24 Salt Effects on Plants

An Overview

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24.1 SALINITY STRESS

Plants as sessile organisms are constantly exposed to different constraining environmental conditions, such as drought, salinity, extreme temperatures, UV radiation, heavy metals and hypoxia. These factors, collectively referred to as abiotic stresses, limit plant growth, and in agricultural crops, decrease productivity and yield. Resistance or sensitivity to these factors is very important, given that they can affect different stages of plant growth and development, and may act synergistically with other abiotic stresses (Chinnusamy et al. 2004). Therefore, the discovery of mechanisms underlying tolerance to abiotic stresses and adaptations to these are a major focus of contemporary research.

In parallel with climate change, which will affect crop production worldwide, there is an increasing need to maintain food security for a growing global population. The quality of land and the availability of water will be critical for agriculture in the future. The Food and Agriculture Organization estimates that salinity, as one of the major abiotic stress factors of plants, currently affects more than 6% of the world's land area (Ilangumaran and Smith 2019). However, much of this land is not used for cultivation; according to the FAO, 450 million ha of irrigated land and 32 million ha under dryland agriculture have been affected by salinity (Munns and Tester 2008). Some reports suggest that the area of salinized land will increase with climate change as the scarcity of water will lead to increased salt concentrations.

24.1.1 SOIL SALINITY

According to the USDA salinity laboratory, a soil is defined as saline when its electrical conductivity of the saturation extract (EC_e) exceeds 4 dSm^{-1} , which is approximately 40 mM sodium chloride (NaCl). Salt-affected lands have been present for thousands of years; the oldest written records date back to ~2400 BCE when salinity was recorded in the alluvial plains of Iraq (Russel et al. 1965). Salinization of soil can be caused by different mechanisms (natural or human activities) that can increase the concentration of dissolved salts. The salts present in soils are predominant NaCl, but may also include sodium nitrate (NaNO_3), sodium sulphate (Na_2SO_4), calcium sulphate (CaSO_4), potassium sulphate (K_2SO_4), magnesium chloride (MgCl_2) and magnesium sulphate (MgSO_4). Salt-affected lands are widespread and occur in all climatic regions, at different altitudes, from below sea level (e.g. the Dead Sea) to mountains above 5000 meters. Natural processes, which lead to the accumulation of the salt in the soil and groundwater, during extended periods of time, result in the formation of salt lakes, marine sediments and salt scalds (Ilangumaran and Smith 2017). However, the process of soil salinization is aggravated and accelerated by cultivation, mainly by crop irrigation, but also by land clearing and inadequate drainage. In general, irrigated lands are more affected by salinity than drylands; this is because the deposition of salt is an inescapable part of the irrigation process. The water used for irrigation can contain calcium (Ca^{2+}), magnesium (Mg^{2+}) and sodium (Na^+). In alkaline soils, after evaporation, Ca^{2+} and Mg^{2+} ions can precipitate as carbonates, leaving sodium as a dominant ion in the soil (Serrano et al. 1999). In addition, irrigation of crops with more water than they can utilize can lead to an increase in the water table, which can mobilize salts from depth in the soil profile towards plant roots.

The capacity of soil to store salt depends on soil type. Sands are more readily leached than clays. Also, sandy soils have low cation exchange capacity compared to clay; negatively charged clay particles are, therefore, able to adsorb Na^+ . If Na^+ is adsorbed to the cation exchange complexes of clays, the soils can become dispersive causing soil aggregates to break down, increasing bulk density and, thus, changing soil porosity. This can affect soil and root aeration. Because of these factors, plants in saline soils can suffer from high levels of sodium ions, water deficits (similar to drought conditions) as well as hypoxia (Carillo et al. 2011).

24.2 EFFECT OF SOIL SALINIZATION ON PLANTS

Soil salinity is a major factor limiting the yield of agriculturally important plants and may well jeopardize the capacity of agriculture to cope with a projected food demand for 10 billion people by 2050 (Flowers 2004; Parida and Das 2005; Munns and Tester 2008; GAP Report 2018).

Plants can be divided into two different groups depending on their ability to survive under saline conditions: glycophytes and halophytes (Munns and Termaat 1986). Most plants, including most crop plants, belong to the first group; their growth is inhibited and they may even be killed by 100–200 mM NaCl. These plants have evolved under conditions of low soil salinity. On the other hand, halophytes can survive high concentrations of salt, up to 400 mM NaCl. Depending on their

salt-tolerance, they can be further subdivided into two groups – obligate and facultative halophytes. Facultative halophytes thrive in less saline habitats, mainly marginal soils representing the border between saline and non-saline soils. On the other hand, obligate halophytes are found in landscapes of continual high salinity.

