Chapter 2: Review of Literature
REVIEW OF LITERATURE

Plants are exposed to several environmental stresses that adversely affect various stages of their growth and development (Madlaung et al., 2004). They overcome environmental stresses by development of tolerance, resistance or avoidance mechanisms. Tolerance allows an organism to withstand the assault unharmed. Resistance involves active countermeasures, while avoidance prevents exposure to the stress. Partly due to their sessile nature, plants have developed sophisticated metabolic responses to tolerate or resist forms of stress, unlike mobile organisms that tend to avoid exposure to stress.

Stress can be either internal or external. Internal stresses have adverse effects on metabolic or genetic regulatory pathways and can result in spontaneous mutations or aberrant cell divisions. External stresses can further be classified as being biotic or abiotic in nature. Biotic stresses include pathogen attack, and competition. Abiotic stresses arise from unfavorable environmental conditions such as sub-optimal temperature, water and nutrient availability or light conditions. Majority of abiotic stress lead to a depression in yield potential due to their common effect on plant water status (Smirnoff, 1998, Knight et al., 2001). Although, water deficit is a normal component of certain developmental processes such as seed development in higher plants, prolonged cellular water deficit results in high solute concentration, alterations in cell volume, shape and cell membrane integrity and denaturation of proteins (Elizabeth, 1997). Together these changes have deleterious effects on the normal metabolic processes and result in a reduction in the yield outputs (Boyer et al., 1982).

The subject of abiotic stress has been reviewed earlier by many workers (Dorothea et al., 2005, Chinnusamy et al., 2005). The present review describes the pathways of sodium ion (Na⁺) entry into the cell and mechanisms for maintenance of sodium homeostasis in the cell.
2.1 Challenges Of Living In A Saline Environment

Salinity refers to the high concentration of certain dissolved ionic species. Salt stress affects the cell or organism primarily in two ways (Greenway et al., 1980):

- High salt concentration lowers the water potential and affects osmoregulation. As a result, plants lose the ability for water uptake leading to conditions resembling physiological drought inside the cells.
- Accumulation of Na\(^+\) in leaf tissues results in necrosis and damage of older leaves. Thus crop yield and net productivity are drastically reduced.

Potassium ions (K\(^+\)) are essential for cellular function and an important coactivator for more than 50 enzymes, a function that cannot be substituted by Na\(^+\) (Bhandal et al., 1998). Metabolic toxicity of Na\(^+\) is largely a result of its ability to compete with K\(^+\) for binding sites. Thus high Na\(^+\):K\(^+\) ratios can lead to aberrations in various enzymatic processes, such as binding of tRNA to the ribosomes that is reliant on high K\(^+\) concentrations and other aspects of ribosome function and protein synthesis (Blaha et al., 2000).

2.2 Pathways Of Sodium Entry

Na\(^+\), unlike K\(^+\) is not essential for growth of most land plants and is cytotoxic at concentrations in excess of about 100 mM. K\(^+\) serves as a dominant counterion for the large excess of negative charge on proteins and therefore is significant in maintenance of charge balances in the cytoplasm (Maathuis et al., 1996) and is an essential cationic inorganic nutrient in most terrestrial plants. Due to the similarity in the ionic radii of the two ions, Na\(^+\) may enter through K\(^+\) transporters and not only lead to accumulation of Na\(^+\) into the plant cells but also impairs K\(^+\) acquisition (Niu et al., 1995).

Transport of metals and alkali cations across the plasma membrane and organellar membranes is essential for plant growth, development, signal
transduction, nutrition and also for use of plants in toxic metal phytoremediation (Barkla et al., 1996). Cells expend as much as 50% of their total intracellular energy reserves to maintain ionic gradients (Nelson et al., 1994).

Transmembrane ionic movement is a well-balanced event with the net influx adjusted to accommodate cellular requirements, and creating ion homeostasis. Under physiological conditions, homeostatic concentrations of ions in the cytosol are K⁺: 100-200 mM; Na⁺: 1-10 mM and Ca²⁺: 100-200 mM (Bush et al., 1995). Transport of ions down the thermodynamic gradient is passive, whereas transport against the gradient requires active transport. Protons form the currency for proton electrochemical gradient that mediates the transport process in plants and fungi (Sze et al., 1999), whereas animal cells use Na⁺ as the driving force. Since ions are hydrated in solution and do not readily traverse the hydrophobic lipid bilayer of membranes, flux across the plasma membrane and tonoplast occurs via specialized transport proteins that can be generally categorized as pumps, carriers and channels (Amtmann et al., 1999)

The following section focuses on the transport of ions (Schachtman et al., 1999) particularly Na⁺ and K⁺ across biological membranes (Figure 1).

2.2.1 Pumps: Transport of substrates against an electrochemical gradient by direct utilization of metabolic energy is facilitated by pumps. The turnover rates are low around $10^2$ per second. The H⁺-ATPases in the plasma membrane and tonoplast and H⁺-pyrophosphatases in the tonoplast are the two primary pumps that couple the free energy of ATP hydrolysis, to vectorial H⁺ transport leading to generation of electrochemical gradient (Gaxiola et al., 2002). The H⁺ pump in the plasma membrane is a P-type ATPase (Ratajczak et al., 2000) that establishes a difference in pH of about 1.5 to 2.0 units (pH =5.0 to 5.5 in the apoplast) and is primarily responsible for the negative membrane potential of -120 to -200 mV across membrane under physiologically steady state conditions. The two H⁺ pumps present in the
 Despite the occurrence of these transporters, the presence of a Na⁺-ATPase and ATPase activity in the cytoplasmic membrane has been reported. The presence of Na⁺-ATPase activity in the cytoplasmic membrane has been reported in all cases. However, the presence of Na⁺-ATPase activity in the cytoplasmic membrane has been reported in all cases.

Figure 1: Transporters involved in the entry of Na⁺ into the cytoplasm.

- The Na⁺-ATPase on the plasma membrane pumps Na⁺ out of the cytoplasm.
- The Na⁺-ATPase on the inner membrane pumps Na⁺ into the cytoplasm.
- The Na⁺-ATPase on the outer membrane pumps Na⁺ into the cytoplasm.

From Hoque et al., 2001.
2.2.2 Carriers: The coupled uphill transport of one solute with the downhill movement of another, either in the same direction (symporters) or in opposite direction (antiporters) is mediated by carriers. They transport substrates against a concentration gradient and are energized via coupling to an electrochemical gradient and have high turnovers of $10^2$ - $10^3$ per second (Serrano et al., 1999). A brief description of the carriers involved in $K^+$ and $Na^+$ uptake is given below.

