Role of Ion Transporters in Salinity Resistance in Plants

Mirvat REDWAN, Francesco SPINELLI and Stefano MANCUSO

LINV — Department of Agrifood Production and Environmental Sciences — University of Florence, Viale delle Idee 30, 50019 Sesto F.no, Florence, Italy

Salinity is one of the most severe factors that limits the yield of filed, because most of crops employed in agriculture is salt sensitive. This losses in yield is mainly due to drought and high salinity of field and this situation will be worst with global climatic changes. In particular the toxicity of Na^+ is due to its ability to inhibit enzyme function directly by binding to inhibitory sites or indirectly by displacing K^+ from activation sites. Plants have evolved a wide range of mechanisms to adapt and mitigate this kind of abiotic stress. Here we reviewed sensing and signaling mechanisms of salt stress and the tolerance strategies in plants.

Keywords : cell signalling, ion transporters, salt stress, tolerance mechanisms

INTRODUCTION

Salinity is an increasing problem that affects vast areas of the world (irrigated, arid and semiarid areas) in particular where precipitation is insufficient to leash salts from the root zones (Leland and Eugene, 1999). About 6% of the global land area is affected by salinity (Flowers and Yeo, 1995) and salt affected soils include about one third of the world's irrigated soils presently and that portion is expanding (Robinson and Downton, 1984; Chauhan et al., 1987).

Because of the intensive irrigation practices secondary salinization is increased in the agricultural soils. And in combination with the competition of fresh water among municipal, industrial and agricultural sectors this problem (salinity) becomes more sever in particular with the growing water shortage problem, where more than 80 countries suffer from water shortage each year (Gleick, 1993), taking into consideration that agriculture consumes more fresh water than any other human activity (Falkenmark et al., 1987).

A definition for saline soils adopted from FAO (1997) is those soils that have an electrical conductivity of the saturation extract (EC_e) of 4 dS m^{-1} or more. Regardless of the specific nature of cations or anions the soil was considered saline when contains excessive amounts of salts in general. However, Na^+ and Cl^- are considered the most important and are toxic to plants (Hasegawa et al., 2000). The toxicity of Na^+ is due to its ability to inhibit enzyme function more specifically either directly by binding to inhibitory sites or indirectly by displacing K^+ from activation sites (Serrano, 1996). Moreover Na^+ is considered cytotoxic at cytosolic concentration in excess of about 100 mM (Serrano et al., 1999). The competition between Na^+ and K^+ in the cytosol is likely to be more critical factor in determining Na^+ toxicity than cytosolic Na^+ concentration *per se*. Salinity has a major impact on plant growth, and usually during the first several days of the stress the inhibition of growth occurs and primarily restricted to the shoot (Munns and Termaat, 1986). It was found that leaf growth is more sensitive to salinity than root growth under salt stress, this can be explained or by water deficit or a specific salt toxicity in the shoot or in the root, but in case of long term salinity large amounts of salt is brought into the shoot, especially into the leaves, by prolonged transpiration which in turn result in damaging leaves, this process must eventually limit the supply of assimilates to the growing regions and might be the main factor determining yield. Another hypothesis for the mechanism by which salinity reduces shoot growth. One suggestion is that salinity reduces photosynthesis which in turn limits the supply of carbohydrates needed for growth (Yeo and Flowers, 1985; Munns and Schachtman, 1993). A second is that salinity reduces shoot growth by reducing turgor in expanding tissues which are not able to fully osmoregulate in response. A third is that roots sense salinity and down regulate shoot growth by via a long distance signal (Termaat et al., 1985; Munns and Termaat, 1986; Rengel, 1992). Fourth a disturbance in mineral supply to the shoot, either an excess (Na^+ or Cl^-) or deficiency, might directly affect growth (Abel and MacKenzie, 1964; Läuchli and Wieneke, 1979; Flowers and Yeo, 1981; Jeschke, 1984). It was found also that NaC1 induced disturbance in the supply of carbon to the growing zones of shoots might be associated with the increased starch accumulation in mature leaves (Munns and Schachtman, 1993).

Taking all the previous responses into account and trying to compare between salt tolerant and salt sensitive genotypes we can say that the key to salt tolerance in plants is the ability to comprise successfully between osmotic ad-

Corresponding author : Stefano Mancuso, fax: +39-055-457-017,
e-mail : stefano.mancuso@unifi.it

justment, ion nutrition, maintenance of energy pool and restriction of Na^+ in order to maintain a low Na^+ : K^+ ratio in the cytosol of cells which is a crucial aspect of survival for a plant in saline environment. In general halophytes accumulate higher Na^+ levels in their leaves, than do glycophytes (Flowers et al., 1977). Salt tolerance involves tissues and whole plant integration of many different transport processes as compartmentation of ions and synthesis of organic osmolytes at cellular level.

