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Introduction

1.1 Soil salinity

Soil salinity is a problem in many parts of the world, especially in arid and semi arid regions where soluble salts accumulate in the soil because precipitation is much lower than evaporation. It is estimated that over 30% of the land area of the world is affected by high salinity (Epstein and Rains, 1987; Landis, 1988). Soil salinity can occur naturally as primary salinity or as the result of human activities as secondary salinity. Examples of activitiese that cause secondary salinity are inappropriate irrigation regimes and dam building that affect on the hydro-geomorphy (Prathapar *et al.*, 2005). The salt content of the soil affects the vegetation and can easily be estimated by electrolyte conductivity. According to classification of "U.S. Salinity Laboratory Staff" (1969), salinity has been classified based on electrolyte conductivity and plants growth response (Table 1).

Table 1. Soil salinity rating based on soil extract electroconductivity and growth response of agricultural crops (U.S. Salinity Laboratory Staff, 1969)

EC (dS/m)	Soil salinity rating	Plant growth response
0 to 2	Non-saline	Salinity effects negligible
2 to 4	Weakly saline	Growth of very sensitive crops may be restricted
4 to 8	Moderately saline	Yield of many crops restricted
8 to 16	Strongly saline	Only in tolerant crops satisfactory yield
> 16	Very strongly saline	Only a few very tolerant crops grow satisfactorily

There are three groups of salt affected soils: saline soils, sodic soils and salt-sodic soils. Saline soils have high concentrations of all salts. In sodic soils just the amount of sodium is high, saline-sodic soils are containing high amounts of sodium salts mostly as NaCl. Salt affected soils have been classified by evaluating the pH, EC and SAR (James *et al.*, 1982) (Table 2).

Table 2. Classification of salt affected soils based on saturation extract analysis (adapted by James *et al.*, 1982).

Classification	Electrical conductivity (dS/m)	Soil pH	Sodium absorption ratio (SAR)
Saline	>4	<8.5	<13
Sodic	<4	>8.5	≥13
Saline-Sodic	>4	>8.5	≥13

* The SAR (sodium absorption ratio) is calculated as:

$$SAR = [Na^+] / \sqrt{[Ca^{2+} + Mg^{2+}]}$$

where the ion concentration is based on molarities.

1.2 Water and salt uptake

Nutrients enter the plants *via* roots by water inflow. Generally water moves from a high to low water potential (Ouyang, 2002). The hydraulic gradient between soil solution and xylem sap leads to water influx into the xylem from where it is transported to the leaves which have the lowest water potential.

By increasing salinity the amount of solute in the soil or growth medium will increase and cause water deficit because of a decrease in water potential in the growth medium. When excess Na^+ and Cl^- have been taken up an ion-specific stress develops resulting from low K^+/Na^+ ratios. Persistent salinity raises the amount of Na^+ and Cl^- in the plant to concentrations that inhibit plant growth (Yamaguchi and Blumwald, 2005). High salt concentrations (greater than 0.4 M NaCl) change the hydrophobic and electrostatic balance of protein molecules and inhibit proper activity of most enzymes (Wyn Jones and Pollard, 1983). However, toxic effects on cells occur at much lower concentrations (about 0.1 M) pointing to specific targets of salt toxicity (Serrano, 1996).

Plants can not prevent the passive influx of solutes into the cortex cells of root, but can reduce their entrance into the vascular system by devices like Casparian band, retention of salt in the vacuoles and active exclusion. Na^+ may interfer with sites involved in binding of cations such as K^+, Ca^{2+} or Mg^{2+} (Wyn Jones and Pollard, 1983; Serrano, 1996). Na^+ can move passively through general cation channels into the cytoplasm (Blumwald, 2000; Mansour *et al.*, 2003). Export of Na^+ through Na^+/H^+ antiporters is also known (Hunte *et al.*, 2005). In plants, K^+ is the major cation to maintain cellular ion homeostasis by establishing an electrochemical concentration gradient across the plasma membrane. K^+ contributes in regulating hydraulic and osmotic pressure and balances the pH. Excess Na^+ disturbs these functions. Chloride (Cl^-) is also causing toxicity and osmotic stress in plants (Ruiz *et al.*, 1999), but is one of the main elements to control cell homeostasis and the regulation of cell volume specially by involving volume activated Cl^- channels like CLC-3 (Wang *et al.*, 2000). Cl^- may interfere with anionic sites involved in binding of RNA and anionic metabolites such as bicarbonates, carboxylates and sugar phosphates (ATP, ADP, NADH) (Wyn Jones and Pollard, 1983; Serrano, 1996). Active absorption of Cl^- is very important for normal plant development. By silencing cation-chloride-cotransporter (CCC) in *Arabidopsis*, elongation of inflorescence was severely reduced and leaves showed necrosis (Colmenero-Flores *et al.*, 2007).

