

1. Introduction

1.1 Saline environments

During medieval times, salt was a costly commodity that was weighed gram for gram with gold (Owens, 2001). In spite of manifold positive uses and exploitations, salt was and is still today regarded more a threat than an asset. Soil salinization has endangered ancient as well as modern agriculture and forestry. Although, salt-affected soils and landscapes can be found in almost all climatic regions, the majority of them occur in arid and semiarid areas or in coastal regions (FAO, 1987). According to Szabolcs (1994) and the FAO Land and Plant Nutrition Management Service (1987), more than 800 million ha of land are affected worldwide by soil salinity or sodicity which comprises approximately 6.5% of the total land area (Fig. 2). Out of the 1500 million ha that are cultivated, about 5% are affected by salt (Munns *et al.*, 1999).

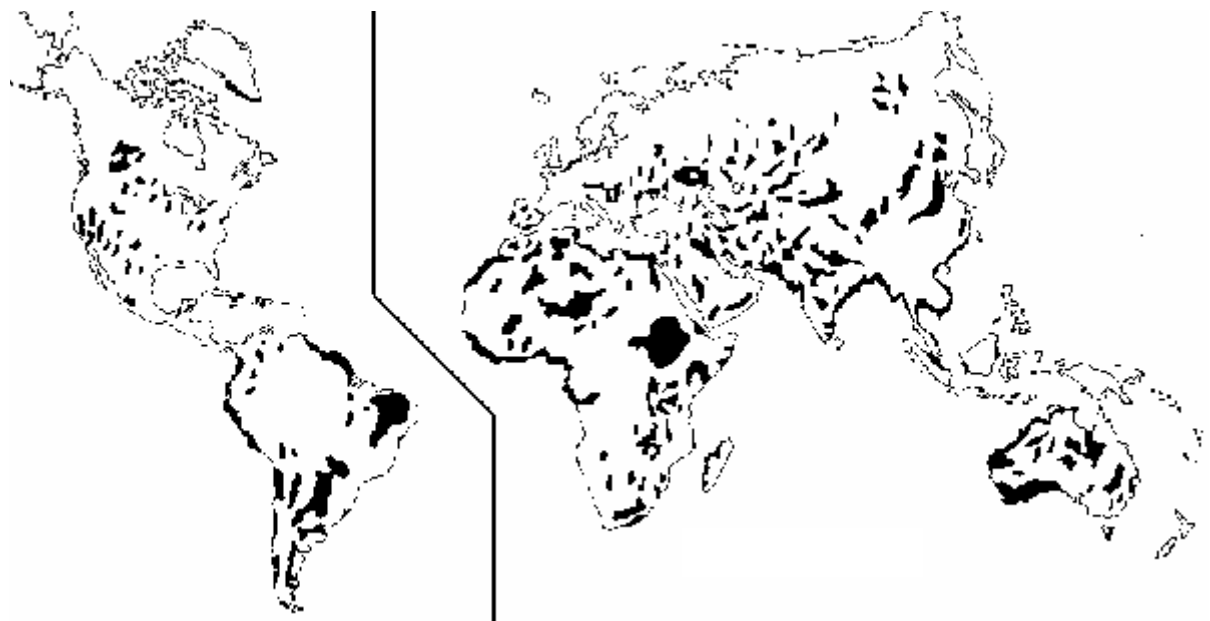


Fig. 2: Global distribution of salt-affected soils (marked black) (after Szabolcs 1985).

Salinisation can either occur naturally (primary salinization) or may be the result of human influence (secondary salinization). Primary salinization can be found all along the sea shores such as salt marshes but may sometimes be extended to adjacent areas up to 5-10 km from the coast due to air-borne salt transport. Following rainfall, the aerosols can deposit salt levels as high as $200 \text{ kg} \times \text{ha}^{-1} \times \text{a}^{-1}$ in some coastal areas (Owens, 2001). Secondary salinization is due

to changes in the hydrologic balance of soils leading to imbalances between water supply and transpiration. This disequilibrium is most commonly caused by land clearing and subsequent replacement of perennial vegetation with annual crops (FAO, 1987). The problem is intensified by a combination of bad water management practices using salt-rich irrigation water and insufficient drainage. When clearing and irrigation come together, soluble salts from the subsoil may be transported with the rising water table to the top of the ground surface. Although the sum of irrigated land is relatively small (ca. 15% of cropped area) it is estimated to produce one-third of the world's food (Munns, 2002). Consequently, salinization of these areas has a significant impact on productivity and food supply. The quantity of salt that is being stored in the soils depends mainly on the soil texture and the soil type, generally being low for sandy soils and high for soils containing elevated amounts of clay and organic substances. Excess soil salinity has devastating effects on both soil structure and plant performance.