UPTAKE AND ACCUMULATION OF NA$^+$

In order to better understand ionic homeostasis in plants under salt stress identification of Na^+ transport pathways was as comprising a key gap, not only that but also the regulation of Na^+ transport across the plasma and vacuolar membranes comprises a critical factor in determining the specific manner in which plant cells handle extra cellular Na^+ loads (Niu et al., 1995). In general living cells in both low and high salt environments try to balance passive influx of Na^+ with Na^+ efflux, either across the plasma membrane back into the apoplast or across the tonoplast into the vacuole. For salt tolerance in addition to the considerable energy required for Na^+ efflux, time is an important factor because the rate of Na^+ uptake will determine how quickly Na^+ reaches toxic levels inside the cell. So it is clear that under high salt conditions limiting Na^+ influx into root cells is fundamental and balancing Na^+ influx with Na^+ export from the cytoplasm back into the apoplast is one way of reducing the Na^+ load (sometimes termed 'futile cycling') (Britto and Kronzucker, 2006; Malagoli et al., 2008). Futile cycling occurs to a varying degree in all plants investigated so far with 78−98% of Na^+ taken up transported back into the environment (Kronzucker et al., 2006; Wang et al., 2006; Malagoli et al., 2008).

The importance of Na^+ export from root cells for salt tolerance is evident in the salt over-sensitivity of mutants that are impaired in the plasma membrane Na^+ /H^+ antiporter SOS1 (Banuelos et al., 1996; Shi et al., 2000). This system seems to be similarly crucial in salt-sensitive and salt tolerant species (Oh et al., 2007). A second strategy for removing Na^+ from the cytoplasm is to compartmentalize it in the vacuoles. Na^+ uptake into the vacuole also requires energy, but has a dual benefit in saline conditions; it avoids Na^+ build-up in the apoplast (Oertli, 1968) and enhances the intracellular solute potential thereby contributing to turgor adjustment. Halophytes exhibit a marked ability for Na^+ accumulation, and their tolerance relies on controlled uptake and compartmentalization of Na^+, K^+ and Cl^- and the synthesis of organic compatible solutes to prevent Na^+ cytotoxicity. Several classes of Na^+ transporters have been identified such as the NHX-, SOS1- and HKT1, they have demonstrated significant involvements in Na^+ sequestration in vacuoles or extrusion from cells or circulation for the alleviation of sodium stress under saline conditions, for example, the Na^+/H^+ antiporter SOS1 mediates the efflux of Na^+ from root cells into the soil or cortical apoplast (Banuelos et al., 1996; Shi et al.,

2000; Rus et al., 2004). To date, the SOS1 (salt overly sensitive) are the best characterized class of transporters attributed with Na^+ exclusion from the cytosol across the plasma membrane. It was found in different plant species such as *Arabidposis* and tomato a significant increase in salt sensitivity and the tendency to accumulate high amounts of Na^+ in the Knockout/Knockdown plants of corresponding SOS1 genes compared with wild types under high salt concentration (Banuelos et al., 1996; Shi et al., 2000; Oh et al., 2009). Together with HKT and CHX-type transporters, it also provides a means for Na^+ transport into and out of the xylem (Shi et al., 2002; Hall et al., 2006; Huang et al., 2006; Munns et al., 2006; Byrt et al., 2007). Much less is known about the transporters responsible for Na^+ uptake into root epidermal and cortical cells. Whereas some HKT transporters change their K^+ and Na^+ selectivity depending on the ionic conditions, similar to multi-ion channel pores (Schachtman and Schroeder, 1994; Rubio et al., 1995; Gassmann et al., 1996; Horie et al., 2001), the only HKT transporter encoded in the *Arabidopsis* genome, AtHKT1, was found to be more Na^+ selective (Gao-Uozumi et al., 2000) and moreover was found that when the encoding genes of some of these transporters is overexpressed the salinity tolerance was improved (He et al., 2005; Agarwal et al., 2013). So Na^+ influx into plant roots can occur through ion channels or other membrane transport proteins that facilitate passive diffusion of Na^+ across the plasma membrane in most cases through K^+ pathways (HKT1), but the main pathway for Na^+ uptake in high salt concentrations is through non selective cation channels (Maathuis and Amtmann, 1999a; Rus et al., 2001; Maser et al., 2002; Demidchik and Maathuis, 2007) which -nonselective cation channels-considered to be partially sensitive to calcium, where the entry of Na^+ into roots was inhibited (Tester and Davenport, 2003). Even of their physicochemical similarities, it is K^+ rather than Na^+ that is essential to plant life, this means that plants in saline habitats have acquired mechanisms that allow the selective uptake of K^+ in the face of considerable competition from Na^+ (Maathuis and Amtmann, 1999b). Plant cells employ primary active transport, mediated by H^+-ATPase, and secondary transport mediated by channels and co-transporters in order to maintain high concentrations of K^+ and low concentrations of Na^+ in the cytosol. The overall sub cellular compartmentation of Na^+ into the vacuole and other organelles, such as mitochondria and plastids also lowers Na^+ concentration in the cytoplasm and at the same time contributes to osmotic adjustment in order to maintain water uptake under salt conditions and the AtNHX family of Na^+/H^+ antiporters which localized in the tonoplast membrane here play an important role in Na^+ compartmentation using the H^+ gradient as a driving force across the membrane under salinity stress (Blumwald, 2000; Bassil et al., 2012). The first functionally-characterized member of this gene family, AtNHX1, contributes to Na^+ and monovalent cation sequestration in plant vacuoles.