1.3 Effects of salt stress in plants

In response to salinity, plants can be divided into halophytes and glycophytes. Halophytes accumulate a high amount of ions in their organisms while glycophytes exclude salt from their tissues. Halophytes have been classified as facultative and obligatory halophytes. Obligatory halophytes are not able to grow in non saline conditions whereas facultative halophytes prefer non saline environment but also can grow and survive in saline areas (Flowers, 1977). Plants differ in salt tolerance. For example, *Atriplex vesicaria* produced high yield in 700 mM NaCl (Black, 1960), while *Atriplex nummularia* died at 600 mM NaCl (Ashby and Beadle, 1957). *Salicornia europaea* plants remained alive in 1020 mM NaCl (Montfort and Brandup, 1927), while species of the halophyte alga *Dunaliella* even survived in 4 M NaCl (Johnson et al., 1927).

Except a small group of obligatory halophytes, salt stress reduces plant growth and productivity (Ashraf and Orooj, 2006; Sudhir and Murthy, 2004). In most glycophytes,

increasing external NaCl concentrations increases the amount of Na^+ and Cl^- in both shoot and roots, whereas K^+ and Ca^{2+} decrease with the progressive rise in salinity (Ashraf and Orooj, 2006). Increasing solutes during salt stress and consequently decreasing water potential in the rhizosphere, decreases or even disrupts water uptake of plants in the early stages of salt stress causing water deficit in the plant. The capability of plants to adjust a proper gradient is different from species to species but generally plants respond to increasing salinity by stomatal closure that is induced by ABA signalling. This is followed by a decrease in evapotranspiration to prevent loss of turgor and recovery of osmotic homeostasis with the accumulation of inorganic ions, amino acids, sugars and other metabolites (Ashraf and Orooj, 2006; Katerji *et al.*, 2000). In sensitive species organic materials contribute more to osmotic adjustment than in tolerant species, where osmotic adjustment is due to Na^+ and Cl^- accumulation (Meloni *et al.,* 2001). One of the common salt stress symptoms is reduction of photosynthesis and transpiration (Sudhir and Murthy, 2004). The chlorophyll content decreased in salt susceptible plants such as tomato, potato and pea, but chlorophyll content increased in salt tolerant plants such as mustard and wheat (Sudhir and Murthy, 2004; Venkatesan *et al*., 2005). The content of carotenoids increased in rice plants under salt stress (Kulshreshta *et al.*, 1987) and decreased in black cumin (Hajar *et al.*, 1996). Photosystem II (PSII) is a pigment-protein complex in the thylakoid membrane mediating non-cyclic electron transport from water to first mobile electron carrier, namely plastoquinone (PQ). Maximum efficiency of photosystem II does not decrease in salt resistant plants and is reduced in salt sensitive species (Wang *et al.*, 2007).

1.4 Salt tolerance mechanisms

The mechanism of salt tolerance differs from species to species (Parida and Das, 2005). However, the sensitivity of cytosolic enzymes to salt is similar in both glycophytes and halophytes, indicating that the maintenance of a high cytosolic K^+/Na^+ ratio is a key requirement for plant growth in soils with a high concentration of salt (Glenn *et al.*, 1999). Maintenance of K^+/Na^+ and Ca^{2+}/Na^+ ratios is one of strategies of salt tolerance (Yeo, 1998). This is possible either by active exclusion of Na^+ from the intracellular space and or by active absorption of K^+ into the cytoplasm. Na^+/H^+ antiporters and K^+/Cl^- cotransporters can help plant to maintain K^+/Na^+ homeostasis (Colmenero-Flores *et al.*, 2007).

Other salt tolerance mechanisms are accumulation of low-molecular-mass compounds like proline, glycine betaine, sugars (like glucose, fructose) and polyols (like mannitol, pinitol) in cytoplasm to establish osmotic homeostasis between vacuole and cytoplasm. This supports continued water influx by lowering the internal water potential (Parida *et al.*, 2004; Zhu, 2001).

Improving salt tolerance in plants is possible in different ways: direct selection of tolerant varieties of a species in saline environments or by mapping quantitative trait loci and subsequent use of selection markers or by generation of transgenic plants by introducing a novel gene or changing the expression level of an existing gene (Yamaguchi and Blumwald, 2005).

