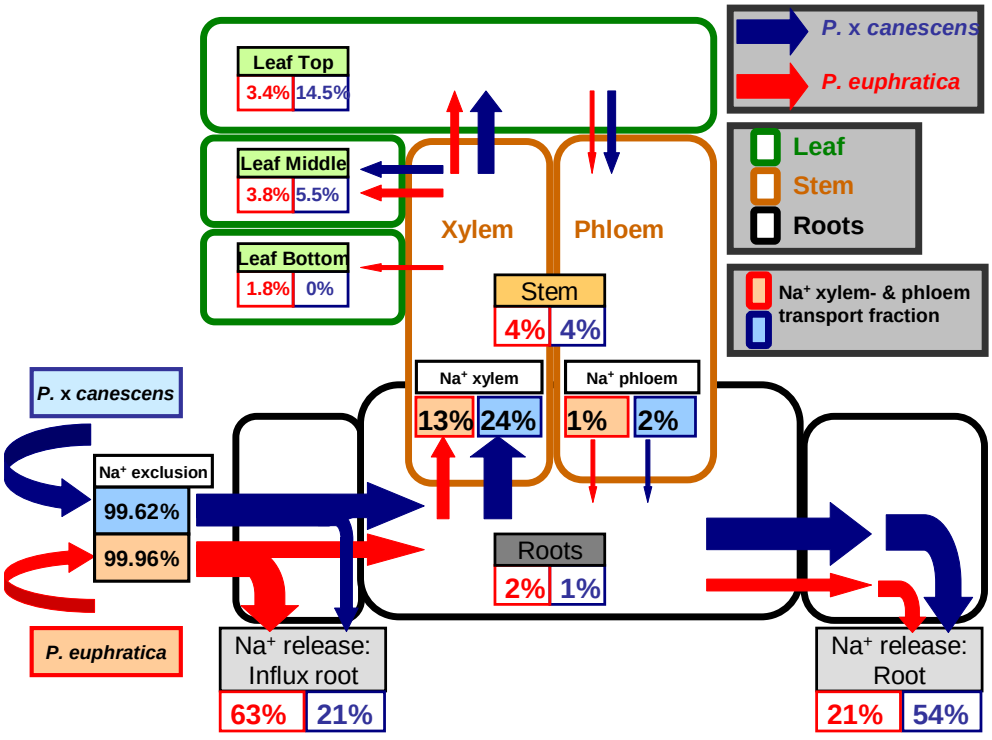

Fig. 3.40: Scheme of Na^+ uptake and Na^+ transport in *P. euphratica* (red) and *P.* x *canescens* (blue). The model is based on data of the split root experiment and leaf feeding experiment for *P.* x *canescens* (see 3.5.1) and *P. euphratica* (see 3.5.2). The Na^+ exclusion was measured during one week. Plants were adapted with weekly increasing NaCl concentration to final concentrations of 150 mM (*P. euphratica*) and 75 mM NaCl (*P.* x *canescens*) (see 2.3). Plants were exposed to their final concentration for two weeks. The thickness of the arrows indicates the amount of translocated Na^+. Data in coloured rectangle and white background display the relative amount of Na^+ at the end of the experiment, respectively the amount of released Na^+ during the experiment. Data in coloured rectangle with coloured background displays Na^+ uptake and Na^+ transport rates.

Both poplar species differ in their Na^+ exclusion rate. *P. euphratica* excluded about 99.96 % of the external Na^+, which is significant higher ($P \leq 0.01$) than in *P.* x *canescens* (Fig. 3.40, Tab. 3.12 and Tab. 3.16). Furthermore, *P.* x *canescens* had a 2-fold increased Na^+ xylem transport rate, compared to *P. euphratica*. These data indicate an enhanced Na^+ exclusion in NaCl tolerant *P. euphratica,* compared to *P.* x *canescens* and a lower Na^+ shoot transport in *P. euphratica* than in *P.* x *canescens*.

The Na^+ release via the influx root in *P. euphratica* was 3-fold increased in comparison with *P.* x *canescens*. In contrast, the Na^+ release via the remaining roots was 2-fold increased in *P.* x *canescens* compared with *P. euphratica*. Since the release of Na^+ via the influx roots seems likely a less energy costing mechanism because Na^+ is not transported through the root and/or shoot of the plant, *P. euphratica* is assumed to have a more effective mechanism of Na^+ release compared to *P.* x *canescens*.

4 Discussion

4.1 The formation and the function of cob roots

One of the most intriguing results of the present study was the observation that *P. euphratica* developed a thickening of roots after long-term exposure to salinity named cob roots. This has not been reported for poplar before. However, in NaCl tolerant cotton (Kurth *et al.* 1986, Zhong and Läuchli 1993) and barely cultivars (Huang and Redman 1995, Shabala *et al.* 2003), increases in root diameter were observed under high salinity. The increased diameter was concluded as an effect of Na^+/Ca^{2+} interaction (Zhong and Läuchli 1993), in which the thickening of roots was induced by decreased Ca^{2+} uptake under salinity (Kurth *et al.* 1986, Shabala *et al.* 2003). For cotton (Kurth *et al.* 1986) and barely (Shabala *et al.* 2003) an ameliorated effect of supplementary Ca^{2+} for root thickening was observed. In the present study it was shown that Ca^{2+} deficiency without NaCl treatment did not result in the development of cob roots in *P. euphratica* (Tab. 3.5 and Fig. 3.7). It was further shown that Cl^- was not the reason for root thickening (Tab. 3.7) but a Na^+-specific effect in *P. euphratica* (Tab. 3.7).

In cotton, anatomical analysis showed that cortical cells of roots exposed to NaCl were longer and narrower, compared to those of the controls (Kurth *et al.* 1986). Such a change in cell shape was not found in *P. euphratica*, in which cortical cells remained their cell shape (Fig. 3.18).

In spite of the increase in root diameter, the number of cortical cell layers was constant in cotton (Kurth *et al.* 1986) and barely (Shabala *et al.* 2003) roots of plants treated with NaCl. In contrast to these findings, the increased number of cortex cell layers was the reason for root thickening in *P. euphratica* in response to salinity (Tab. 3.8). In conclusion the formation of cob roots is different from previously described root modifications in NaCl tolerant plants under salinity.

It was shown in this study that the total cross sectional area of cob roots (0.51 cm^2) was 3-fold increased compared to controls (0.13 cm^2) (Tab. 3.8). Pictures showed that cob roots had a cylindrical form (Fig. 3.17). Assuming the roots as a cylinder with a height of 1 cm, the surface area of the controls is only half of the surface area of cob roots (about 2.51 cm^2 for controls and 5.15 cm^2 for cob roots) and the volume of this cylinder is 3-fold higher for cob roots (0.51 cm^3) than for controls (0.13 cm^3).

As roots are exposed to NaCl, Na^+ uptake per surface area is the same for cob roots and control roots (Fig. 3.19). Assuming a Na^+ influx into control roots of 1 µmol Na^+ per cm^2 surface area and min, the Na^+ uptake into a control root of 1 cm height is about 2.51 µmol Na^+ per min, resulting in a Na^+ accumulation of 19 µmol Na^+ per cm^3 root volume and min. In contrast, assuming the same Na^+ influx and cylinder height, cob roots have a Na^+ uptake of 5.15 µmol Na^+ per min resulting in a Na^+ accumulation of 10 µmol Na^+ per cm^3 root volume and min. This is only the half of the Na^+ accumulation per min than in control roots and indicates that cob roots function in decreasing Na^+ root accumulation.

The dry mass/fresh mass ratio was 0.16 for cob roots and 0.18 for controls (Tab. 3.1). Therefore 1 g root dry mass is about 6.25 g root fresh mass for cob roots and about 5.55 g root fresh mass for controls. The concentration of Na^+ in roots exposed to 150 mM NaCl was about 50 mg per 1 g root dry mass (Fig. 3.6). Assuming the root as a cylinder of 1 cm height and proposing that 1 cm^3 of the root is 1 g fresh mass (about 84 % water), the amount of Na^+ in this cylinder is about 41.9 mmol Na^+ in cob roots and 10.7 mmol Na^+ in controls. Assuming a Na^+ influx of 5 µmol per cm^2 surface area and min that is in the range of the measured Na^+ influx (Fig. 3.19), it would take about 850 min to reach the final Na^+ concentration in control roots and about 1630 min in cob roots, which confirms the previous suggestion that cob roots function in decreasing Na^+ accumulation in the roots. The measured Na^+ influx into cob roots (Fig. 3.19) decreased after 240 min indicating that the final Na^+ concentration in cob roots was reached.