In addition, the role of these vacuolar NHXs in Na^+ compartmentation has been strongly supported by the fact that *Arabidopsis atnhx1* mutant exhibited Na^+ sensitivity

and less vacuolar Na^+/H^+ antiporter activity (Apse et al., 2003) and also overexpression of genes encoding vacuolar AtNHX1 was shown to increase salt tolerance in a range of plant species with accompaniment increase in tissue Na^+ (Apse et al., 1999; Zhang et al., 2001; Agarwal et al., 2013).

So we can summarize with a simple model for mechanisms of Na^+ absorption, recirculation, and extrusion by different classes of Na^+ channels/transporters; Na^+ influx mediated by HKT transporters (Gao-Uozumi et al., 2000; Maser et al., 2002; Sunarpi et al., 2005), and excessive Na^+ in the cytosol is partially transported out of cells by SOS1 antiporters (Shi et al., 2000), and some parte is sequestered in vacuoles by AtNHX1 topnoplast antiporters (Apse et al., 1999). AtHKT1.1, and OsHKT1.5 are present in the plasma membrane of xylem parenchyma cells, and mediate unloading of Na^+ from xylem vessels into xylem parenchyma cells, thus protecting leaves from Na^+ over accumulation and Na^+ damage (leaf Na^+ exclusion) (Berthomieu et al., 2003; Ren et al., 2005; Sunarpi et al., 2005).

NaCl SENSING AND GENES DEFENSE ACTIVATION

It is still unclear the sensing mechanism of salt in plant cell. The dual nature of salt stress, ionic and osmotic, brings a two sensory modalities. For example a high salt concentration in soil produces a hyperosmotic stress at root level. These are distinct from each other, because there are some responses specific for salt and different from purely osmotic stress (Deinlein et al., 2014). The *Arabidopsis thaliana* histidine kinase receptor protein HK1 should be a good candidate for salt receptor. It was demonstrated that HK1 can complement in yeast the loss osmoreceptor SLN1 (Urao et al., 1993) and the overexpression or loss of function of this protein led to a drought associated phenotype (Tran et al., 2007). This sensor is known to be coupled with calcium channel, because after salt exposure, there is a rapid increased of Ca^{2+} ions in cytosol (Ismail et al., 2014). The potential candidates for genetic identities of the Ca^{2+}-permeable channels involved in generating the cytosolic Ca^{2+} signal in *Arabidopsis* under salt stress might include annexin1 as recently suggested (Laohavisit et al., 2013). High cytosolic levels of calcium ions trigger a reactive oxygen species (ROS) production by activating respiratory burst oxidase homolog F (RBOHF) (Julkowska and Testerink, 2015). RBOHF plays a pivotal role in salt response and more in general in abiotic stress response, indeed during pathogen attack the ROS production is due by respiratory burst oxidase homolog D (Montillet and Hirt, 2013). Molecular evidences suggest that ROS acts as a secondary signal during stress response by regulating and modifying gene expression. In particularly ROS influences the gene expression by modifying transcription factors at nucleus level (Apel and Hirt, 2004). Moreover, high level of cytosolic ROS induces mitogen activated protein kinase (MAPK) such as MAPK3/6 and MAPK9/12, this phosphorylation cascade has as a final target transcription factors in nucleus (Kovtun et al., 2000). Other kinases may be involved in transcription factor induction under salt

stress, in particularly calcium dependent-protein kinases (CLDPK) (Harmon et al., 2000; Boudsocq and Sheen, 2013) and calcineurin B-like proteins (CBLs) with CBL-interacting protein kinases (CIPKs) (Weinl and Kudla, 2009). At nuclear level many transcription factors are activated, like MYB20. It was demonstrated MYB20 overexpressed lines are more tolerant to salt in contrast knock-out lines are more sensitive under saline condition (Cui et al., 2013).