1.5 *P. euphratica* and response to salinity and osmotic stress

The genus *Populus* L. from Salicaceae is an important tree model system for the study of tree physiology and genetics and it is famous for its short rotation time and high productivity level (Ceulemans and Deraedt, 1999). Poplars are dioecious, wind pollinated, and are mostly distributed along river floodplains (Bradshaw *et al.*, 2000). *Populus* x *canescens* (Aiton) Sm., an endemic poplar species in Europe is natural hybrid between *Populus alba* and *Populus tremula* that can growth in slightly acidic to slightly alkaline soils and is known as salt sensitive species (Bolu and Polle, 2004). In contrast, *Populus euphratica* (Oliv.), an exotic poplar species in Europe, belongs to the Irano-Turanian and Saharo-Arabian bio- climatic zone (Gruenberg-Fertig, 1996). It is native to northwest China, middle and west Asia (Lledo *et al.*, 1995; Xu, 1988). It can growth in arid environments and it can tolerate a considerable amount of salt in soil but is susceptible to soil water deficit, late frost and pests (Bogeat-Triboulot *et al.*, 2007; Ottow *et al.*, 2005; Wan *et al.*, 2004).

Since both salinity and water deficit induce osmotic stress in plants, the response of *P. euphratica* to osmotic stress induced by withholding water has been analysed in an independent study as well (Bogeat-Triboulot *et al.*, 2007). In this study, anatomical, ecophysiological and molecular responses of *P. euphratica* to gradually developing water deficit were investigated (Bogeat-Triboulot *et al.*, 2007; see App. 7.29). The anatomical studies were contributed as part of this thesis (see App. 7.29) and revealed that water deficit decreased vessel and fibre lumen area and increased fiber cell wall thickness.

P. euphratica showed stomatal closure under water deficit and the compatible solutes (inositol, salicin, glucose, fructose, sucrose, and galactose) increased. Chlorophyll and carotenoid content per leaf area were not affected, but the chlorophyll a/b ratio increased under water deficit. Water deficit led to lipid peroxidation. At the molecular level lipocalins (see 1.6) were upregulated at strong water stress (Bogeat-Triboulot *et al.*, 2007).

These data underline that *P. euphratica* is very drought sensitive. Under field conditions, it was shown that the mortality of plantations in loamy and sandy sites at an early age is very high (Wang *et al.*, 1996; Khamzina *et al.*, 2006). *P. euphratica* is mostly distributed at springs, river banks and valleys, where it forms pre-dominant populations in regions with periodical waterlogging (Wang *et al.*, 1996; Khamzina *et al.*, 2006). It dislikes shade and is intolerant of root or branch competition (Huxley, 1992).

In native forests of *P. euphratica* the salt content in soil can be about 1%, but can reach up to 7% (Ma *et al.*, 1997). Under hydroponic conditions, 3-months-old saplings of *P. euphratica* could cope with up to 450 mM NaCl for one month and removal of the saline medium caused vigorous flushes. Increasing salinity to 600 mM caused mortality of *P. euphratica* (Gu *et al.*, 2004).

Initially, growth of *P. euphratica* increased after irrigating with 100 mM NaCl in soil but decreased later on (Ottow, 2004). When exposed to 150 mM NaCl in the nutrient solution, *P. euphratica* looses turgor pressure within 1h and regains it after one day. Shoot tip water potential dropped in first 30 min and recovered after 12h and decreased again after 2 days. Na^+ and Cl^- accumulated in leaves and roots of salt treated *P. euphratica* with 150 mM NaCl for 9 weeks, K^+ concentration did not change in leaves and roots but decreased in xylem sap, whereas the Ca^{2+} concentrations were decreased in roots and increased in the xylem sap. At the subcellular level, localization of sodium and chloride revealed that both elements were accumulated in the apoplastic space of leaves whereas the potassium concentration was reduced in leaf apoplast and vacuoles (Ottow *et al.*, 2005). Increasing either NaCl (0, 50, 150, 250 mM) or osmotic stress (0, 200, 300 and 400 mM mannitol) in *P. euphratica* caused accumulation of proline in both old and young leaves (Watanabe *et al.*, 2000). The chlorophyll content of *P. euphratica* supplemented with 150 mM NaCl did not change during short term exposure (Ottow, 2004). In a long term experiment, chlorophyll a was enhanced in both low and high salt stress (irrigating with 50 mM and 200 mM NaCl for 10 days) and the content of other pigments (chlorophyll b and carotenoids) was reduced (Ma

et al., 1997). Increasing salinity up to final concentrations of 200 mM NaCl over 16 days increased membrane permeability of a sensitive poplar species, *P. popularis* by 130%, but membrane permeability of *P. euphratica* did not change (Wang *et al.*, 2007).

