# Physiology and molecular biology of salinity stress tolerance in plants

**R. K. Sairam[†,*] and Aruna Tyagi[#]**

[†]Division of Plant Physiology and [#]Division of Biochemistry, Indian Agricultural Research Institute, New Delhi 110 012, India

**The productivity of plants is greatly affected by various environmental stresses. Soil salinity affects plant growth and development by way of osmotic stress, injurious effects of toxic Na$^+$ and Cl$^-$ ions and to some extent Cl$^-$ and SO$_4^{2-}$ of Mg$^{2+}$ and nutrient imbalance caused by excess of Na$^+$ and Cl$^-$ ions. Salinity stress response is multigenic, as a number of processes involved in the tolerance mechanism are affected, such as various compatible solutes/osmolytes, polyamines, reactive oxygen species and antioxidant defence mechanism, ion transport and compartmentalization of injurious ions. Various genes/cDNAs encoding proteins involved in the above-mentioned processes have been identified and isolated. The role of genes/cDNAs encoding proteins involved in regulating other genes/proteins, signal transduction process involving hormones like ABA, JA and polyamines, and strategies to improve salinity stress tolerance have also been discussed.**

EXCESS amount of salt in the soil adversely affects plant growth and development. Nearly 20% of the world's cultivated area and nearly half of the world's irrigated lands are affected by salinity[1]. Processes such as seed germination, seedling growth and vigour, vegetative growth, flowering and fruit set are adversely affected by high salt concentration, ultimately causing diminished economic yield and also quality of produce.

Plants are classified as glycophytes or halophytes according to their capacity to grow on high salt medium. Most plants are glycophytes and cannot tolerate salt-stress. High salt concentrations decrease the osmotic potential of soil solution creating a water stress in plants. Secondly, they cause severe ion toxicity, since Na$^+$ is not readily sequestered into vacuoles as in halophytes. Finally, the interactions of salts with mineral nutrition may result in nutrient imbalances and deficiencies. The consequence of all these can ultimately lead to plant death as a result of growth arrest and molecular damage[2]. To achieve salt-tolerance, the foremost task is either to prevent or alleviate the damage, or to re-establish homeostatic conditions in the new stressful environment. The growth rate must resume, albeit at a reduced rate[1]. However, barring a few exceptions, the conventional breeding techniques have been unsuccessful in transferring the salt-tolerance trait to the target species. A host of genes encoding different structural and regulatory proteins have been used over the past 5–6 years for the development of a range of abiotic stress-tolerant plants. The appreciation is growing that usage of regulatory genes is a more effective approach for developing stress-tolerant plants. Thus, understanding the molecular basis will be helpful in developing selection strategies for improving salinity tolerance. Identification of molecular markers linked to salinity/drought-tolerance traits has provided plant breeders a new tool for selecting cultivars with improved drought-tolerance. The present review deals with proteins/genes, which are induced in crop plants in response to salinity stress. Information on salt-responsive proteins/genes is crucial for improving salt-tolerance through genetic engineering techniques.

## Salt response is a multigenic trait

Salt-stress and dehydration stress show a high degree of similarity with respect to physiological, biochemical, molecular and genetical effects[3]. This is possibly due to the fact that sub-lethal salt-stress condition is ultimately an osmotic effect, which is apparently similar to that brought in by water deficit and to some extent by cold as well as heat stresses[4].

The halophyte *Mesembryanthemum crystallinum* has emerged as a model system for understanding the molecular response to salt-stress. This plant switches from C$_3$ photosynthesis to crassulacean acid metabolism (CAM) in response to salt or drought stress. Organic acids, oxalate and malate are important osmolytes in plants with CAM. A cDNA clone encoding NAPD-malic enzyme has been isolated from common ice plant[5,6]. A large number of genes are concomitantly up- and down-regulated for this switch to be operational[7]. More than a hundred genes are induced and probably transcripts three times that number are repressed in response to salt-stress in *M. crystallinum*[8]. Employing two-dimensional protein gel electrophoresis, it has been noted that application of salt to plants brings about a major change in the protein profile[9]. Therefore, not only is it imperative to ask how many and which genes but also in what hierarchical order do they express. Salinity is a quantitative trait, and arrays of salt-induced genes have been isolated[10].

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*For correspondence. (e-mail: rks_ppl@yahoo.co.uk)

## Salinity stress and plant response

### Compatible solutes

Under osmotic stress, an important consideration is to accumulate osmotically active compounds called osmolytes in order to lower the osmotic potential. These are referred to as compatible metabolites because they do not apparently interfere with the normal cellular metabolism. Molecules like glycerol and sucrose were discovered by empirical methods to protect biological macromolecules against the damaging effects of salinity. Later, a systematic examination of the molecules, which accumulate in halophytes and halo-tolerant organisms, led to the identification of a variety of molecules also able to provide protection[11,12]. Characteristically, these molecules are not highly charged, but are polar, highly soluble and have a larger hydration shell. Such molecules will be preferentially solubilized in the bulk water of the cell where they could interact directly with the macromolecules.