Another very important class of transcription factors are the ABA responsive element-binding protein (AREB1) and ABA insensitive 5 (ABI5), which activate a gene for acclimation and also for plasma membrane ion channel and RBOHF (Umezawa et al., 2013). Activation of acclimation genes array leads to an accumulation of organic osmolytes, such as proline, glycine betaine, sugar alcohols, polyamines, and proteins from the late embryogenesis abundant (LEA) superfamily. This molecule plays a pivotal role in maintaining low intracellular osmotic potential, to preventing the osmotic damage under salt stress (Verslues et al., 2006), in particularly the proline is involved in cell proliferation and cell death (Szabados and Savoure, 2010). Also glycine betaine is proposed as a protectant of membrane structure and major enzyme (Guinn et al., 2011). In the last years emerging the idea that also a chromatin modifications, referred to as epigenetic modifications, contribute to the adaptation potential of plants to different environmental stresses (Zhu et al., 2008). Several research showed that modifications on chromatin are involved in the resistance responses of plants to salt stress in the same generation as the stress occurs. In particularly, hyperosmotic priming was reported for *Arabidopsis* plants that have been treated with mild salt stress in the seedling stage, followed by cultivation under normal media salt free (Sani et al., 2013). During Na^+ stress-free period, no differences between pretreated plants and control plants were detected. Subsequently, after an additional salt stress application, the pretreated group accumulated less Na^+ and thus was more tolerant. This phenotype was attributable to epigenetic histone modifications that mainly affected expression of resistance transcription factors. Moreover the salinity change methylation level of chromatin, specifically a target region of transcription factors (Song et al., 2012).

CONCLUSION AND REMARKS

Salinity stress is well reported to reduce plant growth and to affect plant development. The impact of salt stress on the growth of plant leads in altered plant morphology. The intense research of the last years has shed a light on numerous details of salinity responses and mechanisms of adaptation at molecular level. However, many mechanisms should be elucidated. For example, the regulation of gene expression and signaling cascades that regulate many Na^+ transporters remains unclear. Moreover, it remains to be assessed which of the sodium ions transport processes reviewed here could be employed to enhance plant performance under salt stress. Indeed both molecular breeding and
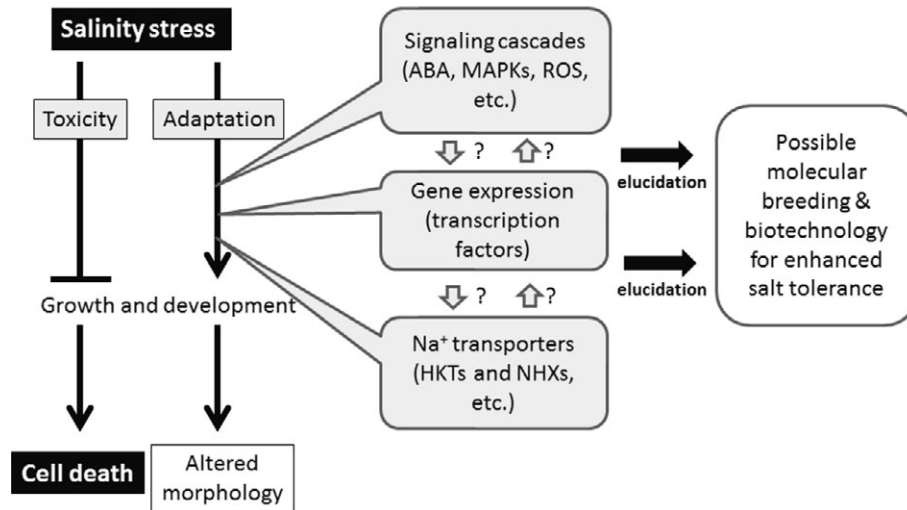
Fig. 1 Ion transporters as possible targets of molecular breeding and biotechnological approaches for conferring the enhanced salinity tolerance in plant cells.

advanced biotechnology methods should be highly helpful to develop crops with enhanced salt tolerance.

As illustrated in the Fig. 1, in this context, it is important to understand the underlying mechanisms that led to salt resistance and adaptation. Basically, molecular signaling components in plant adaptation and response to salt stress involved a network of Na$^+$ transporters, such as HKTs and NHXs, hormones, like ABA, transcription factors, MAPK pathways and ROS. Taken all together these are the major traits that influence plant resistance and adaptation to salt stress. In the next years the major goals will be to understand the cross-talk between different pathways that lead to resistance and adaptation. Moreover the stress tolerant and sensitive plants utilize the same signaling molecules, for that will be important in the future works to dissect and discriminate signals from the signaling molecule.

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