Ma *et al.* (1997) showed that net photosynthesis of *P. euphratica* irrigated with high salt concentration (200 mM) declined but recovered after three weeks close to the control values. No difference in photosynthesis was observed under low salt concentrations (50 mM). Studies on four poplar species (*P.* x *euramericana*, *P. deltoides* x *P. alba*, *P. alba* and *P. euphratica*) in response to salinity revealed that *P. euphratica* retained the highest net photosynthesis rate in 137 mM NaCl among these species (Sixto *et al.*, 2005). By exposing *P. euphratica* to 50 mM NaCl, net photosynthesis rate and transpiration rate of one-year-old seedlings was reduced within 4 hours because of stomata closure and recovered after 24 hours (Wang *et al.*, 2007). Increasing salinity to 200 mM for 16 days did not change the photosynthesis rate and transpiration rate of *P. euphratica* significantly, whereas in the salt sensitive *P. popularis*, irrigation with 150 mM NaCl caused a severe drop of photosynthesis rate and transpiration after 4 days (Wang *et al.*, 2007).

Initially salinity (50 mM NaCl after one day in one year old plants) did not affect maximum photosystem II efficiency of both *P. euphratica* (salt resistant) and *P. popularis* (salt sensitive), but increasing NaCl stress to 200 mM for 12 days reduced maximum photosystem II efficiency *P. popularis* because of reduction in minimal fluorescence level (Fo) and increasing in maximal fluorescence level (Fm), but no change in *P. euphratica* was observed (Wang *et al.*, 2007).

There is some evidence that ABA signalling is involved in early response to dehydration. ABA is transported from the root and through the xylem sap to the leaves and causes stomatal closure before any water deficit in leaves take place (Chaves and Oliveira, 2004). According to Chen *et al.* (2001, 2002), the concentration of ABA in the xylem increased more rapidly in *P. euphratica* than in other more salt-sensitive poplar species. This compilation shows that *P. euphratica* is salt but not drought tolerant. The mechanisms leading to salt tolerance are not understood.

1.6 Lipocalins structure and function

There is some evidence that lipocalins may play an important role under environmental stress. This gene became up-regulated in both osmotic (Bogeat-Triboulot *et*

al., 2007) and salt (Brinker and Polle, 2005) stress in *P. euphratica*. The term lipocalin originated from "Lipo" which means lipid and "Calix" (Latin: *Calyc*, *Calyx*; Greek: *Kalyx*) which means cup-form, describing highly conserved structure and lipid-binding properties of members of this family. They belong to a large family of extracellular proteins which bind with hydrophobic molecules but their function (often putative) is very diverse and in many cases not yet known (Bishop *et al.*, 1995; Bishop, 2000; Flower *et al*., 2000). Lipocalins are present in bacteria, protozoa, plants, arthropods, and cordates (Sánchez *et al.*, 2003). Ligand-binding properties of lipocalins have been summarized by Flower (1996). Three families of ligand binding proteins; ie., lipocalins, fatty acid-binding proteins (FABPs), avidins together with a group of metalloprotease inhibitors (MPIs) and triabin, form the calycin superfamily (Flower *et al*., 2000). In lipocalins three structurally conserved regions (SCRs) related to features of the β-barrel are conserved: SCR1 (strand A and 3_{10}-like helix preceding it; consensus GWxR), SCR2 (portions of strands F and G, and the loop linking them; consensus TDY), and SCR3 (portion of strand H, the beginning of the following helix and the loop in between; consensus R) (Figure 1). Lipocalins are involved in the regulation of cell homeostasis and the modulation of the immune system activation in mammals (Flower, 1996). The plasma membrane anchored lipocalins also seems to have an important role in membrane biogenesis and repair, and in adaptation of cells to high osmotic stress (Bishop, 2000). First plant lipocalins were identified from xanthophyll cycle enzymes such as violaxanthin de-epoxidase (VDE) and zeaxanthin epoxidase (ZEP) that catalyze the addition and removal of epoxide groups (cyclic ether with only three ring atom) in carotenoids of the xanthophyll cycle in plants (Bugos *et al.*, 1998). These two members of lipocalin in compare with ''true plant lipocalins'' (see follow) do not share all three SCRs motifs and other domains except lipocalin are also present in their structural features (Charron and Sarhan, 2006).