Biochemical Mechanisms of Salt Tolerance in Plants: A Review

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Abstract: Among abiotic stresses, high salinity stress is the most severe environmental stress, which impairs crop production on at least 20% of irrigated land worldwide. Understanding the mechanism of stress tolerance along with genes involved in stress signaling network is important for crop improvement. The growth reduction is recorded as a main morphological effect of salinity. This is due to many biochemical mechanisms of the plant. It is thought that excess of salts retards the absorption of water and reduce the growth through osmotic effect. Detrimental effects of salts on growth may be due to the toxicity of specific ions, elevation of osmotic pressure or the increase in alkalinity which may restrict the availability of water or influence cellular physiology and metabolic path ways. Salinity stress response is multigenic, as a number of processes involved in the tolerance mechanism. It affected various compatible solutes/osmolites, polyamines, reactive oxygen species, antioxidant defense mechanism, ion transport and compartmentalization of injurious ions. Various strategies to improve salinity stress tolerance have been discussed in the present review.

Key words: Antioxidative enzymes, ion homeostasis, osmotic stress, salt stress, Arabidopsis thaliana

INTRODUCTION

Different types of edaphic factors affect plant growth. Salinity is one of the most important stress factors which limit the growth and development of plant by altering their morphological, physiological and biochemical attributes. According to the USDA Salinity Laboratory. Saline soils have a high concentration of soluble salts. They are classed as saline when the ECe is ≥ 4 dS m⁻¹.

Ions contributing soil salinity are Na⁺, Cl⁻, Ca²⁺ and Mg²⁺. Approximately 20% of agricultural land and 50% of cropland in the world is salt-stressed (Flowers and Yeo, 1995). Molecular genetic and plant transformation advances have made it feasible to assess biotechnological strategies based on activated signal cascades, engineered biosynthetic pathways, targeted gene or protein expression or alteration of the natural stress responsiveness of genes for development of salt tolerant crops (Hasegawa et al., 2000). 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.

Adaptive mechanism of plant: These are extremely complex and an array of mechanisms appears to be involved in salt tolerance of plants. Mechanisms for tolerance of the salt-specific effects of salinity are of two main types: Those minimizing the entry of salt into the plant, and those minimizing the concentration of salt in the cytoplasm. Overall effect of salt stress on plant and their adaptive mechanism can be study under following heads.

Osmotic effect: Effects of salinity on plants are generally summarized as water stress, salt stress and ionic imbalance stress. Water stress (decrease in water potential) arises as a result of evaporation water used by plants and salt accumulation. Excess quantities of soluble salts in soil solution limit the availability of water to plants. Decrease in plant water potential, which is common response under such situation, must immediately be offset by decrease in osmotic potential, through increased solute content for turgor potential to be maintained. Water potential and osmotic potential of plants become more negative with an increase in salinity, whereas turgor pressure increases with increasing salinity. Osmotic potential of the cell sap changes as to maintain a constant water potential gradient between leaf and soil. Osmotic effects predominate when plant growth is related to osmotic potential of root medium that contains different salts or combination of salts. The reduced growth associated with osmotic stress is
attributed to the build up of osmotic pressure of developing cells to meet the increasing osmotic pressure of the rooting medium and still maintain turgor. Energy expenditure during osmotic adjustment to abiotic stress is one if the main factors for reduced growth (Greenway and Gibbs, 2003).

**Specific ion:** Detrimental effect of salt may be due to the toxicity of specific ion, elevation of osmotic pressure or the increase in alkalinity which may restrict the availability of water or influence cellular physiology and metabolic pathway. Specific ion toxicity is usually associated with excessive intake of chloride, sodium or other ion and hence causes nutritional imbalance (Bernstein, 1964). One of the most detrimental effects of salt stress is to disrupt the ion homeostasis mechanisms of the plant. In particular, the similar radii of Na⁺ and K⁺ make it difficult for transport proteins to distinguish between these two ions. Thus, under conditions of high external Na⁺, there is substantial uptake of Na⁺ through K⁺ transporters or channels (Blumwald et al., 2000).