24.2.1 PLANT RESPONSE TO SALINITY STRESS

Salinity is a complex stress for plants, affecting growth and development in different ways. First, soil salinity disturbs the capacity of roots to extract water because of an osmotic (water) stress. A second stress is cytotoxicity due to the excessive uptake of ions (predominantly sodium and chloride). Third, there can be stress caused by nutritional imbalance. All these effects are typically accompanied by oxidative stress due to the generation of reactive oxygen species (ROS) (Tsugane et al. 1999; Hernández et al. 2001; Isayenkov 2012).

A two-phase model describes plant responses to salinity. During the first phase, osmotic shock causes stomatal closure and inhibition of cell expansion. This phase is immediate, taking place within minutes to hours (Munns and Passioura 1984; Munns and Termaat 1986; Rajendran et al. 2009). A second phase of response takes longer, days to weeks; in this phase, the cytotoxicity of ions slows down metabolism, causing senescence and cell death (Roy et al. 2014; Isayenkov and Maathuis 2019). Plants can adapt to salinity through three mechanisms: control of ion uptake, osmotic adjustment and detoxification.

24.2.1.1 Control of Ion Uptake

Overall, the most important strategy for plants is to control the uptake of ions rather than dissipating energy on detoxification and subsequent damage repair.

Ion uptake into plants can be through two different pathways – the symplastic and the apoplastic pathway (Gao et al. 2007; Maathuis et al. 2014; Negrao et al. 2017; Isayenkov and Maathuis 2019). The apoplastic pathway involves the direct flow of ions in cell walls and the extracellular space between the outside of the root and the root xylem (Krishnamurthy et al. 2009). In some monocots, apoplastic flux can be responsible for up to 50% of total Na^+ uptake, as well as up to 50% of Cl^- translocation to shoots (Shi et al. 2013; Yeo et al. 1987; Kronzucker and Britto 2011). On the other hand, the symplastic pathway requires ions to cross a cell membrane and then move from cell to cell in the symplasm through plasmadesmata. The uptake of Na^+ and Cl^- into roots into the symplastic pathway is catalyzed by different transporters. The most investigated amongst these belong to the group of nonselective cation channels. Another subclass of transporters is HKT (Isayenkov and Maathuis 2019). Arabidopsis possess only the subclass 1, Na^+ selective AtHK1 isoform, but monocotyledonous plants have multiple HKT isoforms. In addition, PIP isoforms as well as low-affinity cation transporter LCT1 and Na^+/H^+ antiporters could be involved in Na^+ transport (Schachtman et al. 1997; Kronzucker and Britto 2011; Huang et al. 2012; Byrt et al. 2017). In contrast to sodium, chloride is an essential nutrient for plants. It can be transported into plant cells by an H^+/Cl^- symport, but the precise mechanism is still unknown. The cation-chloride cotransporters represent another class of potential Cl^- transporters (Zhang et al. 2011).

Although there is a lot of data about mechanisms of salt entering into plants, how Na^+ and/or Cl^- are sensed by plants still remains unknown. In animals, primary Na^+ sensors are specific Na^+ selective ion channels, with Na^+ binding sites. These sites modulate the gate and may act as the reporters of Na^+ concentrations in body fluids. These binding sites can also modulate transporter activity. However, similar mechanisms have not yet been identified in plants (Isayenkov and Maathuis 2019). It is speculated that Ca^{2+} signaling could play an important role in early signaling events, but all upstream components are still unknown (Choi et al. 2014; 2016). It seems that the SOS pathway is a key regulator of Na^+ homeostasis in plant cells, but other components are ROS as well as cyclic nucleotides, such as cGMP (Kiegle et al. 2000; Donaldson et al. 2004). It has been shown that glycosyl inositol phosphorylceramide (GIPC) – sphingolipid in plant plasma membrane – could sense salts (Jiang et al. 2019). MOCA1 as a glucuronosyltransferase for GIPCs in the plasma membrane is required for salt-induced depolarization of the cell-surface potential, Ca^{2+} spikes and waves, Na^+/H^+ antiporter activation, and regulation of growth. Na^+ binds to GIPCs to gate Ca^{2+} influx channels.

24.2.1.2 Osmotic Adjustment

One of consequences of increased salt concentration in the soil is osmotic shock. Salt decreases the water potential of the soil; if this becomes more negative than the water potential of the roots, there can be tissue dehydration. Osmotic shock can decrease the rate of plant growth and development, even causing cell and plant death. One way of estimating the osmotic component of salt stress is necessary to compare the effects of salt exposure to exposure to an equi-osmolar quantity of an inert osmoticum such as polyethylene glycol. Many studies have shown that osmotic stress has more influence on plant growth than ion toxicity (Castillo et al. 2007; Zhao et al. 2010; Isayenkov and Maathuis 2019).