The biochemical pathways producing them are now better known. Genes rate-limiting these steps have been cloned and transferred into crop plants to raise the level of osmolytes. Osmolytes for which some progress has been made are indicated in Table 1.

*Mannitol*: Tobacco plants have been modified by introduction of *Escherichia coli mtlD* gene, which encodes mannitol-1-phosphate dehydrogenase[13]. It is not normally produced by wild-type tobacco. However, many organisms, including some plants synthesize and accumulate mannitol. Transgenic tobacco plants synthesize mannitol-1-phosphate from fructose-6-phosphate. In the absence of salt-stress, wild and transformed plants have similar height and fresh weight gains, but in the presence of 250 mol $m^{-3}$ salt, the *mtlD* gene-transformed plants have a growth advantage over the wild type in terms of better height grain, less fresh-weight loss and more new leaf and root production. Binzel *et al.*[14] found that tobacco cells adapted to 428 mM NaCl could maintain cytosolic $Na^+$ and $Cl^-$ level at less than 100 mM. Though mannitol only partially decreases the amount of inorganic ion accumulation in the cytosol, its protective effect as a compatible solute may be sufficient to give marginal growth advantage observed in transformed plants. Su *et al.*[15] obtained three rice transgenic lines with bacterial *mtlD* and demonstrated that biosynthesis and accumulation of mannitol in plants are correlated with salt-stress tolerance of plants. These solutes are widely believed to function as a protector or stabilizer of enzymes or membrane structures that are sensitive to dehydrations or ionically induced damage. *Arabidopsis thaliana* plants transformed with bacterial *mtlD* encoding mannitol-1-phosphate dehydrogenase have higher mannitol content and were able to withstand NaCl salinity up to 400 mol $m^{-3}$, whereas the wild type seeds ceased to germinate at 100 mol $m^{-3}$ NaCl[16].

**Table 1.** Important osmolytes that accumulate in plants during drought and salinity

| Carbohydrate | Nitrogenous compound | Organic acid |
|---|---|---|
| Sucrose | Proteins | Oxalate |
| Sorbitol | Betaine | Malate |
| Mannitol | Glutamate | |
| Glycerol | Aspartate | |
| Arabinitol | Glycine | |
| Pinitol | Choline | |
| Other polyols | Putrescine | |

*Pinnitol/ononitol*: The cyclic sugar alcohols, pinnitol and ononitol are stored in a variety of species, which are consistently exposed to saline conditions or accumulate in tolerant species when exposed to saline environments[17]. Facultative halophyte such as *M. crystallinum* accumulates these compounds only when subjected to water and salinity stresses. The proposed synthetic pathway consists of methylation of myo-inositol to the intermediate ononitol followed by epimerization to pinnitol[18]. An inositol methyl transferase (*Imt*) cDNA was isolated from transcripts induced in *Mesembryanthemum* plants by NaCl[19]. Transgenic tobacco for inositol methyl transferase has been obtained[20]. Similar to plants transformed with mannitol-1-phosphate dehydrogenase, growth of wild and *Imt*-transformed plants is not distinguishable in the absence of stress, but the latter have growth advantage over control plants in the presence of salt.

*Sorbitol*: This sugar alcohol of glucose is found in a variety of plant species, usually as a constituent of seeds. Sorbitol accumulation has been reported in seeds of many crop plants[21]. In Rosaceae species, it functions as a translocated carbohydrate and is also reported in vegetative parts in the halo-tolerant *Plantago maritima*[22]. Increasing salinity from 0 to 400 mol $m^{-3}$ resulted in an eightfold increase of sorbitol concentration in shoot tissues and a 100-fold increase in root tissues. Accumulation in *P. maritima* serves an osmo-regulatory function and its accumulation in plant seeds suggests that it may contribute to the desiccation tolerance of the mature embryo. *In vitro* protection by sorbitol of the restriction enzyme *pst*1 from desiccation has been reported earlier[23]. The conversion of glucose to its sugar alcohol is catalysed by aldose reductase. An aldose reductase-like protein accumulates during the period of embryo maturation in barley when desiccation tolerance is obtained[24].

*Proline*: In organisms ranging from bacteria to higher plants, there is a strong correlation between increased cellular proline levels and the capacity to survive both water deficit and the effects of high environmental salinity. It may also serve as an organic nitrogen reserve that can be utilized during recovery. In *Lathyrus sativus*, a hardy grain legume which can withstand drought, high proline accumulation was observed in leaves and roots under water stress[25].

Although proline can be synthesized from either glutamate or ornithine, glutamate is the primary precursor in osmotically stressed cells. The biosynthetic pathway consists of two important enzymes, viz. pyrroline carboxylic acid synthetase and pyrroline carboxylic acid reductase. Transcripts corresponding to both cDNAs accumulate in response to NaCl treatment. Both these regulatory steps are keys to developing strategies for overproducing proline in a selected plant species.