Effect of NaCl salinity in rice causes higher salt concentration in older leaves that may result from exclusion of specific ion from the xylem vessels younger leaves (Yeo, 1998). Overall salt tolerance of any crop species may depend upon successful combination of several such mechanism acting in concert as ion toxicity and mineral nutrient deficiencies. In moderate salt stress conditions, leaves are smaller, thicker and usually dark green in colour. Upon salinization sodium moves first to older leaves which in turn protect these younger leaves (Yeo and Flowers, 1982).

**Compartmentalization and osmolytes:** Ion uptake and compartmentalization are essentials because the salt stress disturbs ion homeostasis. Plants restrict the excess salts in the vacuole or compartmentalize the ions in different tissues to facilitate their metabolic functions (Zhu, 2003). There are several possible strategies that plants could employ to avoid a damaging decrease in the K⁺/Na⁺ ratio; reduce entry of Na⁺ into the cell, remove Na⁺ from the cell, or compartmentalize Na⁺ into the vacuole where it cannot disrupt cellular function (Surekha et al., 2005).

Na⁺ and Cl⁻ are energetically efficient osmolytes for osmotic adjustment and are compartmentalized into the vacuole to minimize cytotoxicity (Niu et al., 1995). Movement of ions into the vacuole may occur directly from the apoplast into the vacuole through membrane or a cytological process that juxtaposes the plasma membrane to the tonoplast (Hasegawa et al., 2000). A major category of organic osmotic solutes consists of simple sugars (mainly fructose and glucose), sugar alcohols (glycerol and methylated inositol) and complex sugars (trehalose, raffinose and fructans). Others include quaternary amino acid derivatives (proline, glycine betaine), tertiary amines (1, 4, 5, 6-tetrahydro-2-methyl-4-carboxyly pyrimidine) and sulfonim compounds (dimethyl sulfonium propionate) (Zhifang and Loescher, 2003).

Glycine betaine preserves thylakoid and plasma membrane integrity after exposure to saline solutions or to freezing or high temperatures. 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 (Rhodes et al., 1989).

Proline increases with increase in salinity as an adaptive change in metabolic pattern. Polyols serve as compatible solutes, as low-molecular-weight chaperones and as scavengers of stress-induced oxygen radicals (Bohnert et al., 1995). High level of salt tolerance (growing, completing normal development, flowering and producing seeds) in mature transgenic plants with 300 mM NaCl in the nutrient solution, demonstrate a major role in developing salt-tolerant plants by means of introducing mannitol biosynthesis using M6PR (Zhifang and Loescher, 2003).

Effect of salt stress on amino acids in leaves of chickpea (Cicer arietinum L.) showed that the arginine and asparagine showed a marked decline in their contents with increase in salinity while threonine exhibit a considerable enhancement in its content at 4 dS m⁻¹ EC thereafter it decline with increase in salinity. A sharp increase in the contents of was observed. Isoleucine, leucine, aspartic acid and proline showed a continuous enhancement up to 16 dS m⁻¹ EC (Mudgal, 2004).

Salt stress is complex and imposes a water stress. This water deficit leads to the formation of Reactive Oxygen Species (ROS) such as superoxide, hydrogen peroxide (H₂O₂), hydroxyl radical (OH⁻) and singlet oxygen (¹O₂). The activities of the antioxidative enzymes such as catalase (CAT), ascorbate peroxidase (APX), guaiacol peroxidase (POD), glutathione reductase (GR) and superoxide dismutase increase under salt stress (Mittova et al., 2002, 2003).

**Oxidative stress:** Salt stress, like other abiotic stresses, can also lead to oxidative stress through the increase in Reactive Oxygen Species (ROS), such as superoxide (O₂⁻), hydrogen peroxide (H₂O₂) and hydroxyl radicals (OH⁻), which are highly reactive and may cause cellular damage through oxidation of lipids, proteins and nucleic acids (Pastori and Foyer, 2002; Apel and Hirt, 2004). It has been demonstrated that salinity induces oxidative stress.
in plant tissues and lipid peroxidation has frequently been used as an indicator of oxidative stress when plants are subjected to salinity. This has been shown for *Morus alba* (Ramajulu and Sudhakar, 2001), *Lycopersicon esculentum* (Mittova et al., 2002), *Oryza sativa* (Vaidyanathan et al., 2003) and *Gossypium hirsutum* (Meloni et al., 2003).