The data present in this study point out that the development of cob roots is ion- and species specific adaptation in *P. euphratica* due to high Na^+ concentrations that decreases the Na^+ root accumulation.

4.2 Na^+ and Ca^{2+} uptake and transport in *P. euphratica* and *P.* x *canescens* under salinity

The main objective of this study was to investigate Na^+ uptake and Na^+ transport in *P. euphratica* in comparison to *P.* x *canescens*. Differences between both poplar species were investigated at different Na^+ control points at which Na^+ transport is regulated on the whole plant level (Munns *et al.* 2005, Munns *et al.* 2006) to compare both plant species differing in NaCl tolerance. Since Ca^{2+} supply is crucial for Na^+ transport and Na^+ resistance in plants,

Ca^{2+} uptake and Ca^{2+} shoot distribution were also analysed, regarding its influence on Na^+ resistance of *P. euphratica*.

4.2.1 Na^+ uptake in *P. euphratica*

Na^+ uptake is a result of the unidirectional Na^+ influx and Na^+ efflux from root tissue. Therefore, decreased Na^+ influx is suggested to be an important mechanism for NaCl tolerance in plants (Schubert and Läuchli 1990, Tester and Davenport 2003).

To answer the question, whether cob root development is an adaptive modification on roots of *P. euphratica* due to high Na^+ concentrations, the unidirectional Na^+ influx in control roots and cob roots was determined. NaCl adapted and non adapted *P. euphratica* root tips were exposed to labelled 150 mM NaCl for 1 min (Fig. 3.19). These short-term conditions were chosen, since it was shown in excised *Arabidopsis* root tips that unidirectional Na^+ influx took place only during the first 2 min of exposure (Tester and Davenport 2003) because later Na^+ efflux from root tissue to the external solution and Na^+ efflux to the vacuoles takes place. This observation is in agreement with results present in this study, in which Na^+ influx under short-term conditions up to 5 min was linear increasing with time for NaCl adapted and non adapted root tips, indicating an unidirectional Na^+ influx (Fig. 3.19).

Unidirectional Na^+ influx in excised *Arabidopsis* root tips exposed to 50 mM NaCl was about 1.8 µmol g^{-1} root FM min^{-1} and about 5 µmol g^{-1} root FM min^{-1} for excised Arabidopsis root tips exposed to 200 mM NaCl (Essah *et al.* 2003). These data were mentioned to be in the range of former experiments measuring unidirectional Na^+ influx in wheat (1.5 µmol g^{-1} root FM min^{-1}; Davenport and Tester 2000) and unpublished data for wheat and rice (0.5 – 2 µmol g^{-1} root FM min^{-1}) (Essah *et al.* 2003).

Wang *et al.* (2006) measured the unidirectional Na^+ influx in NaCl acclimated excised roots tips of *A. thaliana* exposed to 100 mM NaCl. Na^+ influx was 0.66 µmol g^{-1} root FM min^{-1} in *Arabidopsis* and 0.31 µmol g^{-1} root FM min^{-1} in its halophyte "relative" *T. halophila*. The unidirectional influx into the halophyte was the half of the influx in NaCl sensitive *Arabidopsis*, indicating that Na^+ influx is crucial for NaCl tolerance in plants, since Na^+ influx was 2-fold reduced in NaCl tolerant *T. halophila.*

The unidirectional influx was around 1.4 µmol g^{-1} root FM min^{-1} for control and 0.96 µmol g^{-1} root FM min^{-1} for NaCl adapted roots of *P. euphratica* exposed to 150 mM NaCl for the 1 min (Fig. 3.19). As the external NaCl concentration was 3-fold higher for *P. euphratica* (150 mM NaCl) compared to *A. thaliana* (50 mM NaCl) and the Na^+ influx was lower for *P.*

euphratica compared to *A. thaliana* this result showed an increased protection against unidirectional Na^+ influx in root tissue of *P. euphratica* compared to NaCl sensitive *A. thaliana*.

EDX-TEM analysis showed that Na^+ concentrations in cortical cell walls were increased compared to Na^+ concentrations in the stelar cell walls of *P. euphratica* roots under salinity (Chen *et al.* 2002, Chen *et al.* 2003). This resulted in a decrease in Na^+ concentrations from cortex to the stele (Chen, pers. communication). In *P.* x *canescens* a decrease in Na^+ cell wall concentrations from the outer epidermis to the stele was also observed (Langenfeld-Heyser *et al.* 2007). These results indicate that either the endodermis functions as a barrier for Na^+ transport into the stele in poplar and therefore protects the stele against Na^+ influx or that an increased Na^+ xylem transport out of the stele takes place or that both mechanisms function in poplar. Läuchli *et al.* (2005) determined the Na^+ concentration in roots of two durum wheat species that differed in their ability to exclude Na^+ by using x-ray microanalysis and concluded that the outer two cell layers of the cortex (epidermis and hypodermis) were the major Na^+ control points that protected the roots of the tolerant species against radial Na^+ influx.

4.2.2 Comparison of Na^+ in *P. euphratica* and *P.* x *canescens*

Since the present study showed decreased Na^+ influx in NaCl adapted *P. euphratica* (Fig. 3.19), Na^+ uptake in both poplar species was analysed to determine differences in Na^+ uptake and Na^+ exclusion. The Na^+ exclusion rate is the fraction of Na^+ that is excluded by the plant from the transpiration water. Na^+ exclusion for *P.* x *canescens* exposed to 50 mM NaCl for one week was about 99.599 % of Na^+ in the nutrient solution (Tab. 3.12). In contrast, the Na^+ exclusion for *P. euphratica* exposed to 2-fold increased external NaCl concentrations for the same time was significantly higher at about 99.96 % of Na^+ in the nutrient solution (Tab. 3.12, Tab. 3.16, see 3.5.2). This indicates that Na^+ exclusion is crucial for NaCl tolerance and that Na^+ xylem loading is a major control point for Na^+ transport in poplar.

This conclusion is furthermore corroborated by determination of the relative Na^+ uptake. *P. euphratica* incorporated around 1.8 % of the total Na^+ during one week of exposure (Tab. 3.15) and *P.* x *canescens* about 6.9 % (Tab. 3.11). As a consequence *P.* x *canescens* accumulated in the same period of time higher Na^+ concentrations, even though exposed to 2-fold lower Na^+ concentrations in the nutrient solution. Since the Na^+ exclusion rates were higher and the Na^+ uptake was lower in *P. euphratica* than in *P.* x *canescens*, the present

results demonstrated that the ability to exclude Na^+ from plant tissue and decrease Na^+ uptake in NaCl tolerant *P. euphratica* was increased, compared to *P.* x *canescens*. Due to this observation, Na^+ exclusion is concluded to play a crucial role in Na^+ resistance in *P. euphratica.* These data are in agreement with observations by Chen *et al.* (2003), who found that the net Na^+ uptake in the whole plant and Na^+ shoot transport in *P. euphratica* was lower under salinity, compared to salt sensitive *P. tomentosa*, while both species were exposed to NaCl. Therefore NaCl tolerance in *P. euphratica* was mainly attributed to its greater capacity of Na^+ exclusion (Chen *et al.* 2001, Chen *et al.* 2003).

Using the same calculation as described by Chen *et al.* (2003), net Na^+ uptake was 9.38 µmol per g root DM and h in *P. euphratica* exposed to 100 mM and 150 mM NaCl for one week each, which is about 10-fold higher than determined by Chen *et al.* (2003) (about 0.88 µmol per g root DM and h for *P. euphratica*). Na^+ concentration in leaves (about 4.2 mg per g DM) and roots (about 3.5 mg per g DM) were also 10-fold lower in *P. euphratica* in the quoted report than in the present study (Fig. 3.4, Fig. 3.6).

4.2.3 Comparison of Ca^{2+} in *P. euphratica* and *P.* x *canescens*

Since Ca^{2+} nutrition is crucial for NaCl tolerance in plants and its uptake is inhibited in many species under salinity (Rengel 1992, Cramer 1997, Lazof and Bernstein 1999), Ca^{2+} uptake was measured in *P. euphratica* under salinity. In *P. euphratica* Ca^{2+} uptake was 2-fold decreased in NaCl treated plants compared to controls (Fig. 3.12). Chen *et al.* (2001) found increased Ca^{2+} xylem sap concentrations in *P. euphratica* in response to NaCl exposure. Therefore the authors concluded that increased Ca^{2+} root uptake was initiated by NaCl treatment. This would be in contrast to the results obtained in the present study. Ca^{2+} concentrations in roots of *P.* x *canescens* decreased (Fig. 3.6). In contrast, no significant decline in Ca^{2+} concentrations in roots of *P. euphratica* was found (Fig. 3.6). This is in agreement with results of Chen *et al.* (2001), who found no influence on Ca^{2+} concentrations in roots of salinized *P. euphratica* but declined Ca^{2+} concentrations in roots of a NaCl sensitive *P. tomentosa*. Because Ca^{2+} uptake was decrease in *P. euphratica*, a higher Ca^{2+} accumulation in roots of *P. euphratica* compared to *P.* x *canescens* is concluded, since autoradiograms of both poplar species showed that Ca^{2+} recirculation from the shoot to the roots did not occur (Fig. 3.16).