2.2.2.1 High affinity potassium carriers: $Na^+$ acts as a competitor of $K^+$ uptake suggesting that uptake mechanisms for both cations are similar. Plant roots utilize a high affinity carrier for $K^+$ uptake from external concentrations in the range of 10-30 $\mu$M (White et al., 1999). Two families namely KUP-HAK gene family ($K^+$ uptake transporter-high affinity $K^+$ transporter), and HKT1 have been identified. Members of the KUP-HAK gene family initially identified in barley and Arabidopsis are transporters that mediate $K^+$ and $Rb^2+$ uptake. Although the exact mechanism by which these transporters move $K^+$ is still unclear, it is believed to occur through coupling with the $H^+$ gradient. KUP-HAK transporters are extremely selective for $K^+$ ($K^+/Na^+$ ratio of 1000) and are competitively blocked by $Na^+$ in millimolar concentration ranges (Maathuis et al., 1999). HKT1 transporters are one of the pathways of $Na^+$ entry into plants (Rus et al., 2001).

2.2.2.2 Low affinity cation carriers: Uptake at high external $K^+$ concentration (millimolar) is mediated by low affinity carriers that have less pronounced $K^+/Na^+$ selectivity. In plants these low affinity uptake systems are implicit to $Na^+$ influx and $Ca^{2+}$ inhibits the process. Low affinity cation transporter, LCT1 is suggested to play a significant role in $Na^+$ uptake at high salt concentration (Schactman et al., 1997).

2.2.3 Channels: Cation channels are integral membrane proteins that catalyze passive movements of cations through transmembrane pores with turnover rates of $10^6$-$10^8$ per second (Demidchick et al., 2002). Various channels involved in $K^+$ and $Na^+$ uptake are described below.
2.2.3.1 \(K^+\) inward rectifying channels (KIRC): These channels mediate the low affinity \(K^+\) uptake. Most KIRC are highly selective for \(K^+\) over \(Na^+\) and other divalent cations and usually do not contribute much to \(Na^+\) uptake. The inward rectifier AKT1 (Cao et al., 1995) is the major route for \(K^+\) uptake from soil to the root epidermis. AKT2 and AKT3 are phloem specific while AKT6 is flower specific. The KAT1 and KAT2 are involved in the stomata opening (Nakamura et al., 1995).

2.2.3.2 \(K^+\) outward rectifying channels (KORC): These mediate the outward \(K^+\) movement. These are not involved in the accumulation of sodium into the cytosol (Wegner et al., 1994).

2.2.3.3 Voltage Independent Channel (VIC): VIC is non-selective to most monovalent and in some cases to divalent cations. The affinity for \(K^+\) is only slightly higher than \(Na^+\) affinity \((P_{K}/P_{Na} = 1.49)\) and is strongly blocked even by low concentrations of external \(Ca^{2+}\). Conditions with high external \(Na^+\) lead to massive influx of \(Na^+\) through VIC. The probability of VIC channel being opened decreases 10-15 fold with a 10-fold increase in external calcium concentration (Demidchick et al., 2002).

2.2.3.4 Cyclic Nucleotide Gated Channels (CNGC): The nucleotide sensitive channels form a major pathway for \(Na^+\) influx. There are about 20 candidate genes in \textit{Arabidopsis} genome that encode putative CNGC having cyclic nucleotide and calmodulin binding domains. Addition of millimolar concentration of cAMP or cGMP to isolated protoplasts of \textit{Arabidopsis} caused an instant decrease (within seconds) by almost 10\% in the open probability of \(Na^+\) permeable CNGC (Maathius et al., 2001). Expression of CNGC3 in yeast increased \(Na^+\) uptake, while \textit{Arabidopsis} knockout mutant in CNGC1 was more tolerant to moderate \(Na^+\) concentrations.
2.3 Strategies For Overcoming Stress

Described below are a few of the strategies adopted by plants to overcome salinity stress.

2.3.1 K$^+$ entry to Balance Na$^+$/K$^+$ Ratio: The transcript levels of several K$^+$ transporter genes are either down or up-regulated by salt stress (Tester et al., 2003). Salt stress increases the transcript level of *Arabidopsis* root K$^+$ transporter gene, *AtKCI* (Pilot et al., 2003). Salt stress up regulates the expression of an AKT/KAT family member; KMTI and various HAK/KUP type genes in ice plant KMTI (Su et al., 2001) Homologues of HKT1 have been found in rice, barley and *Arabidopsis*. Heterologous expression of HKT1 in *Xenopus* oocytes allowed its functional characterization and showed mixed ion selectivity. In wheat, TaHKT1 functions as Na$^+$/K$^+$ symporter at low micromolar Na$^+$ concentrations but works as a Na$^+$ uniporter at high millimolar Na$^+$ concentrations. HKT1 mediated K$^+$ transport depends on the presence of Na$^+$ with two K$^+$ transported per Na$^+$. The K$^+$ binding site has a $K_M$ of about 3 μM and the Na$^+$ binding site has a $K_M$ of 200 μM. At higher than ambient Na$^+$ concentrations, both HKT1 binding sites are occupied by Na$^+$ and therefore, HKT1 type systems may be relevant for Na$^+$ uptake rather than K$^+$ uptake (Rubio et al., 1995).

The *in planta* function of AtHKT1 as an effector of Na$^+$ influx has been confirmed by T- DNA insertional mutations (Rus et al., 2001). AtHKT1 is the only member of the *Arabidopsis* gene family that mediates highly selective Na$^+$ and low affinity K$^+$ uptake (Uozumi et al., 2000). This is in contrast with the function of wheat HKT1 that mediates high affinity K$^+$ uptake energized by either H$^+$ or Na$^+$. Residues presumed to affect K$^+$ binding in TaHKT1 are variant in AtHKT1, and these differences may contribute to the unique cation specificities of wheat and *Arabidopsis* proteins. It is difficult to reason out the retention of AtHKT1 function in glycophytes. However, the recent evidence that vacuolar pH may be controlled by Na$^+$/H$^+$ exchange activity, which
requires intracellular Na\(^+\) uptake in the absence of high Na\(^+\) external. The activity of these transporters under high saline environments is a subject of great interest (Rus et al., 2004). In Oryza sativa there are two forms of HKT1. The OsHKT1 is a Na\(^+\) transporter like AtHKT, but OsHKT2 behaves as a symporter or uniporter as does TaHKT1 (Horie et al., 2001). Interestingly, HKT1 expression in rice is repressed during salt stress and a salt sensitive rice variety was shown to maintain higher levels of HKT1 expression than salt tolerant variety (Golldack et al., 1997).