To overcome the effects of salinity-induced oxidative stress, plants make use of a complex antioxidant system, which is composed of low-molecular mass antioxidants (glutathione, ascorbate and carotenoids) as well as ROS-scavenging enzymes, such as: superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), guaiacol peroxidase (GPX) and glutathione reductase (GR). The SOD may function as a ROS scavenger, by converting $O_2^{-}$ to $H_2O_2$ (Alshe et al., 2002). Recent studies have demonstrated that overexpression of mitochondrial Mn-SOD in transgenic *Arabidopsis thaliana* (Wang et al., 2004) and chloroplastic Cu/Zn-SOD in transgenic *Nicotiana tabacum* (Badawi et al., 2004) can provide enhanced tolerance to salt stress. Similar results have been found in *Morus alba* (Ramajulu and Sudhakar, 2001), *Tritium aestivum* (Sairam and Tyagi, 2004) and *Lycopersicon sp.* (Mittova et al., 2002). Bacterial catalase and Glutathione-S-Transferase (GST)/glutathione peroxidase (GPX) can increase the performance of plants under stress (Roxas et al., 2000).

**Metabolic adaptations:** Cellular level adaptations are the main responses amenable to molecular analysis and have led to the identification of a large number of genes induced by salt. Functional groups responsible for encoding salt-stress proteins are:

- Genes for photosynthetic enzymes
- Genes for synthesis of compatible solutes
- Genes for vacuolar-sequestering enzymes
- Genes for radical-scavenging enzymes (Kawasaki et al., 2001)

High salt (NaCl) uptake competes with the uptake of other nutrient ions, especially K+, leading to K+ deficiency. Increased treatment of NaCl induces increase in Na+ and Cl– and decrease in Ca++, K+ and Mg++ levels in a number of plants. Salinity enhances the content of Na+, Ca++, Cl– and the ratio of K+/Na+ decreases in *Vicia faba* and Chickpea (Mudgal et al., 2009). Salinity stress causes an increase in levels of Na+ and Cl– in guava and the highest ion accumulation is found in the leaves followed by the roots; the Ca++ levels are stable in the roots but decrease in stems and leaves and the K+ content is reduced with increased levels of salinity, particularly in the leaves. On the other hand, Mg++ levels are not affected by salinity in stems and roots but decrease in the leaves of guava. There is a positive relationship between Na+ and Cl– and a negative relationship between Na+ and K+ concentration in roots and leaves. Mg++ concentration in leaves and roots does not vary with the concentration of Na+ and the concentration of Ca++ does not vary with that of Na+ in the leaves but shows an inverse relationship in the roots (Beckman et al., 2008).

**Nitrate Reductase Activity (NRA)** of leaves decreases in many plants under salt stress (Abrol and Kumar, 1981). The NRA decrease in root and shoot of pea plant leading to accumulation of NO3– and NH4+ nitrogen (Garg et al., 2001). Salinity inhibits nitrogen fixation by reducing nodulation and nitorgenase activity in chickpea (Sousi et al., 1999; Mudgal et al., 2009) (Table 1).

### Table 1: Effect of salt stress on nitorgenase activity (ARA) μ mole ethylene/mg nodule dry wt. h in three cultivars of chickpea

<table>
<thead>
<tr>
<th>Variety</th>
<th>EC of saline water (mM)</th>
<th>30</th>
<th>60</th>
<th>90</th>
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</tr>
<tr>
<td>16</td>
<td>0.081±0.005</td>
<td>0.103±0.005</td>
<td>0.069±0.003</td>
<td></td>
</tr>
<tr>
<td>PUSA-939 Control</td>
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<td>0.088±0.004</td>
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<td>Kabuli</td>
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<td>BG-256  Control</td>
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<td>0.022±0.002</td>
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</table>

Values are shown as Mean±SD of three sets of experiments with triplicates in each set (Mudgal et al., 2009).

**EFFECT OF SALINITY ON DIFFERENT GROWTH STAGES**

**Effect on germination and seedling growth:** Germination is the series of steps that proceed to protrusion of the radical. The phase of germination and seedling growth is critical one. Germination under saline conditions is generally affected due to high osmotic pressure of the solution. This osmotic pressure results in less imbibition of water. Due to capillary rise of salts, the concentration of salts is more at seed depth than at lower levels in soil profile. Salinity has been shown to affect time and rate of germination (Poljakoff-Mayber and Gale, 1975; Mudgal, 2004) (Fig. 1).