1.2 Salt resistance: a widespread property in Nature?

Plants and other organisms differ extremely in their capacity to live or survive under saline conditions. Halophiles are salt-loving organisms well adapted to hypersaline environments. This group includes both prokaryotic and eukaryotic organisms with the ability to balance the osmotic and detrimental effects of salts (DasSarma and Arora, 2002). Well known examples include archaeal *Halobacterium* species, cyanobacteria such as *Aphanothece halophytica* and the green alga *Dunaliella salina*. Only very few plants are able to withstand comparably high salt concentrations. A variety of plants can survive in moderately saline soils. So called halophytes are plants that show an increase in biomass production when growing on saline soils. Most plants, however, belong to the group of glycophytes which show reduced growth, leaf damage and an increase in root/shoot ratio under the influence of accelerated salt concentrations in their environment (Munns and Termatt, 1986). Remarkably, nearly all salt-tolerant plants are angiosperms (Greenway and Munns, 1980).

1.3 The impact of salt stress on plant performance

Salinity imposes a wide range of stresses upon a plant. The most common effect of soil salinity on the performance of glycophytes is growth inhibition (Tester and Davenport, 2003).

Inhibition might be caused by primary effects such as osmotic stress, ion disequilibrium and ion toxicity or by secondary effects like oxidative stress and/or a reduction of photosynthesis (Hasegawa *et al.*, 2000). Most stress symptoms are interrelated and are difficult to distinguish. This is especially true for osmotic and ion specific stress responses. Growth reduction within the first hours of salt stress was considered to depend on the osmolarity of the external solution rather than on the Na^+ concentrations in growing tissues (Munns, 2002). In the context of whole-plant adaptability, osmotic stress seems to impose a rather unspecific stress closely related to drought stress (Munns, 2002). Various salts can be elevated in soils but not all have a negative effect on growth. High concentrations in Na^+ and Cl^- are regarded the major cause of plant injury. Apart from a few woody perennials like citrus and grapevines, Na^+ seems to be the primary cause of ion-specific damage (Tester and Davenport, 2003). Another major effect of high soil salinity is the attenuated nutrient acquisition since elevated Na^+ concentrations inhibit the uptake of essential nutrients like K^+ , Ca^{2+} and Mg^{2+} (Hasegawa *et al.*, 2000). Altered nutrient uptake by the roots will eventually lead to changes in ion composition within cells causing various metabolic disorders in plants. Under physiological conditions, plants try to maintain a high cytosolic K^+/Na^+ ratio of about 100-200 mM K^+ in relation to 10 mM Na^+ (Binzel *et al.*, 1988). Since Na^+ directly competes with K^+ for binding sites important for cellular function, Na^+ concentration above ca. 100 mM or relatively low K^+/Na^+ ratios may interfere with a wide range of enzymatic reactions leading to a disruption of protein synthesis (Wyn Jones and Pollard, 1983). More than 50 enzymes are activated by K^+ (Bhandal and Malik 1988). In addition, a certain K^+ level is needed for the binding of tRNA to ribosomes (Blaha *et al.*, 2000).

Leaves are frequently injured by salinity simply because Na^+ and Cl^- are accumulated by the strong transpirational pull and successively accumulate in the leaf when the water evaporates. It is assumed that only minor amounts of Na^+ are recirculated by the phloem (Tester and Davenport, 2003), triggering the problem. One, yet controversial hypothesis, is that Na^+ accumulates in the apoplast causing a steep concentration gradient between apoplast and cytoplasm eventually leading to cell dehydration and massive Na^+ influx into the cell (Oertli, 1968; Flowers *et al.*, 1991; Mühling and Läuchli, 2002).

1.4 Strategies to survive high salinity

When exposed to high NaCl concentrations, plants can either try to restrict uptake (*Excluder*) or take up the salt and accumulate it in different plant organs (*Includer*). Salt resistance can be

subdivided into salt-avoidance and salt-tolerance (Flowers *et al.*, 1977). The classification depends on the strategy that a certain plant follows in surviving excessive salt concentrations in its environment. Salt avoider are salt resistant by preventing toxic amounts of salt (mainly NaCl) from entering their sensible meristematic tissues, growing leaves and especially young fruits. On the contrary, salt-tolerant plants have evolved adaptive mechanisms to cope with increased salt concentrations within their biosynthetic apparatus. Adaptive traits have been recognized on the whole-plant as well as on the cellular and subcellular level (Tester and Davenport, 2003).