A decline in macronutrient (including Ca^{2+}) concentration in *P. euphratica* under salinity was not observed (Fig. 3.6, see 7.2). This is in agreement with observations by Chen *et al.* (2002),

who found reduced K^+ and Mg^{2+} levels in cortical root cell wall and vacuoles of *P.* cv. Italica and *P. popularis* but no decline in K^+, Ca^{2+} and Mg^{2+} concentrations in *P. euphratica*. These data point out that *P. euphratica* was able to maintain Ca^{2+} root concentrations compared to NaCl sensitive poplar species under salinity.

Because *P. euphratica* is able to maintain Ca^{2+} root concentration under salinity, the increased number of cortical cells in cob roots may function as storage tissue for Ca^{2+} and other macronutrients, compensating the decrease in Ca^{2+} uptake and maintaining Ca^{2+} shoot transport under salinity.

In few plant species, but neither in halophytes nor trees, cluster roots were observed. Cluster root formation is an adaptation for nutrient acquisition under low N, P, Fe or K^+ supply (Shane and Lambers 2005). Similar to cob roots, the cortex width is increased in cluster roots resulting in an increased cortex/stellar area ratio that was assumed to function as a storage tissue (Shane and Lambers 2005). Since Ca^{2+} concentrations in roots of *P. euphratica* were unchanged under salinity (Fig. 3.6) cob roots are suggested to function as a storage tissue. Because Ca^{2+} functions in maintaining the structural integrity of root tissue and in membranes (Hanson 1984, Cramer *et al.* 1985, Cramer 2002) the unchanged Ca^{2+} concentrations in cob roots of *P. euphratica* further indicate a higher ion selectivity than in roots of *P.* x *canescens*. Since this higher nutrient selectivity in *P. euphratica* compared to NaCl sensitive poplar species was suggested to afford NaCl resistance of *P. euphratica* (Chen *et al.* 2005) cob roots may play a role in increasing NaCl tolerance.

4.2.4 Comparison of Na^+ xylem transport in both *P. euphratica* and *P.* x *canescens*

In the present study it was examined, whether both poplar species differ in their Na^+ distribution pattern in the shoot and Na^+ xylem transport rate. The Na^+ xylem transport rate per wood area and time was 2-fold higher in *P.* x *canescens* than in *P. euphratica* (Tab. 3.19), although the latter was exposed to 2-fold higher Na^+ concentrations in the nutrient solution. Chen *et al.* (2003) compared Na^+ uptake of *P. euphratica* and NaCl sensitive *P. tomentosa* both exposed to soil irrigated with 100 mM and later 200 mM NaCl for altogether 20 days. Based on the measured Na^+ ion accumulation in the shoot and the dry mass of root tissue, the Na^+ transport to the shoot was calculated. *P. euphratica* had significant lower Na^+ transport rates (0.62 µmol * g^{-1} root DM * h^{-1}) than in NaCl sensitive *P. tomentosa* (1.05 µmol * g^{-1} root DM * h^{-1}). In *P. euphratica*, Na^+ leaf and Na^+ root concentrations (see 4.2.2) as well as Na^+ xylem transport rates (9.41 µmol * g^{-1} root DM * h^{-1}) were about 10-fold higher in this

present study than in data presented by Chen *et al.* (2003). External NaCl concentrations that were about 1.5-fold increased in the present study compared to the quoted report and different experimental conditions, in which Chen *et al.* (2003) placed the plants into soil that may function as a buffer against high NaCl concentrations were assumed to explain the obvious differences in *P. euphratica* Na^+ tissue concentration and Na^+ shoot accumulation.

However, both studies showed that Na^+ xylem transport rates were lower in *P. euphratica* compared to those of NaCl sensitive poplar species (Tab. 3.19), indicating that *P. euphratica* had a greater ability to exclude Na^+ from the shoot, compared to *P.* x *canescens*.

Because it has been proposed that the ability of plants to maintain Ca^{2+} shoot transport under salinity is an index for salt tolerance of plants (LaHaye and Epstein 1971), Ca^{2+} shoot distribution and Ca^{2+} leaf concentration were determined in both poplar species. Autoradiograms of *P.* x *canescens* revealed labelled Ca^{2+} present in new leaves developed after a chase period of two weeks in which the plants were exposed to salinity (Fig. 3.10). In contrast, labelled Ca^{2+} was not present in new leaves of *P. euphratica* (Fig. 3.11) indicating differences in Ca^{2+} shoot distribution in both plants. Since element analysis showed typical Ca^{2+} concentration of about 10 – 15 mg Ca^{2+} per g DM in new leaves of *P. euphratica* (Fig. 3.5) and showed decreases in Ca^{2+} concentrations of older leaves, these results indicate an increased Ca^{2+} transport to new leaves after exposure to NaCl concentrations. This result is in agreement with the results of Chen *et al.* (2001), who measured increased Ca^{2+} xylem sap concentrations *P. euphratica* in response to initial salinity.

To confirm the suggestion of increased Ca^{2+} transport to young leaves of *P. euphratica*, plants were labelled during NaCl exposure. Imaging of the Ca^{2+} distribution (Fig. 3.14) indicated a preferential allocation to young leaves under salinity. In the absence of salinity Ca^{2+} was mainly accumulated in old leaves in the middle shoot part (Fig. 3.13, Fig. 3.14). These results point out that *P. euphratica* changed the pattern of Ca^{2+} distribution in the shoot of NaCl treated plants.

Elevated Ca^{2+} leaf concentrations decrease the displacement of Ca^{2+} with Na^+ at the membrane surface resulting in a higher membrane integrity, ion transport regulation and ion selectivity (Hanson 1984, Cramer *et al.* 1985). This higher ion selectivity in new leaves of *P. euphratica* resulted in decreased Na^+ concentration in developing tissue, since an increased protection of developing leaves is crucial for NaCl tolerance in plants (Jeschke 1984).

Such an influence of salinity on the Ca^{2+} transport and Ca^{2+} supply to plant tissues and its interaction with Na^+ distribution has not been studied before. In two wheat species differing in NaCl resistance the translocation of Ca^{2+} was more suppressed by NaCl concentrations in the

NaCl sensitive than in the tolerant species, indicating that Ca^{2+} transport in processes under salinity in wheat contributed to NaCl tolerance (Davenport *et al.* 1997). Hu and Schmidhalter (1998) found an increased Ca^{2+} concentration in developing leaves of wheat under salinity. Since this result is in disagreement with observation in other plants it was concluded that salinity has different effects on the distribution of macronutrients (Ca^{2+}, K^+ , Mg^{2+}) according to plant species and growth media (Hu *et al.* 2005).

The results presented in this study indicate that NaCl tolerance in *P. euphratica* is attributed to the plants ability to change its pattern of Ca^{2+} distribution under salinity and maintain Ca^{2+} transport into new leaves developed during salinity.

4.2.5 Comparison of Na^+ phloem transport in *P. euphratica* and *P.* x *canescens*

To analyse, whether Na^+ recirculation in poplar takes place and whether Na^+ release contributes to NaCl tolerance split root and leaf feeding experiments were conducted for both poplar species. Na^+ recirculation in plants back to the roots is still an unresolved topic (Apse & Blumwald 2007). For lupine (Munns *et al.* 1988), bean (Jacoby 1967), sweet pepper (Blom-Zandstra *et al.* 1998), and maize (Lohaus *et al.* 2000), Na^+ phloem transport to the roots was reported. In contrast, Davenport *et al.* (2005) observed no Na^+ retranslocation from shoot to the roots in two durum wheat species, differing in their NaCl tolerance. Although Na^+ phloem transport was found in different plants, its importance for NaCl resistance has been questioned since it was estimated to be too low compared with Na^+ xylem transport to contribute significantly to remove Na^+ from the plant (Davenport *et al.* 2005). To analyse the influence in *A. thaliana* exposed to 75 mM NaCl, Na^+ release via the roots were measured (Sunarpi *et al.* 2005). Based on their data and assuming a transpiration rate, Davenport *et al.* (2007) calculated that 12.5 – 40 % of xylem-delivered Na^+ could be removed via the phloem in *Arabidopsis*. This can be assumed to be a significant contribution to NaCl tolerance. In a NaCl tolerant cereal barely the Na^+ export from leaves via the phloem was calculated to be about 10 % of the Na^+ xylem transport (Munns *et al.* 1986).

