A wide range of environmental stresses (such as high and low temperature, drought, alkalinity, salinity, UV stress and pathogen infection) are potentially harmful to the plants (Van Breusegem et al. 2001). Salt stress in soil or water is one of the major stresses especially in arid and semi-arid regions and can severely limit plant growth and productivity (Allakhverdiev et al. 2000, Koca et al. 2007).

According to the incapacity to grow on high salt medium, plants have been classified as glycophytes or halophytes. Most plants are glycophytes and cannot tolerate salt stress (Sairam and Tyagi 2004). The deleterious effects of salinity on plant growth are associated with: (1) low osmotic potential of soil solution (water stress), (2) nutritional imbalance, (3) specific ion effect (salt stress) or (4) a combination of these factors (Ashraf 1994). During the onset and development of salt stress within a plant, all the major processes such as photosynthesis, protein synthesis and energy and lipid metabolisms are affected. The earliest response is a reduction in the rate of leaf surface expansion followed by cessation of expansion as the stress intensifies but growth resumes when the stress is relieved (Parida and Das 2005).

Resistance to environmental stress occurs when a plant withstands the imposed stress that may arise from either tolerance or a mechanism that permits escape from the situation. Although whole plant mechanism can contribute to the avoidance of stress during the plants life cycle, tolerance can also occur at the cellular level. Plants are either dormant during the salt episode or there must be cellular adjustment (Yokoi et al. 2002) and the response is species and genotypes dependent and depends on the length and severity of the salinity, the age and stage of development, the organ and the cell type and the sub-cellular compartment. An example of avoidance at the cellular level is the process of osmotic adjustment, where the osmotic potential of the cell is lowered in order to favour water uptake and maintenance of turgor (Bray 1997).

Conventional selection and breeding techniques have been used to improve salinity tolerance in crop plants (Ashraf 2002). The agronomical parameters used for salt tolerance are yield, survival, plant height, leaf area, leaf injury, relative growth rate and relative growth reduction (Ashraf and Harris 2004).

But, the conventional breeding techniques have been unsuccessful in transferring salt tolerance to the target species (Sairam and Tyagi 2004). Many investigators are of the view that plant species should possess distinctive indicators of salt tolerance at whole plant, tissue or cellular level.
(Ashraf 2002, Munns 2002). Nonetheless, parallels have been drawn between different biochemical indicators and plant tolerance. For example, there is strong evidence that glycinebetaine (quaternary ammonium compound) and proline (amino acid) play an adaptive role in mediating osmotic adjustment and protecting the subcellular structures in stressed plants. In a number of studies a positive correlation between the accumulation of these two osmolytes and stress tolerance has been recorded (Yamada et al. 2003, Yang et al. 2003). Due to increased environmental stress the balance between the production of reactive oxygen species (ROS) and the quenching activity of the antioxidants is upset, often resulting in oxidative damage (Spychalla and Desborough 1990). ROS can be important mediators of damage to cell structures, nucleic acids, lipids and proteins (Valko et al. 2006). The hydroxyl radical is known to react with all components of the DNA molecule, damaging both the purine and pyrimidine bases and also the deoxyribose backbone (Halliwell and Gutteridge 1999).

While determining the role of various antioxidants in salt tolerance of tomato, Mittova et al. (2002) demonstrated that higher salt tolerance of wild tomato (*Lycopersicon pennellii*) as compared to cultivated tomato (*Lycopersicon esculentum*) could be correlated with increased activities of SOD (superoxide dismutase), APX (ascorbate peroxidase), and POD (guaiacol peroxidase).

This review provides information on biochemical parameters, which are used as stress indicators at the cellular level. The overproduction of osmoprotectants, increasing expression of antioxidant enzymes helps the plant to withstand the environmental stress.