1.4.1 Adaptations on the whole-plant level

Salt avoidance can be achieved by building-up transport barriers in the root and/or within the shoot. Effective filtrating systems are known to operate in some mangroves which are capable of keeping their vascular system almost free of harming salts (Suárez and Sobrado, 2000). Transport barriers have been described for various crop plants which readily take-up salt but hold it back in the roots (Nevo *et al.*, 1992). For a wide range of monocotyledonous species a correlation has been found between moderate salt resistance and the ability to avoid salt by excluding Na^+ from the leaf blades or even from the entire shoot (Schachtman *et al.*, 1992; Flowers and Hajibagheri, 2001; Zhu *et al.*, 2001). However, this correlation cannot be considered a general rule. The connection between low shoot Na^+ concentration and salt resistance does not hold for most dicotyledonous halophytes. Glenn *et al.* (1999) proved certain halophytes to contain high Na^+ concentrations in the shoot without dying. However, it is unlikely that dicotyledonous halophytes have principally higher transport rates than glycophytes. Instead, they simply live longer than their salt-sensitive relatives on saline substrates accumulating larger amounts of Na^+ . Yet, halophytes end up with shoot Na^+ concentrations that are lethal for most glycophytes. In contrast to dicotyledonous species, monocotyledonous halophytes follow a different strategy taking up less Na^+ than dicotyledonous halophytes adjusting the osmotic balance through pronounced sugar synthesis (Tester and Davenport, 2003).

Most plants avoid salt stress by minimizing the initial entry of Na^+ and Cl^- . In these cases, uptake control and distribution processes may contribute effectively to the tolerance level. Thus, restricting initial uptake and by targeting the incoming salt to tissues less sensitive are thought to be important strategies to the plant. Additionally, maintaining a sufficient K^+ uptake and supply in combination with a high K^+/Na^+ ratio seems to be at least as important as low Na^+ levels *per se*. This hypothesis has been widely proven because many aspects of

Na^+ toxicity are based on K^+ shortage (Maathuis and Amtmann, 1999; Cuin *et al.*, 2003). Initial entry into the roots is based on passive influx due to the electrochemical gradient caused by differences in Na^+ concentrations and voltage (Cheeseman, 1982). Since Na^+ is also excluded, the final concentration of Na^+ is a result of influx and efflux ratios. Schubert and Lauchli (1990) proposed that maximizing Na^+ efflux would minimize plant damage. It is noteworthy, that most plants seem to be unable to inhibit high unidirectional Na^+ influx into the root but are on the other hand capable of strong active Na^+ efflux across the plasma membrane out of the root reducing the total Na^+ concentration considerably (Tester and Davenport, 2003). Roots can regulate the Na^+ concentration by transporting Na^+ into the xylem and subsequently into the shoot. Another strategy might be recirculation of Na^+ via the phloem back to the roots (Munns *et al.*, 1988; Blom-Zandstra *et al.*, 1998). It also seems as if the extent of this recirculation is directly correlated to salt resistance. Comparing salt-resistant *Lycopersicon pennellii* with salt-sensitive *L. esculentum* revealed that the salt resistant tomato had a higher recirculation rate (Perez-Alfocea *et al.*, 2000). In salt resistance, the protection of younger leaves may play an important role (Jeschke, 1984). An intensive up movement in the xylem and a corresponding down movement within the phloem was proposed by Wolf *et al.* (1991). However, to what extent recirculation occurs must await further study. Within leaves, high amounts of Na^+ were found in bundle sheath cells indicating a putative involvement in minimizing Na^+ accumulation in the leaf mesophyll (Karley *et al.*, 2000). A few halophytes, such as salt cedar (*Tamarix spp.*), the salt bush (*Atriplex spp.*) and some mangroves, route the Na^+ and Cl^- to specialized salt glands on their leaf surfaces (Atkinson *et al.*, 1967). Salt glands are located within the epidermis of the leaf and act as gates removing salt to the surface where it forms crystals that are washed away with the next rain. Interestingly, this apparent effective strategy is not widespread but instead is restricted to only a few plant species (Tester and Davenport, 2003). Since salt glands co-secrete a remarkable amount of water, they are only found in plants that are growing under sufficient water supply as can be found in salt marshes. Within drier areas, salt hairs and hydathodes can be found which waste less water. Development of leaf succulence is another effective way to counteract salt stress (Tester and Davenport, 2003). Increasing the number of cells or the cellular volume dilutes the salt and may increase CO_2 absorption per unit of leaf area (Shannon *et al.*, 1994). A quite drastic way of salt exclusion is leaf shedding when a critical salt concentrations has been reached. This strategy is known from trees like the mangroves but has been also observed in tiny plants such as *Thellungiella halophila* (salt cress), a close but extremely salt resistant relative of *Arabidopsis thaliana* (Inan *et al.*, 2004).