A main result of the present study was that Na^+ transport from the shoot to the roots was found in both poplar species. Since the treated leaf was harvested after 24 h for all treatment and based on the assumption that Na^+ transport flow did not change during the chase period of 48 h, the relative Na^+ phloem transport rate to the roots was 3.3 % for *P. euphratica* and 5.2 % for *P.* x *canescens*, based on the measurements of radioactivity in the plants at harvest (Fig. 3.29 and Fig. 3.38).

These calculation indicates that Na^+ phloem transport from the shoot to the roots is too low to significantly contribute to transport Na^+ from the shoot to the root in both poplar species, since less than 6 % of the label were transported in a downward direction from the exposed leaf in *P.* x *canescens*. NMR flow imaging showed a phloem/xylem volume flow ration of 0.05 at day and 0.19 at night in poplar (Windt *et al.* 2006) that are assumed to contribute to the determined low Na^+ phloem transport rates. These data further indicate that the relative Na^+ phloem transport is similar in *P.* x *canescens* compared to *P. euphratica* and that Na^+ phloem transport and Na^+ recirculation does not contribute to higher NaCl tolerance in *P. euphratica* and *P.* x *canescens*. This is in contrast to observation in *Lycorpersion pennelli*, a NaCl tolerant wild tomato species, in which the Na^+ transport via the phloem was enhanced, compared to domesticated tomato species (Perez-Alfocoa *et al.* 2000, Tester and Davenport 2003).

Measurements of the radioactive label at harvest showed an increased fraction of label present in the top shoot part for all NaCl adaptation regimes in *P. euphratica* (45 – 75 %; Fig. 3.38) compared to *P.* x *canescens* (18 – 25 %; Fig. 3.30). These data indicate that a higher portion of Na^+ is transported to the top of the shoot in *P. euphratica* than in *P.* x *canescens*. Since young, developing leaves function as a phloem sink and high Na^+ phloem concentrations may be a threat for these leaves (Mühling and Läuchli 2002). This indicates that a relative higher Na^+ phloem transport in *P. euphratica* than in *P.* x *canescens* to the top shoot part may be the cause of this higher Na^+ fraction.

In *P.* x *canescens* Na^+ phloem transport rates declined with increasing NaCl concentrations in the nutrient solution (Tab. 3.20). NaCl in the nutrient solution reduces the plants ability to take up water, which is described as the osmotic or water-deficit effect of salinity (Munns 2005). For both poplar species a decrease in water uptake under salinity compared to controls was observed (data not shown). This decrease in water uptake is assumed to result in lowered xylem and phloem water fluxes. Since Na^+ movement is depending in part of water flux (Munns et al. 2005), a reduction of phloem water fluxes is proposed to also decrease Na^+ phloem transport rates resulting in declined Na^+ phloem transport rates.

The results present in this study showed that Na^+ recirculation takes place in both poplar species but Na^+ phloem transport rates are assumed to be too low to contribute to significant Na^+ remove from the shoot of the plant to the roots.

One aim of this study was to analyse the role of HKT1;1 in Na^+ phloem transport of both poplar species, since AtHKT1;1 was described to play a major role in plant NaCl tolerance

and Na^+ phloem transport within the plant (Berthomieu *et al.* 2003, Sunarpi *et al.* 2005, Rodriguez-Navarro & Rubio 2006, Rus *et al.* 2004, Davenport *et al.* 2007).

AtHKT1;1 was observed to be the gene being responsible for elevated Na^+ shoot concentration in two different *Arabidopsis* populations and that the loss of AtHKT1;1 expression in roots was responsible for elevated Na^+ shoot concentration (Rus *et al.* 2006). Results present in this study showed that transcript level of HKT1;1 in the roots of both poplar species under salinity were not changed (Fig. 3.31 and Fig. 3.39). AtHKT1;1 was also shown to play an important role in Na^+ recirculation in the phloem (Berthomieu *et al.* 2003, Sunarpi *et al.* 2005, Rodriguez-Navarro and Rubio 2006). In the present study transcript levels of HKT1;1 in bark tissue of *P.* x *canescens* were 34-fold downregulated in plants exposed to 75 mM NaCl suggesting a significant decrease of HKT1;1 transport activity (Fig. 3.30). This assumption was corroborated by the findings that Na^+ phloem transport rate in *P.* x *canescens* declined under NaCl treatment (Tab. 3.20). This suggest that HKT1;1 may play a role in regulating Na^+ recirculation via the phloem. This observation is in contrast to a recent report that questions the function of *AtHKT1;1* in Na^+ recirculation in the phloem of *Arabidopsis* based on Na^+ flux measurement using radioactive tracer in hkt1;1 mutants (Davenport *et al.* 2007). To analyse these contrasting results regarding the function of HKT1;1 in poplar, further research should be conducted.

4.2.6 Na^+ accumulation and Na^+ release in roots of *P. euphratica* and *P.* x *canescens*

Na^+ release via the phloem and the roots against an ion gradient implies energy costs for the plants (Tester and Davenport 2003). On the one hand, the Na^+ release via the roots reduces the plants content of cell toxic Na^+. On the other hand, the emitted ions increase the external Na^+ concentration, leading to enhanced osmotic and Na^+ ion specific effects on the plants (Munns 2005). Especially for perennial plants that may live for longer periods of time compared to annual species, Na^+ exclusion is an important mechanism (Munns *et al.* 2005). This is in agreement with the observation that especially salt tolerant woody perennials like citrus and grapevine tend to retain Na^+ in woody roots and stem (Tester & Davenport 2003) rather than to recirculate high amounts of Na^+.

In the present study autoradiograms of *P. euphratica* showed that Na^+ was present in the whole root system 3 weeks after application of the label to one part of the root (Fig. 3.36). In contrast to this, the label was present only in a small part of the coarse roots in the transition

area to the stem in *P.* x *canescens* (Fig. 3.28). These data show that *P. euphratica* has an increased capability to retain and bind Na^+ in the roots.

Both poplar species differ in their Na^+ release. *P. euphratica* released about 20 % of the total amount of Na^+ release via the non-labelled root part and about 80 % via the labelled root part (Fig. 3.33). This is in contrast to *P.* x *canescens* that released nearly 80 % via the non-labelled root part (Fig. 3.25). This result indicates that a large portion of Na^+ is transported through *P.* x *canescens*, suggesting a more effective Na^+ transport in *P. euphratica* that released the highest fraction of Na^+ uptake via the roots in which Na^+ was incorporated.

Na^+ phloem transport rate were similar for *P.* x *canescens* and *P. euphratica* (Tab. 3.20) and relative Na^+ phloem transport rates were too low to significantly contribute to Na^+ retrieval from the shoot (under 6 %, see 4.2.5). Therefore, Na^+ retrieval from the shoot to the roots is not the only cause for the high fraction of Na^+ release via the non-labelled root part in *P.* x *canescens* since about 80 % were released via the non-labelled root part. This suggest that lateral Na^+ transport in the roots system as well as Na^+ recirculation from the shoot to the roots takes place in both poplar species.

5 Summary

The salinization of soils caused by increasing NaCl concentrations is a major problem for today's agriculture. Since *P. euphratica* is able to grow on sodic soils, it has been used for reforestation in saline areas. Because of this attribute there is a strong interest in elucidating this plants´ mechanisms and strategies to cope with high external NaCl concentrations. Therefore, the main objectives of this study were to analyse the uptake, transport and allocation of Na^+ in *P. euphratica* and *P.* x *canescens*. This study also addresses the question whether Na^+ recirculation in poplar takes place. Since the maintenance of Ca^{2+} transport under salinity was proposed to be decisive for NaCl tolerance of plants, a further aim of this work was to investigate Ca^{2+} uptake and Ca^{2+} distribution in poplar.