2.3.2 Anatomical Modifications for Sodium Partitioning in Cells and Tissues: The mechanism by which Na\(^+\) is partitioned in cells to allow normal metabolism in the cytosol depends upon the nature of tissue explants involved, particularly the anatomy of the tissue. A few of these strategies are described below:

2.3.2.1 Salt excretion: Most halophytes bear characteristic salt glands that function to remove salt from the leaf surface (Yeo et al., 1998). These glands remove both water and salt from the leaf, and to counter this water loss such plants have adapted themselves in restricted habitats with readily available water source, such as salt marshes (Glenn et al., 1999). Some sequester salt in specialized anatomical structures that can be later shed off. Leaf surfaces in marsh plants bear bladder hairs that consist of several stalk cells and a bladder cell. The stalk cells transport ions into the vacuole of the bladder cell, which eventually undergoes death and falls off the plant (Seaman et al., 2001).

Porteresia coarctata, a wild relative of rice can grow in 25% seawater, and has specialized salt secreting micro hairs (Flowers et al., 1990). The relatively low abundance of halo tolerant plants may be indicative of the fact that there is a significant disparity in the input energies for construction and/or operation of strategies to evade or withstand salt.
2.3.2.2 Switch from C3 to CAM: Halophytes can increase their water use by lowering stomatal conductance. Plants such as *Mesembryanthemum crystallinum*, switch from C3 to CAM, and increase their water use efficiently (Bohnert., 1995). The CAM pathway is characterized by closure of stomata during daytime thereby reducing transpiratory water loss. CO₂ taken up at night is assimilated as oxaloacetate by the enzymatic action of phosphoenolpyruvate carboxylase (PEPC), and finally into malate. The malate is stored in the vacuole from where it is mobilized during daytime, providing CO₂ for the action of Ribulose-1, 5-bisphosphate carboxylase/oxygenase. The CAM-specific isoforms of PEPC (encoded by *pepc1*) show stress-induced induction (Adams et al., 1992). Thus the CAM pathway helps water conservation and represents a mechanism for CO₂ concentration that prevents or ameliorates photo inhibitory condition.

2.3.2.3 Entry of Na⁺ into the roots: In certain crop species regulation of Na⁺ transport to the shoot often differentiates the tolerant and the non-tolerant varieties. It has been shown that Na⁺ toxicity can be ameliorated by the external addition of upto 10 mM of calcium (Epstein et al., 1998). This effect of extra cellular Ca²⁺ on Na⁺ and K⁺ transport has been attributed to the activity of SOS signaling pathway. Salinity induced rise in cytosolic calcium, activates SOS3, which causes changes in expression and activity of Na⁺ and K⁺ transporters primarily the Na⁺/H⁺ antiporter. In wheat, extra cellular calcium inhibits unidirectional sodium influx through a non-selective cation channel, suggesting that the effect of calcium on modification of ion channel activity and consequent Na⁺ influx may be direct and not through cytosolic signaling (Davenport and Tester, 2000). However, there is little effect of external calcium on salt tolerance in instances where Na⁺ enters through leaks in the endodermis as seen in rice. The contribution of this apoplastic flow to total Na⁺ influx varies between plant species. Halophytes have anatomical adaptations to minimize this mode of salt entry. It has been reported in some cases that the width of the casparian band is two- three times greater in halophytes when compared to non- halophytes. The inner
layers of cortical cells can differentiate into second endodermis and can prevent leakage of sodium.

2.3.3 Na\(^+\) Entry into the Vacuoles: Electrochemical H\(^+\) gradients generated by H\(^+\) pumps at the plasma membrane H\(^+\)-ATPases and the tonoplast, H\(^+\)-ATPase, H\(^+\)-Ppiase provide energy used by the plasma membrane and tonoplast bound Na\(^+\)/H\(^+\) antiporters to couple the passive movement of H\(^+\) to the active sequestration of Na\(^+\) inside the vacuole. Plant vacuolar Na\(^+\)/H\(^+\) activity was first measured in tonoplast enriched membranes isolated from red beet storage tissue (Barkla et al., 1991).

The cation proton antiporter, (CPAI) family of Arabidopsis cation /H\(^+\) antiporters include 8 members named AtNHX1-8 (Maser et al., 2001). AtNHX7 has been identified as AtSOS1 and shown to be a plasma membrane antiporter (Shi et al., 2002). AtNHX8 is highly similar to AtNHX7 and is also most probably a plasma membrane antiporter. AtNHX1-6 has significant similarity to the endosomal NHX from yeast. Based on their amino acid similarity, the vacuolar AtNHX antiporters can be grouped into two subgroups AtNHX1-4 and AtNHX5-6. While the first group is 75% similar, the second group is< 40% similar to the first. Despite this fact, AtNHX5-6 is able to complement the yeast mutant strain. Further GFP and immunological studies have shown that AtNHX1, 2 and 5 are localized in the vacuole (Yokoi et al., 2002).

The overall structure of AtNHX1 is distinct from the human Na\(^+\)/H\(^+\) antiporter, NHE1. It comprises of nine transmembrane domains, a hydrophilic C-terminal domain and three hydrophobic regions that do not appear to span the tonoplast membrane but appear to be membrane associated (Wakabayashi et al., 2000). Whereas the N-terminal of AtNHX is facing the cytosol, almost the entire C-terminal is present in the vacuolar lumen. The orientation of transmembrane domains 1, 2 and 10-12 of AtNHX1 (corresponding to TM2-3, 11 and 13 of NHE1 respectively) appear to be similar to that in NHE1, whereas TM4, 7 and 8 of AtNHX 1(TM5, 8 and 9 of...
NHE) are embedded in the opposite direction in the membrane. In NHE1, it has been shown that the C-terminal hydrophilic region is cytosolic, and that it participates in the regulation of Na⁺/H⁺ antiporter. However for AtNHX it has been shown that the entire C-terminal region is hydrophilic and resides in vacuolar lumen. Deletion of the hydrophilic C-terminal region leads to a drastic increase in the relative rate of Na⁺/H⁺ transport and the ratio of Na⁺/K⁺ transport was observed to be twice that for the unmodified AtNHX1 (Yamaguchi et al., 2003). The AtNHX1 transcript level was up regulated by NaCl, KCl or ABA, but not by cold/ dehydration treatments. AtNHX1 was expressed at a significantly high level in leaves upon NaCl stress, particularly in older leaves. The expression in root hair was strongly up regulated by salt stress as suggested by promoter GUS analysis (Shi and Zhu, 2002).