Plants can regulate their osmotic potential through the process of osmotic adjustment, which prevents macromolecules from denaturation. One of the most common mechanisms of osmotic adjustment is the synthesis and accumulation of compatible organic solutes – organic acids (e.g. malate, aspartate and oxalate), amino acids and their derivatives (e.g. glutamate, proline and glycinebetaine), carbohydrates (sucrose) and polyols (e.g. sorbitol, mannitol and pinitol). These molecules act as the protectors of macromolecules under conditions of increasing ionic strength. Apart from their role as osmoprotectants, some of those molecules can also serve as sources of nitrogen. Thus, proline accumulated as a free amino acid for osmotic adjustment can also regulate the availability of nitrogen. In addition, some of these osmolites can stabilize cell membranes, preventing and reducing the damage caused by high salt levels (Iqbal et al. 2014; Vaishnav et al. 2016).

24.2.1.3 Detoxification

One secondary effect of salt stress and other abiotic stress is the occurrence of oxidative stress. Oxidative stress occurs because of an imbalance between the production and scavenging of ROS. These include hydrogen peroxide (H_2O_2), the hydroxyl radical ($\text{OH}\cdot$), singlet oxygen ($^1\text{O}_2$) and the superoxide anion radical ($\text{O}_2^{\cdot-}$) (Sharma et al. 2012). Apart from ROS, the production of reactive nitrogen species can also

cause oxidative stress. Many ROS are able to propagate themselves, as well as producing other ROS, leading to the damage of macromolecules in the cell; indeed, cell death can be caused by lipid peroxidation, protein oxidation and the oxidation of nucleotids in nucleic acids. Plants have developed mechanisms to protect them from ROS that are both non-enzymatic (e.g. the production of glutathione, ascorbate, tocopherols, carotenoids, phenolics) and enzymatic. Enzymatic defense mechanisms include the synthesis of enzymes that regenerate the reduced forms of antioxidants, such as ascorbate peroxidase (APX) and glutathione reductase, and ROS-interacting enzymes (ROS scavengers), such as superoxide dismutase, catalase (CAT), non-specific peroxidase (POD) and APX (Jovanović et al. 2011).

24.3 HOW TO PRODUCE SALT-TOLERANT PLANTS

A variety of strategies can be used to improve the tolerance of plants to different abiotic stresses, and all of them have been extensively studied because of the economic importance of plant tolerance to agriculture. These vary from traditional breeding and genetic engineering to the use of chemical (priming agents) and biological strategies (biofertilizer).

The traditional approach, i.e. breeding and selection for higher yields, is time consuming and labor intensive (Vaishnav et al. 2016). However, the development of new molecular techniques, such as the use of QTLs and molecular markers, gene mapping etc. have accelerated the development of marker-assisted breeding (Agarwal et al. 2013).

The development of molecular biology and genetic engineering provide the most progressive approach for producing salt tolerant plants. Halophytes (salt-tolerant plants) and glycophytes (salt-sensitive plants) can be used to identify genes associated with the salt response. Once identified and characterized, these genes can be transferred into different plants, in order to improve their tolerance against salt stress. There have been many attempts to obtain transgenic plants tolerant to salinity. Zhang et al. (2019) developed transgenic salinity-tolerant rice varieties using CRISPR/Cas9 targeted mutagenesis. Experimental data have shown that such genes are involved in different activities during salt stress, such as ROS scavenging, and the biosynthesis of compatible osmolytes and protective proteins (Ashraf and Akram 2009). Although the use of transgenic approaches has great potential for the development of salt tolerant varieties, a lot of ethical issues will need to be solved (Vaishnav et al. 2016).

Another method for improving plant growth in saline environments may be to prime plants with exogenous chemicals; in this case, chemical products of plant metabolism produced at low concentration in plants could be applied to enhance the salt tolerance of plants. These compounds, such as nitric oxide, H_2O_2 , salicylic acid, jasmonates, proline and glycine betaine, could be applied to plants to control downstream processes. One major problem with the use of these compounds is that their use is not yet cost-effective. Furthermore, it is possible that these chemicals might cause a number of other environmental problems.

One of the most promising approaches for the enhancement of plant tolerance to salinity is the use of biocontrol agents such as plant growth promoting bacteria

(PGPB) (Russo et al. 2010; Vejan et al. 2016; Numan et al. 2018; Abbas et al. 2019; Egamberdieva et al. 2019).

24.4 FUTURE PERSPECTIVES

Salinity as a major limiting factor for crop productivity affects almost 30% of the irrigated land of the world. Every year this area increases by 1–2% (FAO 2014). Climate change, with an increase in temperature as well as with a decrease in average annual rainfall, will worsen the situation. Studies on plant responses to salinity have to be done not only with model plants (such as *Arabidopsis*), but also with other plant species, especially with agriculturally important and woody plants. Although many studies on salinity tolerance have focused at the agronomic, physiological and biochemical level, this focus is now increasingly moving towards the molecular level providing valuable information about candidate genes that could be used in salt resistant plants. New molecular methods will accelerate the development of transgenic salt-tolerant plants, with desirable traits. However, the use of transgenic plants will bring new problems, not only with consumers but also with legal issues. Another feasible strategy to mitigate salinity impacts on crop production could be the use of halotolerant PGPB, which may help plants thrive in more extreme environmental conditions.

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