The influence of salinity on the performance of *P. euphratica* and the NaCl sensitive *P.* x *canescens* was analysed. Both poplar species were exposed to up to 150 mM NaCl and growth measurements were carried out. *P.* x *canescens* was very sensitive and died at 150 mM NaCl whereas *P. euphratica* showed growth reduction but no significant leaf injury. Element analysis of old and young leaves revealed no significant differences between old leaves of *P.* x *canescens* and *P. euphratica*, but a higher Na^+ accumulation in new leaves of *P.* x *canescens* than in those of *P. euphratica*. This suggests that *P. euphratica* is able to protect young, developing leaves against excess Na^+. Long term exposure to NaCl levels leading to death of *P.* x *canescens* resulted in *P. euphratica* in morphological adaptation: roots of *P. euphratica* showed thickenings with increased root diameter. This increase was caused by an increased number of cell layers in the cortex and not by an increased cell volume. Since Ca^{2+} deficiency and Cl^--ions could be eliminated as the cause of this root swelling, and *P.* x *canescens* showed no thickening of roots under the same NaCl regime; thus the induction of "cob-like" thickening was concluded to be a Na^+ ion- and species-specific adaptation in *P. euphratica* roots.

To determine the function of cob roots for Na^+ uptake under salinity, Na^+ influx in adapted and non-adapted root tips of *P. euphratica* was measured using radioactive labelling. A decreased Na^+ influx in adapted roots in relation to the fresh mass of the roots was measured, indicating that cob roots decrease Na^+ accumulation of *P. euphratica*. Because cob roots of *P. euphratica* decreased Na^+ uptake, their function for NaCl tolerance was tested. *P. euphratica* plants were adapted to high Na^+ concentrations, subsequently grown in Na^+-free hydroponic

solution to remove excess sodium and then exposed to NaCl shock. Measurements of radioactively labelled Na^+ uptake during the NaCl shock treatment showed an increased uptake in non-adapted plants compared to adapted plants. Chlorophyll fluorescence measurements and the plants performance showed an increased NaCl tolerance in pre-treated plants. These results demonstrate that cob roots function in increasing NaCl tolerance of *P. euphratica*.

Because of the differences in salt sensitivity, *P.* x *canescens* and *P. euphratica* plants were exposed to maximum external NaCl concentrations which they could withstand for longer periods of time. To determine Na^+ uptake, Na^+ release and Na^+ xylem transport rates under salinity, split root experiments were conducted for both species, in which radioactive labelling was added to one root part and Na^+ translocation to the other parts was determined during a chase period. Na^+ uptake was 3-fold higher in *P.* x *canescens* than in *P. euphratica*, even though *P. euphratica* was exposed to 2-fold higher external NaCl concentrations than *P.* x *canescens*. The higher uptake of Na^+ resulted in a 2-fold enhanced Na^+ xylem transport per wood area in *P.* x *canescens* than in *P. euphratica*.

Furthermore, both poplars differ in their strategy of Na^+ retention and Na^+ release. Analysis of Na^+ allocation by imaging of the $^{22}Na^+$ distribution revealed that *P. euphratica* retained Na^+ in roots. In contrast, the $^{22}Na^+$ label was present only in a small part of the coarse roots of *P.* x *canescens*. However, *P.* x *canescens* released a main fraction of incorporated Na^+ into a non labelled root compartment, indicating Na^+ recirculation. These results also demonstrate an increased Na^+ accumulation and Na^+ binding to roots of *P. euphratica*, suggesting that roots may function as a Na^+ storage tissue.

To investigate Na^+ phloem transport, leaf feeding experiments were conducted using radioactive $^{22}Na^+$. Since $^{22}Na^+$ was detected in the shoot and in phloem sap collected below the feeding leaf, Na^+ recirculation via the phloem took place in both poplars. Na^+ phloem transport rates per bark area were 3-fold higher in *P. euphratica* than in *P.* x *canescens* under salinity. In *P.* x *canescens* phloem transport decreased under salinity and this was accompanied by decreased transcript levels of HKT1;1 in bark tissue of *P.* x *canescens*. This suggests that HKT1;1 plays a role in Na^+ phloem transport in bark tissue of poplar.

The maintenance of Ca^{2+} shoot transport is crucial for plants under salinity. Therefore Ca^{2+} uptake and Ca^{2+} distribution were analysed in both poplar species. Autoradiograms of both poplar species were used to image the distribution of radioactive Ca^{2+}. Neither *P.* x *canescens* nor *P. euphratica* transported previously incorporated Ca^{2+} into new leaves formed during subsequent exposure to salinity. When the radioactively labelled $^{45}Ca^{2+}$ was added during NaCl exposure, a diminished Ca^{2+} uptake was detected. However, this Ca^{2+} was preferentially allocated to new leaves in *P. euphratica*, indicating that this species adapted its pattern of Ca^{2+} distribution, to sustain Ca^{2+} nutrition to the developing organs.

In conclusion, the present study shows multiple differences in Na^+ uptake and Na^+ distribution between *P. euphratica* and *P.* x *canescens* under salinity. It was shown that *P. euphratica* had a higher Na^+ exclusion and a lower Na^+ xylem transport rate than *P.* x *canescens* pointing out that Na^+ xylem loading is a major control point for Na^+ transport in poplar. Further experiments are required to elucidate the molecular basis of these differences because Na^+ xylem loading is a major reason for differences in NaCl tolerance in the two poplar species.

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7 Appendices

7.1 Biomass of poplar

7.1.1 Dry mass of *P. euphratica* for NaCl stress experiment with the same external NaCl concentration (see 3.1.1, 3.2.1 and 3.2.2)

Tab. 7.1: DM in mg of *P. euphratica* plants exposed to weekly increasing NaCl concentration, starting at 25 mM NaCl to a final concentration of 150 mM NaCl (see 2.3.1). Plants were harvested weekly. Data indicates means ± SD; n = 3 – 4; ** indicates significant differences at $P \leq 0.01$.

P. euphratica			
Leaves		**Control**	**NaCl**
1. Week	[mg DM]	505.09 ± 210.28	574.48 ± 344.27
2. Week	[mg DM]	624.49 ± 252.25	576.00 ± 100.76
3. Week	[mg DM]	576.30 ± 177.25	653.32 ± 72.15
4. Week	[mg DM]	1161.01 ± 665.15	571.52 ± 200.22
Stem		**Control**	**NaCl**
1. Week	[mg DM]	303.33 ± 100.17	313.33 ± 162.89
2. Week	[mg DM]	340.00 ± 158.75	330.00 ± 113.58
3. Week	[mg DM]	346.67 ± 70.95	496.67 ± 98.66
4. Week	[mg DM]	672.50 ± 346.35	390.00 ± 188.68
Roots		**Control**	**NaCl**
1. Week	[mg DM]	196.82 ± 74.63	203.89 ± 109.48
2. Week	[mg DM]	399.90 ± 214.30	220.33 ± 22.24
3. Week	[mg DM]	332.92 ± 83.81	356.40 ± 37.33
4. Week	[mg DM]	697.44 ± 315.58	366.90 ± 126.58
Whole plant		**Control**	**NaCl**
1. Week	[mg DM]	1005.24 ± 375.80	1091.71 ± 614.80
2. Week	[mg DM]	1364.39 ± 623.53	1126.33 ± 222.47
3. Week	[mg DM]	1255.88 ± 296.08	1506.39 ± 177.20
4. Week	[mg DM]	2530.95 ±1304.06	1328.41 ± 468.98

7.1.2 Dry mass of *P.* x *canescens* for NaCl stress experiment with the same external NaCl concentration (see 3.1.1, 3.2.1 and 3.2.2)

Tab. 7.2: DM in mg of *P.* x *canescens* plants exposed to weekly increasing NaCl concentration, starting at 25 mM NaCl to a final concentration of 150 mM NaCl (see 2.3.1). Plants were harvested weekly. Data indicates means ± SD; n = 3 – 4; ** indicates significant differences at $P \leq 0.01$.