In context of the whole plant under salt stress, it is likely that SOS1 and AtNHX1 co-ordi rate function to confer salt tolerance. SOS1 expressed preferentially in the parenchyma cells surrounding the vascular tissue, controls amount of Na⁺ to be transported to the shoot for compartmentation in leaf vacuoles through vacuolar antiporters like AtNHX1. Strong expression of SOS1 but lack of expression of AtNHX1 in root tips suggest that the meristematic tissues strongly rely on Na⁺ efflux rather than Na⁺ compartmentation to maintain low cytosolic Na⁺ concentration due to the fact that these cells lack developed vacuoles. The role of AtNHX1 under normal conditions remains to be answered (Shi and Zhu, 2002). Ubiquitous and relatively high basal level of expression indicates that AtNHX1 has important house keeping physiological functions in Arabidopsis even in the absence of stress (Yamaguchi et al., 2001).

2.3.3.1 Regulation of the vacuolar Na⁺/H⁺ exchange in Arabidopsis by SOS pathway: The tonoplast Na⁺/H⁺ activity is reported to be significantly reduced in sos2 mutants and unchanged in sos3 mutants in comparison to wild type plants. It has been suggested that the activity of the vacuolar Na⁺/H⁺ antiporter is controlled by SOS2 kinase. However NHX activation was not due to phosphorylation by SOS2. Direct SOS2 stimulation
of transport activity in the absence of a corresponding in vitro phosphorylation of the transporter has been shown for the CAX, a Ca\(^{2+}\)/H\(^+\) antiporter in *Arabidopsis*. It may be possible that an unidentified intermediate probably exists between SOS2 and NHX1 (Qui *et al.*, 2004).

2.3.4 Role of Calcium in Conferring Salinity Tolerance: Transduction pathways that regulate plant ion homeostasis during salt stress have been well studied. The identification of Salt Overly Sensitive (SOS) pathway mutants in *Arabidopsis* has helped in revealing the mechanisms involved in the plants response to ionic stress. The SOS pathway as it operates in response to stress via calcium signaling is described in the following section.

**SOS pathway:** One of the responses of plant cells to salt stress is the transient generation of a cytosolic calcium and subsequent activation of Ca\(^{2+}\) sensor protein expression (Knight *et al.*, 1997). SOS3 is a calcium sensor essential for transducing stress induced Ca\(^{2+}\) signal. SOS3 encodes a calcium binding protein with an N-terminal myristolyation motif and three calcium binding EF hands (Zhu *et al.*, 2003). The *Arabidopsis thaliana* SOS3 (Ishitani *et al.*, 2000) gene product shares homology with the regulatory subunit of yeast calcineurin B, CNB. A loss of function mutation that reduces the calcium binding capacity of SOS3 (sos3-1) renders hypersensitivity to salt, a defect that can partially be rescued by high levels of Ca\(^{2+}\). SOS3 binds Ca\(^{2+}\) with low affinity compared to other calcium binding proteins like calmodulin. SOS3 physically interacts with and activates a protein kinase encoded by *Arabidopsis* SOS2 gene (Halfter *et al.*, 2000). SOS2 is a serine / threonine protein kinase with an amino terminal catalytic domain and a carboxyl terminal regulatory domain. SOS2 is similar to the yeast SNF1 and mammalian AMPK protein kinase (Liu *et al.*, 2000).

Under normal conditions the catalytic and regulatory domains interact with each other probably preventing substrate phosphorylation by blocking substrate access. A FISL motif in the regulatory domain of SOS2 is necessary and sufficient for interaction with SOS3 and deletion of this motif
makes the SOS2 kinase constitutive (Guo et al., 2001). The FISL motif is located near the kinase domain of SOS2. Replacement of Thr 168 in the kinase domain with Asp also leads to production of a constitutively active kinase. Salt stress induced Ca\(^{2+}\) increase relieves the kinase from inhibition by SOS3 binding to the FISL motif in the regulatory domain (Figure II).

![SOS signaling pathway in Arabidopsis](image)

**Figure II: SOS signaling pathway in Arabidopsis.** Salt stress is perceived by increases in calcium levels that activate SOS3. The transcript levels of SOS1 are regulated by SOS2-SOS3 kinase complex. Further SOS2 may regulate the activity of AtNHX and also negatively influence At HKT1 (potassium transporter). Dotted lines with bar indicate inhibition. (Adapted from Chinnusamy et al., 2004)

The SOS3/SOS2 kinase complex regulates the transcript levels of SOS1 and also activates the Na\(^+\)/H\(^+\) antiporter. SOS1 encodes a plasma membrane Na\(^+\)/H\(^+\) antiporter with a very long predicted cytoplasmic tail. Salt stress induced up regulation of SOS1 transcript is diminished in sos2 and sos3
mutants. The SOS3-SOS2 complex was found to phosphorylate SOS1 directly (Quintero et al., 2002).

The myristoylation of SOS3 has been shown to be critical for salt tolerance, because a G2A mutation that disrupts myristoylation causes salt hypersensitivity (Ishitani et al., 2000). It has been suggested that the constitutive myristoylation of SOS3 allows recruitment of SOS2 to the plasma membrane, and bringing it to its target SOS1 (Quintero et al., 2002). Na+ entry into root cells during salt stress appears to be mediated by AtHKT1, a low affinity transporter (Uozumi et al., 2000). The athkt1 mutation suppresses the sos3 mutation (Rus et al., 2001), suggesting that SOS3/SOS2 kinase complex may prevent Na+ influx by inactivating the HKT1 protein or by down regulating HKT1 gene expression during salt stress (Figure II). In addition the activity of vacuolar Na+/H+ antiporter may also be activated by SOS3 SOS2 kinase complex (Qiu et al., 2004).

2.3.5 Counteracting Na+ Toxicity via Osmoprotectants and Scavenging ROS: In many cases salinity also leads to generation of osmotic stress in plants. Therefore, in addition to regulating Na+ homeostasis stress, in some plants is counteracted by producing osmoprotectants, which ameliorate negative effects of reactive oxygen species (ROS).