**Effect of salinity on nodulation:** Salinity interfered with the nodule initiation in chickpea, cowpea and mung bean and also caused a reduction in number, weight as well as nitrogen fixing efficiency of nodules (Balarubramanian and Sinha, 1976). Salinity causes a
Table 2: Effect of salt stress on number of nodules/plant (NN), nodules dry weight (mg plant⁻¹) (NDW) and root dry weight (mg plant⁻¹) (RDW) in three cultivars of chickpea.

<table>
<thead>
<tr>
<th>Variety</th>
<th>EC</th>
<th>NN</th>
<th>NDW</th>
<th>RDW</th>
<th>30 Days after sowing</th>
<th>NDW</th>
<th>RDW</th>
<th>60 Days after sowing</th>
<th>NDW</th>
<th>RDW</th>
<th>90 Days after sowing</th>
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<td>75.0±1.64</td>
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<td>70</td>
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<td>30.1±1.44</td>
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<td>47.6±1.56</td>
<td>70.4±1.50</td>
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<td>23.4±1.23</td>
<td>80.8±1.18</td>
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<td>30.1±1.44</td>
<td>57±1.02</td>
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<td>45.1±0.83</td>
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<td>PUSA-939</td>
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</table>

Values are shown as Mean±SD of three sets of experiments with triplicates in each set (Mudgal, 2004).

Fig. 1: Effect of salt stress on percent germination in ten cultivars of chickpea (Mudgal, 2004)

significant decrease in leghaemoglobin content as compared with control up to 125 DAS which decrease with aging of nodules probably because of irreversible oxidation of leghaemoglobin. Tu (1981) observed that inhibition of colonization of the root by rhizobium strain was the main reason for poor nodulation under salt stress. It is due to shrinkage of root hairs. Martin and Ruiz-Torres (1992) found that N₂O accumulation was significantly higher in C₄ (barley and wheat) than C₃ (maize and sorghum) cereals. Salinity show inhibitory effect on nodulation of chickpea even at 4.0 dS m⁻¹ significantly. Salt stress decrease shoot and root dry weight, total number of nodule per plant, nodule weight and average nodule weight (Table 2) (Elsheikh and Wood, 1990, Mudgal, 2004). Although, nodules were observed in inoculated plants grown at 6 dS m⁻¹ but nitrogen fixation was completely inhibited. The findings indicate that symbiosis is more salt sensitive than both rhizobium and host plant.

Effect of salinity on nodulation: The overall effect of salinity on Chickpea is reduction in growth, number of lateral branches, number of leaves and dry weight of shoot, root and leaves, with increasing electrical conductivity of saline water in all the three varieties (PUSA-1053, PUSA-939 and BG-256). Salinity response among genotypes was compared and found that there was about 50% reduction is shoot growth at 16 dS m⁻¹ EC as compared to control (Mudgal, 2004). A consequence of grain development in all monocarpic crops is the senescence of leaves and eventually the death of plants. Longer the retention of green leaf area in post-anthesis phase, generally higher is grain yield (Yoshida, 1981). Growth of the plants progressively decreased, flowering delayed, number of flower decrease and pod setting was reduced with increase in salinity level in pea and chickpea (Mudgal, 2004). Salinity reduced the number of pods, more than the seed. Purely on structural consideration, grain yield per plant or per unit area in chickpea dependent primarily upon number of pods bearing nodes per plant or per unit area which can be achieved either from multibranching or from extra plant height (Mudgal, 2004). In chickpea the traditional bushy type cultivars were characterized by multibranching habit in the form of primary, secondary, tertiary and late order branch (Mudgal, 2004).