P.* x *canescens			
Leaves		**Control**	**NaCl**
1. Week	[mg DM]	292.19 ± 146.36	227.53 ± 58.68
2. Week	[mg DM]	813.56 ± 68.77	256.46 ± 110.13**
3. Week	[mg DM]	766.87 ± 188.55	430.96 ± 191.22
4. Week	[mg DM]	976.89 ± 379.06	585.79 ± 97.15
Stem		**Control**	**NaCl**
1. Week	[mg DM]	166.67 ± 98.66	120.00 ± 43.59
2. Week	[mg DM]	583.33 ± 98.66	153.33 ± 83.86**
3. Week	[mg DM]	786.67 ± 219.62	240.00 ± 139.52
4. Week	[mg DM]	390.00 ± 245.90	345.00 ± 91.83
Roots		**Control**	**NaCl**
1. Week	[mg DM]	155.51 ± 21.10	178.22 ± 69.97
2. Week	[mg DM]	688.02 ± 79.63	234.98 ± 139.45**
3. Week	[mg DM]	727.90 ± 302.63	336.68 ± 249.24
4. Week	[mg DM]	701.64 ± 247.09	502.10 ± 46.66
Whole plant		**Control**	**NaCl**
1. Week	[mg DM]	614.36 ± 248.32	525.75 ± 170.21
2. Week	[mg DM]	2084.92 ± 183.27	644.78 ± 332.79**
3. Week	[mg DM]	1981.43 ± 708.74	1007.64 ± 572.90
4. Week	[mg DM]	2068.53 ±719.58	1432.89 ± 219.46

7.1.3 Dry mass of *P. euphratica* exposed to high NaCl concentration (see 3.2.4)

Tab. 7.3: DM in mg of *P. euphratica* (3.2.4). Plants were exposed to weekly increasing concentration of NaCl starting with 25 mM, to a final concentration of 150 mM NaCl (see 2.3). Afterwards, plants were exposed to 150 mM NaCl for two weeks. Plants were harvested weekly, starting after the exposure to 100 mM NaCl (1. week). Data indicates means ± SD; n = 3 – 4; * indicates significant differences for $P \leq 0.05$ and ** indicates significant differences at $P \leq 0.01$.

P. euphratica			
Leaves		**Control**	**NaCl**
100 mM NaCl	[g DM]	0.14 ± 0.02	0.09 ± 0.02*
150 mM NaCl	[g DM]	0.15 ± 0.03	0.11 ± 0.01*
150 mM NaCl	[g DM]	0.21 ± 0.09	0.15 ± 0.03**
Stem		**Control**	**NaCl**
100 mM NaCl	[g DM]	0.08 ± 0.03	0.05 ± 0.02
150 mM NaCl	[g DM]	0.08 ± 0.01	0.08 ± 0.02
150 mM NaCl	[g DM]	0.09 ± 0.05	0.09 ± 0.04
Roots		**Control**	**NaCl**
100 mM NaCl	[g DM]	0.03 ± 0.00	0.03 ± 0.01
150 mM NaCl	[g DM]	0.03 ± 0.00	0.04 ± 0.10
150 mM NaCl	[g DM]	0.05 ± 0.02	0.04 ± 0.01
Whole plant		**Control**	**NaCl**
100 mM NaCl	[g DM]	0.25 ± 0.05	0.17 ± 0.05
150 mM NaCl	[g DM]	0.27 ± 0.03	0.23 ± 0.03
150 mM NaCl	[g DM]	0.42 ± 0.06	0.28 ± 0.07*

7.1.4 Fresh mass of *P. euphratica* and *P.* x *canescens* for split root experiment (see 3.5.1)

Tab. 7.4: FM in g of *P. euphratica* and *P.* x *canescens* in split root experiment (see 3.5.1). Plants were adapted to a final concentration of 150 mM NaCl for *P. euphratica* and 75 mM NaCl for *P.* x *canescens* with weekly increasing NaCl concentration (see 2.3). Afterwards, plants were exposed to its final NaCl concentration for three (*P. euphratica*), respectively two weeks (*P.* x *canescens*). Data indicates means ± SD; n = 6 – 8.

P. euphratica		
Leaves	[g FM]	2.93 ± 1.40
Stem	[g FM]	1.20 ± 0.54
Roots	[g FM]	2.08 ± 0.69
Whole plant	[g FM]	6.21 ± 2.40
P.* x *canescens		
Leaves	[g FM]	1.74 ± 1.19
Stem	[g FM]	0.80 ± 0.38
Roots	[g FM]	2.90 ± 1.50
Whole plant	[g FM]	5.44 ± 3.00

7.1.5 Fresh mass of *P. euphratica* and *P.* x *canescens* for leaf feeding experiment (see 3.5.2)

Tab. 7.5: FM in g of different tissues in *P. euphratica* and *P.* x *canescens* (see 3.4.3). Plants were adapted to its final concentration of 25 mM NaCl and 150 mM NaCl (*P. euphratica*), respectively 75 mM NaCl (*P.* x *canescens*) with weekly increasing NaCl concentration (see 2.3). Data indicates means ± SD, n = 3 plants. Different letters indicate significant differences at $P \leq 0.05$.

P. euphratica		**0 mM**	**25 mM**	**150 mM**
Leaves	[g FM]	3.84 ± 1.03^{a}	5.56 ± 1.55^{a}	3.83 ± 0.71^{a}
Bark	[g FM]	0.47 ± 0.18^{a}	0.65 ± 0.07^{a}	0.55 ± 0.14^{a}
Wood	[g FM]	0.99 ± 0.37^{a}	1.20 ± 0.38^{a}	0.72 ± 0.11^{a}
Roots	[g FM]	1.79 ± 0.48^{a}	2.43 ± 0.84^{a}	1.87 ± 0.45^{a}
Whole plant	[g FM]	7.09 ± 1.86^{a}	9.84 ± 2.46^{a}	6.97 ± 0.72^{a}
P.* x *canescens		**0 mM**	**25 mM**	**75 mM**
Leaves	[g FM]	2.40 ± 1.33^{a}	1.43 ± 0.39^{a}	3.11 ± 0.71^{a}
Bark	[g FM]	0.47 ± 0.25^{a}	0.33 ± 0.09^{a}	0.71 ± 0.13^{a}
Wood	[g FM]	0.60 ± 0.39^{a}	0.33 ± 0.06^{a}	0.74 ± 0.11^{a}
Roots	[g FM]	2.72 ± 1.37^{ab}	1.59 ± 0.30^{a}	4.39 ± 0.80^{b}
Whole plant	[g FM]	6.19 ± 3.31^{a}	3.68 ± 0.78^{a}	8.96 ± 1.56^{a}

7.2 Ion concentration in leaves and roots of poplar under salinity

7.2.1 Element concentration in leaves of *P.* x *canescens* under salinity (see 3.2.1)

Tab. 7.5: Element concentration in new and leaves of *P.* x *canescens*. Plants were treated as described under 3.2. Data are means ± SD; n = 3 – 4 plants.

P.* x *canescens		**25mM**		**50mM**		**100mM**		**150mM**	
		Mean	**sd**	**Mean**	**sd**	**Mean**	**sd**	**Mean**	**sd**
New leaves	**Na**	18.81	3.43	44.68	8.90	54.75	7.82	55.19	6.31
Salt	**K**	49.81	6.60	42.82	8.13	29.92	6.47	30.15	5.12
	Ca	15.92	4.04	4.45	0.80	7.16	2.20	9.28	0.83
	Mg	13.87	1.72	7.21	1.58	6.28	2.69	4.62	0.74
	Mn	0.00	0.00	0.00	0.00	0.07	0.08	0.19	0.03
	Fe	1.59	0.41	0.85	0.23	0.99	0.68	0.26	0.08
	Al	0.00	0.00	0.00	0.00	0.83	1.65	0.32	0.27
	P	4.63	0.84	6.38	0.83	6.25	1.23	6.55	2.98
	S	2.77	0.29	2.45	0.21	2.95	0.81	1.64	0.73
Old leaves	**Na**	7.66	1.48	29.45	8.80	35.31	21.58	26.89	9.93
Salt	**K**	28.54	3.13	16.32	5.00	14.37	4.41	18.66	2.64
	Ca	47.96	4.92	43.03	7.29	41.38	18.20	45.74	7.96
	Mg	14.87	1.10	10.46	0.80	8.25	2.48	7.85	2.01
	Mn	0.24	0.04	0.20	0.04	0.23	0.09	0.32	0.03
	Fe	0.47	0.10	0.38	0.07	0.36	0.11	0.36	0.01
	Al	0.26	0.26	0.16	0.28	0.10	0.12	0.19	0.05
	P	3.54	0.83	4.94	0.83	5.83	3.36	10.60	1.32
	S	2.40	0.66	3.13	0.48	2.10	0.34	3.36	0.50
New leaves	**Na**	4.09	1.98	1.91	1.33	6.00	6.06	2.88	3.41
Control	**K**	65.63	3.37	62.66	3.07	57.60	11.89	53.06	4.71
	Ca	12.64	1.95	19.49	3.66	20.17	5.95	28.61	2.21
	Mg	14.61	1.11	14.67	1.33	15.29	0.34	15.02	0.65
	Mn	0.00	0.00	0.03	0.06	0.11	0.04	0.11	0.02
	Fe	0.87	0.11	0.61	0.36	0.34	0.14	0.20	0.03
	Al	2.16	3.75	0.63	1.09	0.49	0.85	0.14	0.03
	P	4.75	0.63	6.29	1.99	7.40	1.75	9.66	1.58
	S	3.12	0.58	3.53	0.50	4.52	0.70	4.21	0.81
Old leaves	**Na**	0.68	0.10	0.60	0.46	0.75	0.56	1.04	0.57
Control	**K**	19.09	3.17	17.12	3.66	12.85	3.11	16.18	3.71
	Ca	59.47	3.86	63.27	3.40	69.41	3.79	66.27	3.73
	Mg	19.45	2.83	18.28	1.45	16.32	0.92	15.86	0.80
	Mn	0.34	0.08	0.26	0.01	0.29	0.02	0.28	0.02
	Fe	0.49	0.09	0.28	0.02	0.25	0.01	0.21	0.04
	Al	0.47	0.44	0.19	0.19	0.15	0.14	0.17	0.13
	P	2.70	0.29	6.85	1.01	5.95	1.21	7.22	2.31
	S	3.02	0.82	3.87	0.96	2.45	0.67	2.97	0.41

7.2.2 Element concentration in leaves of *P. euphratica* under salinity (see 3.2.1)

Fig. 7.6: Element concentration in new and old leaves of *P. euphratica*. Plants were treated as described under 3.2. Data are means ± SD; n = 3 – 4 plants.