2.3.5.1 Osmotic adjustment and osmoprotection: Osmoprotectants or compatible solutes are highly soluble, neutral or zwitterionic compounds (Yancey et al., 1982). Examples include a range of compounds such as simple sugars (fructose, glucose), sugar alcohols (mannitol), complex sugars (trehalose) and quartenary amino acid derivatives (proline, glycine betaine etc.). In many species of halophytic Poaceae and Chenopodiaceae are found to accumulate Glycine Betaine (GB) (Seaman., 2001). GB is accumulated by a taxonomically restricted range of species. In higher plants it is synthesized from choline via betaine aldehyde using choline monoxygenase (CMO) and betaine aldehyde dehydrogenase (BALDH) in chloroplast (Rathinasabapathi et al., 1997). Some bacteria directly, convert choline to betaine in a single step.
reaction catalysed by choline oxidase, Cod. CodA from *Arthrobacter globiformis*, has been expressed in *Arabidopsis*, a non betaine accumulator, in chloroplast using a transit peptide. High concentrations of betaine upto (50 mM) could be produced in these transgenic *Arabidopsis* plants rendered the ability to withstand salt and freezing tolerance (Hayashi et al., 1997).

Proline is another osmoprotectant. The enzyme, υ'-pyrroline-5-carboxylate synthetase, p5cs catalyses the conversion of glutamate to υ'-pyrroline-5-carboxylate, which is then reduced to proline. The p5cs gene from *Vigna aconitifolia* containing the catalytic activities of υ-glutamyl kinase and glutamic-υ- semialdehyde has been used to transform tobacco (Kishor et al., 1995). Transgenic plants over expressing P5CS produced 10-18 fold more proline under drought stress, showed enhanced biomass production and flower development under salt stress.

Mannitol, a sugar alcohol (Stoop et al., 1996) has been over expressed in both *Arabidopsis* and tobacco by over expressing the bacterial, *MltD*, encoding Mannitol 1-phosphate dehydrogenase. Transgenic tobacco plants with high concentration of mannitol concentrations displayed salinity tolerance (Tarczynski et al., 1993). The hydroxyl content of transgenic plants was also lower suggesting that the protection could result from mannitol mediated scavenging of oxygen radicals. The key target for hydroxyl radical produced by illuminated thylakoids is the Calvin cycle enzyme phosphoribulokinase. Inactivation of this enzyme is prevented by mannitol and could explain the in vivo protective effect of transgenic plants (Shen et al., 1997).

Trehalose, a non-reducing disaccharide has been over expressed in tobacco, by introduction of TPS1 gene encoding Trehalose-6-phosphate synthetase gene from yeast (Romero et al., 1997). The transgenic tobacco plants when assessed for drought tolerance, showed improved water retention and desiccation tolerance.
2.3.5.2 Reactive oxygen species: High salinity can lead to water deficit conditions. The primary effect of water deficit is alteration in stomatal conductance. Reduced stomatal aperture leads to lower intracellular CO₂ concentrations which result in the enhancement of the photorespiratory pathway, which produces H₂O₂ (Zhu, 2001). Decrease in carboxylation equally affects energy transduction through and from the photosystems in the thylakoids membranes of chloroplast (Smironoff, 1993). This may lead to over reduction of the photo systems and subsequent damage to the proteins as active radical oxygen species increase. These toxic molecules damage membranes; membrane bound structures and macromolecules especially in the mitochondria and chloroplast. Antioxidant systems in plant consist of enzymes that scavenge oxygen radicals, such as SOD, peroxidases, catalases and glythathione reductases. The SOD are essential components in almost all plant antioxidants defenses, catalyzing the dismutation of two superoxide radicals into oxygen and H₂O₂. The SOD isoenymes can be divided into three different classes according to their metal co factors requirements copper/Zinc, Manganese and iron (Fe). Plants generally contain Fe SOD and Cu/Zn SOD in chloroplast, Mn SOD in mitochondria and Cu/Zn SOD in cytosol. SOD from Arabidopsis has been expressed in transgenic tobacco (Slooten et al., 1995). When targeted to the chloroplast this enzyme protected both the plasmalemma and the photosystem II against super oxide generated during illumination of leaf discs impregnated with methyl viologen by scavenging radicals. However, salt stress at the whole plant level was not greatly altered.

Catalase is the enzyme that destroys H₂O₂. Reduction of Cat1 activity by introduction of an antisense construct resulted in plants with 10% of wild type activity (Willekens et al., 1997). Plants generated had apparently a normal phenotype at low light intensity but developed necrotic spots at high light intensity. Further these plants were more sensitive to ozone, high salinity, and peroxide stress (Chamnongpol et al., 1997).
2.4 Molecules/Proteins Up Regulated During Salt Stress

Salinity stress induces a series of responses. Proteins involved in the plant response to stress can be broadly classified as components of the signal transduction pathway and end products of molecular events that directly reduce the specific stress. Transcription factors, protein kinases, 14-3-3 proteins, MAP kinases, calcium-dependent protein kinase (CDPK) etc fall into the first category. An increase in activity of these genes enables the plant to overcome multiple stresses (Holmberg et al., 1998, Munns, 2002). The second group includes enzymes responsible for synthesis of compatible solutes, reactive oxygen species (ROS) and other protective proteins.

2.4.1 Role of Transcription Factors: Genes encoding transcription factors transiently induced early during salt stress in turn activate the expression of target genes (Zhu, 2001). DREB2A and DREB2B that bind the DRE (drought responsive element) are activated by osmotic stress while DREB1 is induced by cold stress (Liu et al., 1998). The DREB1 transcription factors activate the DRE/CRT class of stress responsive genes (Figure III). These include the dehydration (RD), cold regulated (COR), cold inducible (KIN), low temperature induced (LTI) genes, which have consensus DRE in their promoters. Several basic leucine Zipper (bZIP) transcription factors can bind ABRE and activate the expression of ABRE responsive genes examples of which include Em, Rab16 and Rab 17 etc (Nakashima et al., 2000).

Some stress responsive genes such as RD22 do not have typical DRE/CRT elements and therefore may be activated through different mechanisms. A MYC transcription factor, RD22BP1 and the MYB transcription factor, AtMYB22 are shown to bind cis elements of the RD22 promoter (Zhu, 2002). In addition to transcription factors that directly bind the cis-elements of stress responsive genes, efficient transcriptional activation needs additional co-factors that can be important in determining the levels of expression. When over expressed in Arabidopsis, the soybean gene SCOF1, which encodes a Zn finger protein, can activate COR gene expression and increase freezing
tolerance. The SCOF1 protein does not bind directly to either DRE or ABRE elements. SCOF1 interacts with another G-box binding bZIP protein, SGBF1. SGBF1 can activate ABRE-driven reporter gene expression in Arabidopsis leaf protoplasts.