Effect on nitrogen metabolism: Salinization also results in reduction of sulphur containing amino acids. There is a tendency for amino acids (argenine and glutamic acid), amides and toxic diamines (putrescine and cadaverine) to accumulate in tissue of stress affected plant due to depression of diamine oxidase activity (Stewart and Lee, 1974). Cellular uptake of sodium and chloride occurs at two main sites, at the plasma membrane of root epidermis or cortical cells and the plasma membrane of xylem parenchyma cells (Kuiper, 1968). The former regulate the
entry of NaCl to root and the latter regulate its entry to shoots. There are two ways in which NaCl would be restricted from entering root epidermal/cortical cells. One would be restriction to passive uptake and the other would be active efflux. For Cl, a passive restriction is feasible, because the negative cell electric potential provide on electrochemical gradient working against Cl entry (Kuiper, 1968). The permeability properties of plasma membrane would then be crucial. In case of sodium, active efflux may be the main process (Kuiper, 1968).

Plant in saline habitat accumulated lysine, proline, asparagine, glutamine, aspartic acid, glutamic acid, alanine, tyrosine and valine. The increased concentration of amino di caboxylic acid and amides under such circumstances indicate incomplete utilization of nitrogen entering the root due to disturbed protein synthesis (Munns, 2002). Ammonia, lysine and proline have toxic effect and their increased quantities cause production of abnormal metabolites (Munns, 2005). The harmful effect of NaCl on the nitrogen metabolism in plants is especially pronounced in the aerial parts. Salt stress in legume has also been associated with elevated peroxidase level (Munns et al., 2006; Munns and Tester, 2008).

Nitrogen fixation was highest at 50-60 DAS. After this there is a fall in acetyl enzyme reductase activity reaching to negligible level at 75 DAS. Nodule leghaemoglobin was preferentially degraded at 60-75 DAS as compared to nodule cytosolic protein during nodule senescence. The nodule peroxidase activity registered a sharp increase as the nodule progressed towards senescence; catalase activity remains unchanged between 45-60 DAS but decline considerably at 75 DAS under salt stress (Sheokhand et al., 1995).

Dehydrogenase oxidoreductase activity (DHA) decline by 71% at 28 dS m⁻¹ EC and by 87% at 40.8 dS m⁻¹ EC. DHA was negatively correlated with pH (r = -0.767 in saline soil), while it was positively correlated with organic compounds (r = -0.812). Saline soil dominated by NaCl and Na₂SO₄ had higher DHA than barren alkaline soil (Batra and Mann, 1997).

Glycine betaine or choline in a minimal medium with added NaCl had a beneficial role in growth of Vicia faba var. major and Cicer arietinum L. (Lee et al., 2001). Salinity generally reduces the lag phase or slowed the log phase of multiplication of rhizobia.

The phosphonol pyruvate carbonylase (PPC) and malate dehydrogenase (MDH) activities in leaves increase in early stages with salt, but fell during the following harvest (Soussi et al., 1998). Glutaminate synthetase in nodules continuously increased at same harvest. Plant under high fertility conditions maintained higher level of soluble protein, free praline, reducing sugars and high activity of nitrate reducetase compared to plants under stressed conditions (Soussi et al., 1998).

**TRANSFORMATION AND SALT TOLERANCE**

The activity of Na⁺/H⁺ antiporters could be limited by their number, or by the H⁺ difference across the membranes (Gaxiola et al., 2001). In that case, increasing the capacity of a proton pump would increase the salt tolerance of the plant. The vacuolar H⁺-PPase, AVP1, which may be important to energize the vacuole membrane under salt stress, was over expressed in Arabidopsis and increased its salt tolerance (Gaxiola et al., 2001). Transformed plants were able to grow in 250 mm NaCl, whereas the wild type died. The enhanced performance of transformed plants may have been caused by their higher accumulation of Na⁺ and K⁺ (Laurie et al., 2002). HKT transporters are probably very important in regulation of K⁺ and Na⁺ transport from root to shoots, but the mechanism is not known. Transgenic studies have yielded somewhat inconsistent results with TaHKT1 (Laurie et al., 2002) and AtHKT1 (Rus et al., 2004). However, a knockout mutant of AtHKT1 clearly increased salt sensitivity (Rus et al., 2004), which shows that the function of HKT1 in the control of K⁺ or Na⁺ transport is important. The HAL1 gene from yeast controls K⁺/Na⁺ selectivity and salt tolerance of yeast cells. Expression in tomato increased fruit yield and enhanced K⁺/Na⁺ selectivity in leaves (Rus et al., 2001). The exact function of this gene in higher plants is not known.