P. euphratica		**25mM**		**50mM**		**100mM**		**150mM**	
		Mean	**sd**	**Mean**	**sd**	**Mean**	**sd**	**Mean**	**sd**
New leaves	**Na**	9.64	2.03	13.67	1.79	20.56	5.90	40.21	7.41
Salt	**K**	63.53	1.85	56.35	3.03	54.57	4.01	41.45	5.04
	Ca	12.69	3.17	15.92	2.77	12.48	0.99	10.47	3.93
	Mg	13.41	0.57	13.36	0.30	12.05	1.08	6.97	1.85
	Mn	0.07	0.06	0.10	0.01	0.09	0.00	0.07	0.02
	Fe	0.45	0.06	0.26	0.02	0.25	0.04	0.31	0.22
	Al	0.21	0.36	0.33	0.29	0.00	0.00	0.52	0.88
	P	8.70	0.60	9.26	0.66	7.80	0.33	5.96	0.75
	S	5.99	0.34	7.40	0.54	5.76	0.63	3.80	0.72
Old leaves	**Na**	7.81	5.49	17.09	2.85	22.74	6.19	42.64	6.79
Salt	**K**	33.09	5.68	33.60	1.91	30.87	4.02	21.65	3.95
	Ca	44.68	10.52	36.01	3.36	34.53	5.20	25.99	7.45
	Mg	13.52	1.76	12.76	0.33	11.35	0.38	8.78	1.38
	Mn	0.25	0.11	0.17	0.01	0.18	0.05	0.17	0.08
	Fe	0.50	0.16	0.22	0.03	0.21	0.04	0.34	0.13
	Al	0.16	0.27	0.15	0.13	0.12	0.12	0.44	0.35
	P	7.40	0.86	9.65	2.45	8.75	1.45	8.92	2.07
	S	10.44	2.62	15.76	0.76	16.21	2.51	9.52	3.52
New leaves	**Na**	5.39	0.65	2.85	1.13	2.19	0.23	3.65	2.07
Control	**K**	64.85	4.55	39.08	4.30	47.72	7.81	60.90	6.54
	Ca	14.50	4.07	38.83	3.65	29.69	6.93	18.78	3.38
	Mg	14.71	0.21	18.47	1.11	19.94	0.96	16.08	1.24
	Mn	0.07	0.06	0.16	0.03	0.14	0.02	0.07	0.01
	Fe	0.47	0.02	0.33	0.06	0.24	0.05	0.22	0.07
	Al	0.00	0.00	0.05	0.05	0.02	0.03	0.05	0.02
	P	6.06	0.66	3.40	0.93	8.52	1.28	7.65	0.83
	S	10.98	1.29	5.37	1.90	7.00	1.29	6.47	0.68
Old leaves	**Na**	1.97	0.97	3.81	1.10	2.43	1.20	2.24	1.76
Control	**K**	34.73	4.61	30.19	3.26	24.29	7.93	39.37	10.26
	Ca	47.63	3.68	50.66	2.46	56.58	9.53	40.52	9.34
	Mg	15.11	0.88	14.25	1.77	15.99	3.21	17.20	1.83
	Mn	0.19	0.04	0.24	0.05	0.32	0.17	0.14	0.06
	Fe	0.37	0.12	0.35	0.10	0.29	0.09	0.19	0.10
	Al	0.00	0.00	0.52	0.45	0.09	0.16	0.35	0.33
	P	6.18	1.67	7.03	3.00	7.35	1.21	7.07	1.12
	S	10.98	2.54	11.59	4.55	9.70	1.09	9.59	2.96

7.2.3 Element concentration in roots of *P. euphratica* and *P.* x *canescens* under salinity (see 3.2.1)

Fig. 7.7: Element concentration in roots of *P. euphratica* and *P.* x *canescens*. Plants were treated as described under 3.2. Data are means ± SD; n = 3 – 4 plants.

		25mM		150mM	
		Mean	**sd**	**Mean**	**sd**
P. euprahtica	**Na**	31.40	2.63	54.64	11.86
Roots	**K**	38.18	1.56	12.31	5.14
Salt	**Ca**	15.87	1.69	20.29	10.78
	Mg	8.12	0.67	5.06	0.35
	Mn	1.35	0.89	0.19	0.07
	Fe	4.59	1.29	6.56	4.88
	Al	0.49	0.45	0.95	0.30
	P	5.14	1.20	2.58	1.07
	S	2.45	0.67	1.97	0.43
P. euprahtica	**Na**	6.35	0.89	11.92	8.08
Roots	**K**	45.56	17.18	35.68	19.02
Control	**Ca**	29.98	16.91	36.22	13.76
	Mg	9.28	0.87	11.87	2.99
	Mn	0.78	0.38	0.82	0.25
	Fe	6.59	0.49	3.19	1.59
	Al	1.47	0.27	0.32	0.37
	P	5.50	2.80	7.88	5.49
	S	2.29	0.94	3.40	1.37
P.* x *canescens	**Na**	33.91	1.80	55.54	8.63
Roots	**K**	21.52	1.79	7.94	3.64
Salt	**Ca**	24.64	5.73	23.33	3.93
	Mg	8.77	0.55	5.40	0.91
	Mn	0.66	0.17	0.30	0.19
	Fe	9.05	3.45	6.28	4.96
	Al	1.45	0.86	1.23	0.94
	P	4.87	0.36	3.87	0.40
	S	2.68	0.27	2.97	0.53
P.* x *canescens	**Na**	2.33	0.46	8.24	2.54
Roots	**K**	41.45	4.89	25.51	8.11
Control	**Ca**	34.81	1.49	47.87	4.15
	Mg	9.64	1.17	9.49	1.75
	Mn	1.13	1.09	1.67	0.78
	Fe	8.98	3.42	6.42	3.67
	Al	1.66	0.94	0.81	0.27
	P	5.00	0.50	2.84	0.35
	S	2.57	0.52	2.30	0.51

7.2.4 Element concentration in new and old leaves of *P. euphratica* under salinity (see 3.2.4)

Fig. 7.8: Element concentration in new and old leaves of *P. euphratica*. Plants were treated as described under 3.2.4. Data are means ± SD; n = 3 – 4 plants.