Besides, the intermediates of proline biosynthesis and catabolism, such as glutamine and δ-1-pyrroline-5-carboxylic acid could increase the expression of several osmotically regulated genes in rice[26]. There is also evidence that degradation of proline in the mitochondria is directly coupled to respiratory electron transport system and ATP production. A pyrroline-5-carboxylate synthetase (P5CS) cDNA from moth-bean was introduced into rice. Expression of this P5CS transgene under the control of a stress-inducible promoter led to stress-induced overproduction of the P5CS enzyme and proline accumulation in transgenic rice plants. Second generation (RI) transgenic plants showed an increase in biomass under salt and water stress conditions[27].

*Glycine-betaine:* Levels of glycine-betaine in *Poaceae* species are correlated with salt-tolerance. Highly tolerant *Spartina* and *Distichlis* accumulated the highest levels, moderately tolerant species accumulate intermediate levels and sensitive species accumulate low levels or no glycine-betaine[28]. Glycine-betaine is synthesized from choline in two steps, the first being catalysed by choline monooxygenase leading to synthesis of betaine-aldehyde, which is further oxidized by betaine-aldehyde dehydrogenase. Salinity stress induces both the enzyme activities[6,29]. Genetic evidence that glycine-betaine improves salinity tolerance has been obtained for barley and maize[28,30]. Isogenic barley lines containing different levels of glycine-betaine have different abilities to adjust osmotically. Transgenic rice plants expressing betaine-aldehyde dehydrogenase converted high levels of exogenously applied betaine-aldehyde to glycine-betaine than did wild-type plants. The elevated level of glycine-betaine in transgenic plants conferred significant tolerance to salt, cold and heat stress.

Huang *et al.*[31] reported metabolic limitation in betaine production in transgenic plants. *Arabidopsis thaliana*, *Brassica napus* and *Nicotiana tobbacum* were transformed with bacterial *Choline oxidase* cDNA. The levels of glycine betaine were 18.6, 12.8 and 13.0 ∝mol g$^{-1}$ dry weight in *A. thaliana*, *B. napus* and *N. tobbacum* respectively, 10–20 fold lower than the levels found in natural betaine producers. A moderate stress tolerance was noted in some transgenic lines based on relative shoot growth in response to salinity, drought and freezing. However, choline-fed transgenic plants synthesized substantially more glycine-betaine, suggesting that there is need to enhance the endogenous supply of choline to support accumulation of physiologically relevant amount of betaine.

## *Polyamines*

A number of stress factors such as potassium deficiency, osmotic stress, low pH, nutrient deficiency or light have been shown to stimulate the accumulation of polyamines, and particularly putrescine in plants. Putrescine accumulation during environmental stress is correlated with increased argenine decarboxylase (ADC) activity in oats. Recent studies with transgenic carrot cells over-expressing ornithine decarboxylase (ODC) cDNA showed that these cells were significantly more tolerant to both salt-stress as well as water stress[7].

Polyamines have recently gained importance in the escape of seedlings from the adverse effect of salinity. Suppression of polyamine biosynthesis by cyclohexylamine has been reported to result in increased ethylene synthesis as well as seed germination[32]. This suggests a cross-linking of pathways of polyamine and ethylene biosynthesis. Lin and Kao[33] reported an increase in the level of spermidine under salinity, but a low level of putrescine in the shoot and roots of rice seedlings. Accumulation of spermidine and spermine with the activity of ADC in rice seedlings plays a specific role in salt-tolerance.

The diamine putrescine and the polyamines spermidine and spermine are present in probably all plants, whereas the diamine cadaverine occurs within the Leguminosae. Putrescine can be formed from argenine and one of the key enzymes is ADC. Polyamines such as spermine and spermidine are derived from methionine and ornithine. The first step is the decarboxylation of ornithine catalysed by ODC. Under salinity and drought conditions, polyamines as well as their corresponding enzyme activities are substantially enhanced[34]. Whereas nuclear DNA is stabilized by histones in eukaryotic organisms, putrescine and polyamines take over the role of histones in bacteria. DNA in plant mitochondria and chloroplasts is regulated and stabilized by putrescine and polyamines. In addition, many steps of protein biosynthesis are stimulated by polyamines, may be through interaction with nucleic acids. In addition, they can stabilize bio-membranes. Transgenic rice for ADC cDNA showed increase in biomass under salinity stress condition compared to the control[35].

Ten-days-old *Zea mays* plants salt-stressed for eight days increased the content of putrescine and spermidine in their roots and leaves, and the increase in leaves was higher than in roots[36]. Lefevre *et al.*[37] studied the relative importance of ionic and osmotic components of salt-stress on modification of free proline level in rice. They suggested that the ionic component by itself might trigger short-term polyamine accumulation.