Figure III: Transcription factors involved during osmotic stress. Osmotic stress results in increase in ABA levels and IP3 levels that lead to rise in calcium levels and enhance expression of MYB/MYC and bZIP factors that bind to the promoters of stress responsive genes. (Adapted from Chinnusamy et al., 2004) Thus SCOF1 indirectly regulates the activity of the cold responsive genes through SGBF1 (Kim et al., 2001). Presently there is no example of a transcription factor encoding a gene induced only in response to salinity stress.

2.4.2 Role of MAPK in Stress Response: MAPK signaling is one of the most important conserved modules for signal transduction in yeast, plants and human (Ligterink et al., 2001). MAPK cascades commonly include three
protein kinases: MAPK kinase kinase (MAPKKK), MAPK kinase (MAPKK) and MAPK, which covalently phosphorylate side chain of serine/threonine residues of specific proteins inside the cells. MAPK catalyzed phosphorylation of substrate proteins leads to turn on or turn off various genes that mediate adaptation to stress responses. These substrates include transcription factors, protein kinases etc. On the basis of sequence similarities at least 20 MAPK, 10 MAPKK and 60 MAPKKK genes have been identified in Arabidopsis (Riechmann et al., 2000, Ichimura et al., 2002). In Arabidopsis drought, high salt and cold conditions induce the expression of MAP kinase, AtMPK3 (Mikolajczyk et al., 2000) and ATMEKKI within 5 minutes and the expression level increases steadily till a maximum peak is reached at 24h. ATMEKKI (Ichimura et al., 1998) is involved in a MAPK cascade responding to drought, high salt and cold. This MAP kinase cascade is composed of ATMEKKI (MAPKKK), MEKI (MAPKK) and ATMPK4 (MAPK) (Huang et al., 2000). Similar to OsMAPK5, AtMPK4 negatively regulates down stream gene expression in biotic stress and positively regulates gene expression in abiotic stress. Tobacco MAPK, SIPK and WIPK are included by salicylic acid and wounding respectively. SIPK (Hoyos and Zhang, 2000) is also activated by hyper osmotic stress, indicating that the same kinase plays a role in hypo and hyper osmotic stress. Nevertheless WIPK is induced by hypo-osmolarity but not hyper-osmolarity.

2.4.3 Role Of Phospholipids In Stress Response: Phospholipase C (PLC) hydrolyses phosphatidylinositol 4,5- bisphosphate (PIP2) upon activation and generates two second messengers, inositol-1,4,5-triphosphate (IP3) and diacylglycerol (DAG). Diacylglycerol and IP3 are second messengers that can activate protein kinase C (PKC) and trigger Ca^{2+} release respectively (Sanders et al., 1999, Schroeder et al., 2001). A cDNA for PLC, AtPLC, was isolated from dehydrated Arabidopsis and was strongly induced by cold, dehydration and salinity at transcriptional level (Hirayama et al., 1995).
Phospholipase D, PLD, is involved in the transduction of stress signals. PLD hydrolyses phospholipids to generate phosphatidic acid (PA), another second messenger that can activate PI-PLC and protein kinase C (Munnik and Meijer, 2001). Like PLC, PLD has a role in the regulation of stomatal aperture during osmotic stress. ABA promoted stomatal closure is shown to be mediated by guard cell PLD activity in response to water stress (Jacob et al., 1999).

2.3.6.4 Role of calcium dependent protein kinases (CDPK): Transient increases in cytosolic Ca\(^{2+}\) are perceived by various calcium binding proteins (Sanders et al., 1999). CDPK and the SOS3 family of calcium sensors (Gong et al., 2004) are major players in coupling this universal inorganic signal to specific protein phosphorylation cascades. CDPK are serine/threonine protein kinases with a C-terminal calmodulin-like domain with up to 4 EF motifs that directly bind calcium. Overexpression of CDPK was found to lead to increased cold and salt tolerance (Sheen et al., 1996). Induction of CDPK in a tolerant rice variety occurs earlier and is sustained for longer duration compared to the sensitive variety (Kwasaki et al., 2001).

2.4.5 Role of Abscisic Acid (ABA): ABA plays a crucial role in abiotic stress. Although the phenomenon of ABA biosynthesis up regulation in response to osmotic stress is common, the signaling pathway that causes such up regulation is unknown (Finkelstein et al., 2002). ABA biosynthetic genes, ZEP (encoded by ABA1 in Arabidopsis and ABA2 in tobacco) are regulated by calcium. In addition ABA can feedback stimulate the expression of ABA biosynthetic genes, through a calcium-dependent-phosphoprotein cascade. Stress inducible genes contain consensus ABA-responsive element, PyACGTGGC, in their promoter region. These ABRE function as cis-elements involved in ABA-regulated gene expression (Guiltinan et al., 1990, Mundy et al., 1990). ABA-dependent signaling activates basic leucine zipper transcription called ABF/ABRE to induce stress responsive gene expression. ABF contain highly conserved basic regions (Figure III) and apart from DNA binding properties they may be involved in nuclear translocation,
transcriptional activation or protein-protein interactions. Further each ABF may function for a specific stress, for example ABF1 expression is induced in cold, ABF2 and ABF3 by salt and ABF4 is induced by cold, salt and drought (Choi et al., 2000).

2.4.6 Role Of Late Embryogenesis Abundant Proteins (LEA): The LEA proteins were first identified as genes expressed during maturation and desiccation phases of seed development (Galau et al., 1986). These were found to be additionally expressed during periods of water loss resulting from water, osmotic and low temperature stress. The majority of lea gene products are predominantly hydrophilic, biased in amino acids composition in lacking in Cys and Trp residues and are proposed to be cytosolic in location. LEA proteins (D19 or group 1) are predicted to have enhanced water-binding capacity. These proteins have a high percentage of charged amino acids and glycine. One member of this group, Em, is approximately 70% random coil, leading to the prediction that it has a high capacity for binding water (Dure et al., 1989). Group 2 proteins have a conserved 15-a.a sequence, EEKKGIMDKIKELPG at the C-terminal and at least once internally. They function to preserve protein structure and thus act as chaperones. One of the LEA proteins (D7 family/ group 3) is predicted to play a role in sequestration of ions that are concentrated during cellular dehydration. These proteins have an 11 amino acid consensus motif TAQAAKEKAGE, repeated as many as 13 times and predicted to form an amphiphilic alpha helix. The hydrophilic face may be important in forming a homodimer and the outside charged face may be involved in sequestering the water deficit induced increase in ion concentration (Ingram et al., 1996).