**SYMPTOMS OF Na⁺ ACCUMULATION**

Salt stress creates both ionic as well as osmotic stress on plants. These stresses can be distinguished at several levels (Tester and Davenport 2003). The root and shoot growth reduces abruptly in salt sensitive plants and this effect does not appear to depend on salt concentration in the growing tissues, it is rather a response to the osmolarity of the external solution (Munns 2002). Na⁺-specific damage is associated with accumulation of Na⁺ in leaf tissues and results in necrosis of older leaves. The time scale over which specific damage is manifested depends on the rate of accumulation of Na⁺ in leaves, and on the effectiveness of Na⁺ compartmentation within leaf tissues and cells. The Na⁺-specific effects are superimposed on the osmotic effects of NaCl (Tester and Davenport 2003), and importantly, show greater variation within species than osmotic effect (Munns 2002).

Deficiency of other nutrients in the soil is due to the high concentration of Na⁺ that interacts with other environmental factors, such as drought, which exacerbate the problem (Silberbush and Ben-Asher 2001). Besides, high Na⁺ hampers the uptake of other nutrients by: (1) Na⁺ interfering with transporters in the root plasma membrane, such as K⁺-selective ion channels, and (2) reduction of root growth by high Na⁺ concentration (Tester and Davenport 2003). Thus the uptake of water, growth limiting nutrients (such as P, Fe or Zn) and the growth of soil microorganisms, such as mycorrhizal fungi can be inhibited.

Leaves are more vulnerable than roots to Na⁺ simply because Na⁺ and Cl⁻ accumulate to higher levels in shoots than in roots (Tester and Davenport 2003). Though Na⁺ is transported to shoots through the rapidly moving transpiration stream in the xylem, it can only return to roots via the phloem. There is limited evidence of extensive recirculation of shoot Na⁺ to root, suggesting that Na⁺ transport is largely unidirectional and results in progressive accumulation of Na⁺ as leaves age (Tester and Davenport 2003).

The high levels of Na⁺ or Na⁺:K⁺ ratio can disrupt various enzymatic processes in the cytoplasm. K⁺ activates more than 50 enzymes and is an essential element in protein synthesis as it binds tRNA to the ribosomes (Blaha et al. 2000). The disruption in protein synthesis appears to be an important cause of damage by Na⁺ (Tester and Davenport 2003). Several studies suggest that the plasma membrane may be the primary site of salt injury (Mansour 1997). Nonelectrolytes and water permeability get altered markedly upon salt exposure.

Osmotic damage (i.e. osmotically driven removal of water from cells) could occur as a result of build up of high concentrations (possibly several hundred mmol) of Na⁺ in the leaf apoplast, since Na⁺ enters leaves in the xylem stream and is left behind as water evaporates (Flowers et al. 1991).

**OSMOLYTES AND OSMOPROTECTANTS**

During stress conditions plants need to maintain internal water potential below that of soil and maintain turgor and water uptake for growth.
This requires an increase in osmotica, either by uptake of soil solutes or by synthesis of metabolic (compatible) solutes. To accommodate the ionic balance in the vacuoles, cytoplasm accumulates low-molecular-mass compounds, the compatible solutes because they do not interfere with normal biochemical reactions (Zhifang and Loescher 2003); rather, they replace water in biochemical reactions. With accumulation proportional to the change of external osmolarity within species-specific limits, protection of structures and osmotic balance supporting continued water influx (or reduced efflux) are accepted functions of osmolytes (Hasegawa et al. 2000). While some compatible osmolytes are essential elemental ions, such as K⁺, the majority are organic solutes (Yokoi et al. 2002). However, the solutes that accumulate vary with the organism and even between plant species and a major category of organic osmotic solutes consists of simple sugars (mainly fructose and glucose), sugar alcohols (glycerol and methylated inositols) and complex sugars (trehalose, raffinose and fructans) (Bohnert and Jensen 1996). Others include quaternary amino acid derivatives (proline, glycine betaine, β-alanine betaine, proline betaine, tertiary amines 1,4,5,6-tetrahydro-2-methyl-4-carboxyl pyrimidine), and sulfonium compounds (choline osulfate, dimethyl sulfonium propironate) (Yokoi et al. 2002).