P. euphratica		**100mM**		**150mM**		**150mM**	
		Mean	**sd**	**Mean**	**sd**	**Mean**	**sd**
New leaves	**Na**	13.17	4.32	8.58	1.48	5.98	1.73
Salt	**K**	33.54	13.42	26.49	4.85	26.27	5.49
	Ca	5.48	1.16	3.28	0.77	2.98	1.06
	Mg	2.38	0.52	2.10	0.38	1.93	0.43
	Mn	0.00	0.00	0.00	0.00	0.00	0.00
	Fe	0.12	0.01	0.15	0.04	0.19	0.04
	Al	0.68	0.45	2.37	1.99	0.00	0.00
Old leaves	**Na**	19.49	10.78	30.04	12.64	18.22	3.65
Salt	**K**	24.12	9.43	25.27	14.83	14.47	5.10
	Ca	14.66	5.11	17.25	1.47	17.04	0.64
	Mg	2.97	0.65	3.34	0.53	2.98	0.75
	Mn	0.11	0.08	0.13	0.03	0.17	0.07
	Fe	0.24	0.05	0.29	0.16	0.46	0.06
	Al	0.32	0.21	0.00	0.00	1.77	3.53
New leaves	**Na**	4.63	2.88	6.03	1.55	5.89	1.78
Control	**K**	30.35	16.29	45.11	9.90	37.45	10.85
	Ca	10.82	3.46	9.78	1.97	7.58	2.67
	Mg	3.18	0.96	4.17	0.39	3.48	0.43
	Mn	0.02	0.04	0.04	0.05	0.04	0.05
	Fe	0.16	0.06	0.36	0.19	0.32	0.08
	Al	1.04	1.03	0.07	0.15	0.38	0.76
Old leaves	**Na**	1.16	0.49	2.40	0.50	2.66	0.84
Control	**K**	23.91	9.47	30.54	10.41	25.94	16.82
	Ca	29.97	3.34	31.50	6.44	29.81	7.20
	Mg	5.00	0.66	5.42	1.35	4.46	1.75
	Mn	0.20	0.02	0.22	0.03	0.24	0.10
	Fe	0.19	0.09	0.31	0.09	0.23	0.07
	Al	1.77	3.11	0.00	0.00	0.00	0.00

7.2.5 Element concentration in roots of *P. euphratica* under salinity (see 3.2.4)

Fig. 7.9: Element concentration in roots of *P. euphratica*. Plants were treated as described under 3.2.4. Data are means ± SD; n = 3 – 4 plants.

P. euphratica		**100mM**		**150mM**		**150mM**	
		Mean	**sd**	**Mean**	**sd**	**Mean**	**sd**
Roots	**Na**	9.80	6.30	16.97	6.44	17.24	9.18
Salt	**K**	5.89	3.46	5.94	3.79	7.20	4.48
	Ca	9.46	4.69	5.18	1.43	6.75	2.86
	Mg	1.32	0.18	1.16	0.32	1.09	0.24
	Mn	0.17	0.06	0.09	0.03	0.14	0.07
	Fe	5.82	0.95	5.19	1.82	5.03	0.99
	Al	0.05	0.10	0.00	0.00	0.08	0.15
Roots	**Na**	4.52	1.93	4.76	2.13	3.67	0.74
Control	**K**	10.00	7.80	11.27	8.62	9.84	4.34
	Ca	13.30	1.09	13.51	7.00	11.09	2.73
	Mg	2.11	1.01	2.21	0.76	1.88	0.33
	Mn	0.54	0.48	0.92	0.54	1.19	0.50
	Fe	5.27	0.86	5.37	0.38	6.65	3.10
	Al	0.00	0.00	0.00	0.00	0.00	0.00

7.3 Alignment of primer

The primer PtHKT1fwd and PtHKT1rev2 (see 3.5.3) were used for QRT-PCR of *P. euphratica*. The primer PtHKT1fwd was obtained from Dr. Peter Ache. The primer PtHKT1rev2 was designed as described under 2.7.5. The sequences of both primers and the sequence of HKT1 were aligned using GeneDoc software (Version 2.6.002).

```
               980         *      1000         *      1020         *      1040         *
HKT1      : CCCTATCAGAAGTTGATGGGTGCATTGTTTCAGGTTGTAAATTCAAGGCATACCGGTGAATCTATTGTCGATCTCTCCATC : 1053
PtHKT1fwd : ------------------------------------------------------------------------------- :    -


              1060         *      1080         *      1100         *      1120         *
HKT1      : ATCTCTCCAGCAATCTTGGTGCTCTTCGTGGTTATGATGTATCTCCCACCATACACTTCATTTATGCCGAAAAAGCAACAG : 1134
PtHKT1fwd : ------------------------------------------------------------------------------- :    -


             1140         *      1160         *      1180         *      1200         *
HKT1      : GAAGAGGTTGATTCAGAAACTGGCCAAAAATGCAAAAACCAAAGGAAGTCTTTGGTTCAGTGCCTGTTGTTCTCACCTTTA : 1215
PtHKT1fwd : ------------------------------------------------------------------------------- :    -


             1220         *      1240         *      1260         *      1280         *
HKT1      : TCTACCTTAGCCATCTTTGTGATTCTCATTTGCGTCTCAGAGAGAGAGAAGTTGAAGAAAGACCCCCTCAACTTCAACGTA : 1296
PtHKT1fwd : ----------------------tctcatttgcgtctcag---------------------------------------- :   17
                                   TCTCATTTGCGTCTCAG

            1300         *      1320         *      1340         *      1360         *      1
HKT1      : CTCAATATCACCATAGAAGTCGTAAGTGCATATGGAAATGTCGGGTTCTCAACTGGCTATAGCTGCAAACGACAACTTGAA : 1377
PtHKT1fwd : ------------------------------------------------------------------------------- :    -


             380         *      1400         *      1420         *      1440         *      14
HKT1      : CCTGATAGCTCGTGCAAGGATGCATGGTTTGGATTTGTTGGAAGGTGGAGTAACATGGGAAAATTTATCCTCATCTTAGTA : 1458
PtHKT1fwd : ------------------------------------------------------------------------------- :    -


             60         *      1480         *      1500         *      1520
HKT1      : ATGTTCTTCGGAAGGCTTAAGAAATTCAGCATCAATGGTGGTAAAGCTTGGAAGCTATCCTAG : 1521
PtHKT1fwd : ------------------------------------------------------------ :    -
```

Fig. 7.1: Alignment of Primer PtHKT1fwd. Both primers were aligned using GeneDoc software (Version 2.6.002).

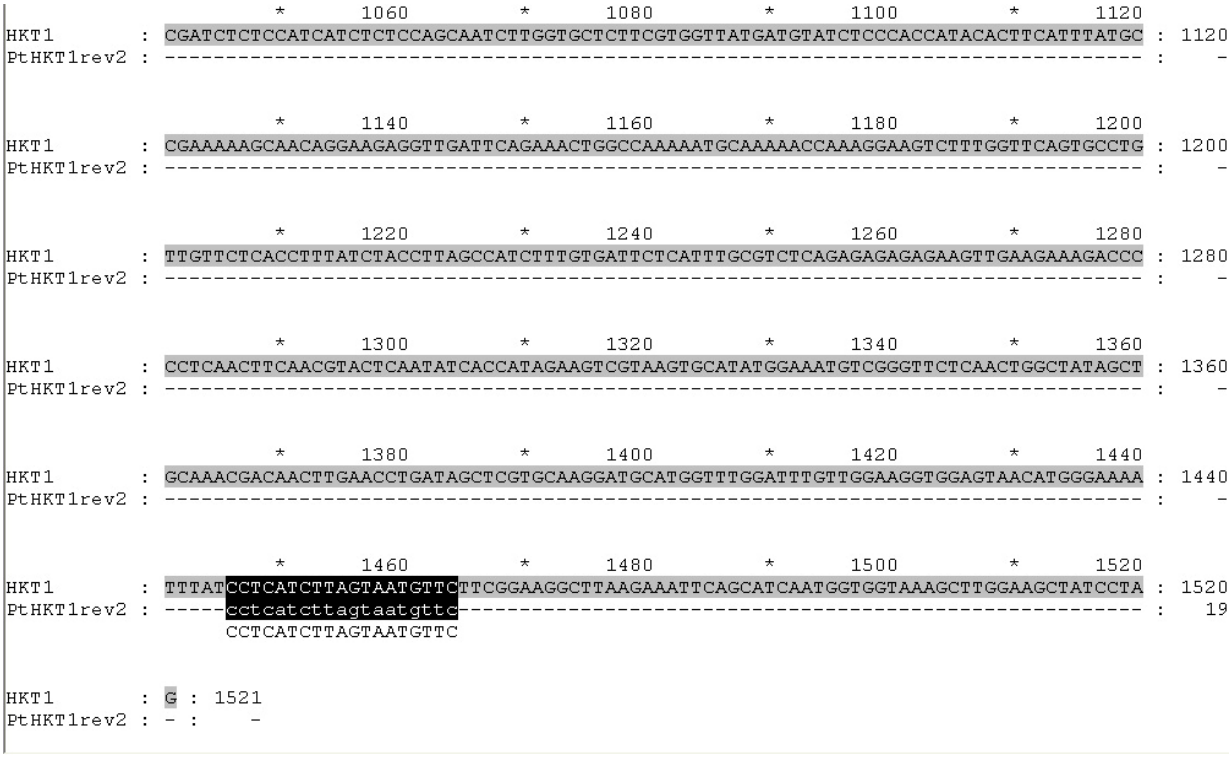

Fig. 7.2: Alignment of Primer PtHKT1rev2. Both primers were aligned using GeneDoc software (Version 2.6.002).

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