2.5 Integrative Mechanisms Of Salinity Tolerance: Clues From Different Organisms

All life forms have evolved from marine organisms. It has been suggested that many years of natural selection in non-saline terrestrial environments
removed the salt tolerance from progenitors of most crop plants. Thus though all primitive organisms have mechanisms to survive under saline conditions (Kenrick and Crane, 1997) higher plants have essentially lost the ability to survive under such conditions during evolution. The following section describes adaptive tolerance mechanisms in different organisms.

2.5.1 Mechanisms in Halophilic Organisms: Halophiles or “salt loving” organisms inhabit hyper saline environments. Archeal halobacterium species, cyanobacteria Aphanothece halophytica and green alga, Dunaliella salina and multicellular eukaryotes species including brine shrimp and brine flies are found in hypersaline environments. Based on the salt requirement the halophiles are classified as slight halophiles that grow optimally at 2-5% NaCl, moderate halophiles at 5-20% NaCl, and extreme halophiles have salt requirements of above 20-30% NaCl.

It has been found that many halophiles have novel characteristics of enzymes. Cloning of the malate dehydrogenase gene from extremely halophilic archeabacterium, Haloarcula marismortui in E coli produced a soluble but inactive protein, that could be activated only on increasing the salt concentration to as high as 3 M (Cendrin et al., 1993). These enzymes seem to be altered in a way of having more acidic residues and consequently become dependent on high Na⁺ concentrations for their activity.

Algae predominantly use polyols as compatible solutes. In green alga, Dunaliella salina, glycerol is synthesized in response to osmotic stress. The cytoplasmic concentration of glycerol can constitute 50% of the dry weight of cells when grown in medium containing 2-5% NaCl.

2.5.2 Mechanisms in E.coli: In E. coli two mechanisms; generation of osmoprotectants such as betaine and regulation of sodium antiporters have been used to adapt to high salinity (Waditee et al., 2002). Two mechanisms are thought to be involved behind the activity of these osmoprotectants:
i. The ability to raise the osmotic potential of the cell
ii. The ability to stabilize membranes and/or macromolecular structures as they have minimal affects on pH or charge balance of the cytosol.

In the *E. coli* the production of Betaine is carried out in a two-step process

\[ \text{Choline} \xrightarrow{\text{betA}} \text{Betaine aldehyde} \xrightarrow{\text{betB}} \text{Betaine} \]

In *E. coli*, three antiporters, NhaA, NhaB and ChaA have been characterized. The NhaA antiporter activity is dependent on environmental pH and is greatly enhanced as the pH increases from neutral to alkaline. It has been shown that the stoichiometry changes from 1.1 at pH=7.4 and reaches 1.4 at pH=8.4 (Noumi et al., 1997). Unlike NhaA (Taglicht et al., 1991), NhaB antiporter is relatively constitutive, although the affinity for Na⁺ increases ten fold with an increase in pH from 7.2 to 8.5. (Pinner et al., 1994). NhaA antiporter exhibits relatively high activity for Li⁺ and Na⁺ transport. ChaA has proton/cation exchange activity with Na⁺ or Ca²⁺ but not with Li⁺ or K⁺ and can exchange Na⁺/H⁺ at alkaline pH of 8.5. The factors that determine ion specificity are unclear.

2.5.3 Mechanisms in Yeast: Two main mechanisms for adaptation to salt stress in yeast include accumulation of a polyol: glycerol and maintenance of ion homeostasis. When exposed to NaCl the cells experience both osmotic stress and ion toxicity. Accumulation of glycerol in response to a low external osmotic potential compensates for the difference between the extra and intra cellular water potential. A brief description of the different mechanisms is given below.

2.5.3.1 Glycerol accumulation high osmolarity glycerol (HOG) pathway: High osmolarity is perceived by the two membrane osmosensors: the protein products of *Slr1* and *Sth1* (Brewster et al., 1993). The SLN1
protein contains an extracellular sensor domain, a cytoplasmic histidine kinase domain, and a receiver domain; YPD and ssk1 receiver signals in the cytosol. The sensor and the receiver domains function like bacterial two component systems.

Early events of the osmosensing begin with the autophosphorylation of Sln1 at the histidine residue followed by a cascade of phosphorylation events (Posas et al., 1996). The downstream osmotic signal transduction pathway is composed of three tiers of protein kinases, namely SSK2 which is a MAPKKK, Pbs (MAPKK) and HOG response MAP (MAPK). Signals transferred via the MAPK cascade finally enhance the expression of glycerol biosynthesis pathway. Glycerol is synthesized from dihydroxyacetone phosphate (DHAP) as follows:

\[
\text{Glycerol-3-phosphate dehydrogenase} \\
\text{DHAP} \rightarrow \text{Gdp1/Gdp22dp2} \rightarrow \text{Glycerol-3-phosphate} \rightarrow \text{Glycerol phosphatase} \rightarrow \text{Glycerol}
\]

The osmotic induction of glycerol biosynthetic genes is mediated by the HOG-MAP kinase-signaling pathway. In addition to induced glycerol production, yeast cells may decrease membrane permeability to glycerol, which leads to an increased retention of glycerol in cells under osmotic stress. To maintain an optimum cytoplasmic concentration of K\(^+\) and a stable K\(^+\)/Na\(^+\) ratio cells employ three distinct strategies.

### 2.5.3.2 Efficient efflux of toxic cations from the cells

Induction of the Ena1 gene encoding a Na\(^+\)-ATPase (Haro et al., 1993) by salt operates through the osmotically activated HOG/MAP kinase and Ca\(^{2+}\) activated calcineurin phosphatase. Mechanism of osmotic regulation of Ena1 promoter is known in considerable detail. HOG1 regulates binding of the bZIP repressor to CRE element (TGACGTCA) and inhibits transcription by recruitment of co-repressor complex. Localized de-acetylation of histones H3
and H2B at the Ena1 promoter inhibits transcription by forming a repressive chromatin structure that decreases TATA-binding protein (TBP) occupancy. The HOG1 counteracts this repression of the Ena1 gene by translocating to the nucleus and phosphorylating the repressor at multiple sites. The phosphorylation event thus prevents the formation of the co-repressor complex thus allowing transcription of Ena1 gene. The HOG-MAP kinase pathway is activated at low salt concentrations NaCl (0.1 –0.3M). Induction of Ena1 gene at high salt concentrations NaCl is mediated by the calcium-activated protein phosphatase, calcineurin and is specific for Na⁺. Calcineurin, consisting of a catalytic subunit CNA and a regulatory subunit CNB requires Ca²⁺ and Calmodulin for activity. The high Na⁺ concentration could raise intracellular Ca²⁺. Regulation of Ena1 is at the transcriptional level involves the Zn-finger transcription factor Crz1/TCn1/hal8.