**ARABIDOPSIS THALIANA AND SALT OVERLY SENSITIVE (SOS)**

Arabidopsis thaliana Salt Overly Sensitive (SOS) pathway have a role in the maintenance of ion homeostasis under saline conditions. The Arabidopsis sos1, 2 and 3 mutants, were isolated in a genetic screen for plants hypersensitive to NaCl. Subsequent work has shown that these three SOS proteins are components of a stress-signaling pathway controlling ion homeostasis (Zhu, 2003). SOS1 plays a crucial role in sodium efflux from root cells and long-distance Na⁺ transport from roots to shoots (Shi et al., 2000; Dong-Hu et al., 2009). The SOS1 is a ser/thr protein kinase capable of autophosphorylating. It has an N-terminal catalytic domain similar to that of yeast sucrose nonfermenting 1 (SNF1) and ma- mmalian AMP-activated protein kinase (AMPK) and a C-terminal regulatory domain (Liu et al., 2000). The SOS1 is a myristoylated calcium-binding protein that is thought to respond to salt-induced Ca²⁺ transients in the cytosol (Liu and Zhu, 1998).
Arabidopsis mutants has demonstrated the existence of the SOS signaling pathway that senses salt stress, either through the Ca²⁺ sensing activity of SOS, or direct sensing of Na⁺ by SOS1 and regulates Na⁺ efflux and other ion transport and salt tolerance mechanisms (Surekha et al., 2005).

STRATEGIES TO IMPROVE STRESS TOLERANCE

Recent advances in molecular biology offer a new opportunity for understanding the genetics of stress-resistance genes. Molecular genetic maps have been developed for major crop plants, including sorghum, maize, rice, barley, wheat and potato which make it possible for scientists to tag desirable traits using known DNA landmarks. Molecular genetic markers reducing need for extensive field-testing, time and space. These biotechnological advances will provide new tools for breeding in stress environment. Biotechnological advances together with molecular genetic markers offer a new strategy known as marker assisted selection. Another molecular strategy which depends on gene cloning and plant transformation technology is genetic engineering of selected genes into elite breeding lines. What makes a particular goal attainable or unattainable in genetic engineering experiments are the availability of the following three inputs:

- The gene of interest
- An effective technique for transferring the desired gene from one species to another
- Promoter sequences for regulated expression of that gene (Sairam and Tyagi, 2004)

Amongst these, the first is considered a rate-limiting factor. Stress-responsive genes can be analyzed following targeted or non-targeted strategy. The targeted approach relies upon the availability of relevant biochemical information (i.e. in terms of defined enzyme, protein, a biochemical reaction or a physiological phenomenon) (Sairam and Tyagi, 2004).

The non-targeted strategy to obtain a desired gene is indirect. This strategy, for instance, includes differential hybridization and shotgun cloning (Sairam and Tyagi, 2004).

CONCLUSION

Several abiotic stresses cause changes in morphological, physiological, biochemical and molecular plant processes. The increasing prevalence of soil salinity is one of the most dangerous obstacles to improving crop productivity and quality. The adverse effects of saline soil include ion toxicity, nutrient constraints, osmotic stress and oxidative stress. Oxidative stress caused by the accumulation of reactive oxygen species those damage membrane lipids, proteins and nucleic acids. Maintenance of a high cytosolic K⁺/Na⁺ concentration is a key requirement for plant salt tolerance. Recent advances in genetic and molecular analysis of Arabidopsis thaliana mutants, ion transporters and stress signaling proteins have improved our understanding of the mechanisms of cellular ion homeostasis and its regulation in plants. Since, Na toxicity is the principal stress component in saline soils, much research has focused on the identification of ion transporters and regulatory mechanisms. SOS signaling pathway, composed of the SOS1, 2 and 3 proteins, has emerged as a key factor in the detection of and tolerance to salt stress. Recent evidence suggests that the SOS pathway may regulate several ion transport mechanisms critical for salt tolerance. It is hoped that systematic genetic, molecular and biochemical studies along with further work on the molecular mechanisms of ion homeostasis and stress sensing and signaling, will uncover the most promising approaches to improve the performance of crop plants under saline conditions. These technologies have mainly been applied to model systems and have greatly enlarged the knowledge of mechanisms of tolerance.

REFERENCES