2.5.3.3 Discrimination among alkali metal cation at the level of influx: The yeast plasma membrane H⁺-ATPase, P⁺-Piase encoded by the PMA1 gene is responsible for the proton gradient maintenance (Andre et al., 1995), while the product of PMA2 gene is induced at low pH when the PMA protein cannot function properly. The TRK proteins are involved in K⁺ uptake but can also transport Na⁺. The membrane potential drives uptake of K⁺ via membrane proteins TRK1 and TRK2. TRK1 is required for high affinity K⁺ uptake while TRK2 for low affinity K⁺ uptake. At high external Na⁺ concentrations Na⁺ can inhibit K⁺ uptake and enter the cell through the potassium channels. Since the high affinity K⁺ transport system shows a higher K⁺/Na⁺ discrimination than the low affinity system, it has been shown that yeast cells subjected to salt stress may shift from low to high affinity K⁺ uptake, allowing cells to accumulate more K⁺ than Na⁺ and maintain a low Na⁺/K⁺ ratio.

2.5.3.4 Selective sequestration of cations in the organelles: Na⁺/H⁺ exchangers comprise a family of membrane proteins that catalyze the counter transport of Na⁺ and H⁺ and regulate transmembrane movement of
salt, water and acid-base equivalents critical for cell volume maintenance, regulation of pH and sodium tolerance.

In *Saccharomyces cerevisiae*, the primary pathway for Na\(^+\) extrusion is mediated by the plasma membrane Na-ATPase; Enal. Additional genes like NhaA, NhX and YJLO94c may also contribute to Na\(^+\) tolerance. Nhal belongs to a family of fungi specific Na\(^+/\)H\(^+\) exchangers that is unrelated in sequence to bacterial or mammalian Na\(^+/\)H\(^+\) exchangers. The Nhal homologue in *Schizosaccharomyces pombe* Sod2 localizes to the plasma membrane and mediates electrochemical H\(^+\) gradients driven Na\(^+/\)H\(^+\) exchange activity.

NHX localizes to the endosomal/vacuolar compartments and is important in development of salt tolerance, pH regulation and vesicular trafficking. The C-terminus of yeast Nhxl interacts with a GTPase activating protein from the Ypt7/ Rab family of GTPases. Both these proteins co-localize in the endosomal or prevacuolar compartment (PVC) (Ali et al., 2004). Nhxl is regulated post-translationally by N-linked glycosylation. The glycosylation site maps to residues in the C-terminal hydrophilic tail portion of the exchanger (Wells and Rao, 2001).

### 2.6 Salt Tolerance In Plants

One of the major reasons attributed to salinity is the continued irrigational practice. Besides the dominant Ca\(^{2+}\) and Mg\(^{2+}\) cations, irrigation water also contains some Na\(^+\). As the water evaporates and transpires, the Ca\(^{2+}\) and Mg\(^{2+}\) tend to precipitate as carbonates leaving Na\(^+\) as the dominant cation in the soil (Flowers et al., 2004). Plants that possess the natural ability to thrive on high saline environments are called halophytes. Taxonomically distinct group of plants dominated by salt tolerant species include mangroves and chenopods. Species such as *Salicornia bigelovii* belonging to Chenopodiaceae are capable of producing biomass and seed equivalent to conventional crops even when the NaCl concentrations are 1.3 M (~twice that of sea water) (Glenn et al., 1991). In contrast, glycophytes are sensitive
to long exposures of even mild salinity. Glycophytes such as rice and beans are harmed by 20-50 mM NaCl (Greenway and Munns., 1980). Higher plants do not have a salt tolerant metabolism even when the organism itself thrives in seawater. The tolerance levels for enzymes \textit{in vivo} are similar between salt sensitive crop species and plants native to salt marshes (Blumwald, 2000). In sharp contrast are halo tolerant bacteria, with proteins that undergo conformational alterations to yield functional higher order structures only in the presence of high NaCl (Dym \textit{et al.}, 1995).

2.6.1 \textbf{Cress Plant:} \textit{Thellungiella halophila}, salt cress commonly used as a genetic model system of halophytes is capable of growing in salt concentrations as high as 500 mM (Bressan \textit{et al.}, 2001). Strikingly it has no anatomical specialized structures like salt glands that would help it cope up with the salt stress (Inan \textit{et al.}, 2004). Micro array analysis of gene expression profiles in salt cress using full length \textit{Arabidopsis} cDNA showed a strong induction of 6 genes in response to high salinity stress while 40 genes were identified as salt stress inducible genes in \textit{Arabidopsis} (Taji \textit{et al.}, 2004). Several genes found to be induced with abiotic or biotic stress in \textit{Arabidopsis} were found to be present under normal growth conditions in salt cress The expression profiles of genes highly expressed in salt cress under normal conditions resembled those of \textit{Arabidopsis} genes induced under abiotic stress rather that biotic stresses (Volkov \textit{et al.}, 2004).

Proline is the most common osmoprotectants known to accumulate in many organisms upon exposure to high salinity, drought and freezing. Proline degradation is catalyzed by proline dehydrogenase (ProDH). The amount of proline in salt cress was equivalent to that in transgenic \textit{Arabidopsis} over expressing antisense AtProDH cDNA.

Levels of SOSI (Shi \textit{et al.}, 2002) transcript in overexpressed transgenic lines is only slightly higher than wild type under normal growth conditions, while these levels are induced upon salt stress. It is suggested that SOSI mRNA is unstable under normal conditions and that salt stress causes a
posttranscriptional stabilization of the transcript (Shi et al., 2003). However SOSI genes are expressed at high levels in salt cress indicating that either the machinery or the set of genes involved in mRNA degradation may be down regulated or the mRNA transcribed is inherently more stable. Thus novel mechanisms to tolerate salt stress seem to exist in halophytes.

2.7 Transgenic Approaches To Develop Salt Tolerant Plants

In the previous sections at appropriate places a brief mention of the transgenic approach that has been adopted to validate the function of a gene has been given. The following table lists some of the mechanisms that have been targeted to achieve salinity stress tolerance using transgenic approach.
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