### Carbohydrates

For the various organic osmotica, sugars contribute up to 50% of the total osmotic potential in glycophytes subject to saline conditions (Cram 1976). The accumulation of soluble carbohydrates in plants has been widely reported as a response to salinity or drought, despite a significant decrease in net CO₂ assimilation rate (Murakeozy et al. 2003). Carbohydrates such as sugars (glucose, fructose, sucrose, fructans) and starch accumulate under salt stress (Parida et al. 2002), playing a leading role in osmoprotection, osmotic adjustment, carbon storage, and radical scavenging. A decrease in starch content and an increase in both reducing and nonreducing sugars and polyphenol levels have been reported in leaves of *Bruguiera parviflora* (Parida et al. 2002). In leaves of tomato the contents of soluble sugars and total saccharides are increased significantly, but the starch content is not affected (Khavarinejad and Mostofi 1998). Ashraf and Tufail (1995) determined the total soluble sugar content in five sunflower accessions differing in salt tolerance; the salt tolerant lines had generally greater soluble sugars than the salt sensitive ones (Table 1).

Trehalose, a disaccharide, accumulates under various abiotic stresses and protects membranes and proteins in cells exposed to stress that cause water deficit and reduced aggregation of denatured proteins (Singer and Lindquist 1998). According to Yamada et al. (2003) trehalose has a suppressive effect on apoptotic cell death. There is now conclusive evidence to suggest that trehalose is present in trace amounts in vascular plants, including major crops, but the actual role of this osmolyte in metabolism is still unclear.

### Proteins

Proteins that accumulate in plants under saline conditions may provide a storage form of nitrogen that is re-utilized later (Singh et al. 1987) and may play a role in osmotic adjustment. They may be synthesized de novo in response to salt stress or may be present constitutively at low concentration (Pareek-Singla and Grover 1997). It has been concluded that a number of proteins induced by salinity are cytoplasmic which can cause alterations in cytoplasmic viscosity of the cells (Hasegawa et al. 2000).

A higher content of soluble proteins has been observed in salt tolerant cultivars of barley, sunflower,
finger millet, and rice (Ashraf and Harris 2004). Agastian et al. (2000) have reported that soluble protein increases at low salinity and decreases at high salinity in mulberry cultivars. Although Ashraf and Fatima (1995) found that salt tolerant and salt sensitive accessions of safflower did not differ significantly in leaf soluble proteins, there are reports of decrease in soluble protein content in response to salinity (Table 2).

In higher plants, osmotic stress induces several proteins in vegetative tissues, which are related to late-embryogenesis-abundant (LEA) proteins. The correlation between LEA protein accumulation in vegetative tissues and stress tolerance indicates its protective role under dehydration stress (Ingram and Bartels 1996). Engineered rice plants over expressing a barley LEA gene, HVA1, under the control of rice actin 1 promoter showed better stress tolerance than did the wild type (Xu et al. 1996).

### Amino acids and amides

Amino acids (alanine, arginine, glycine, serine, leucine, and valine, together with the imino acid, proline, and the non-protein amino acids, citrulline and ornithine) and amides (such as glutamine and asparagines) have also been reported to accumulate in plants subjected to salt stress (Mansour 2000). Total free amino acids in the leaves have been reported to be higher in salt tolerant than in salt sensitive lines of sunflower (Ashraf and Tufail 1995), safflower (Ashraf and Fatima 1995), *Eruca sativa* (Ashraf 1994) and *Lens culinaris* (Hurkman et al. 1991).

Proline, which occurs widely in higher plants and accumulates in larger amounts than other amino acids (Abraham et al. 2003), regulates the accumulation of useable N. Proline accumulation normally occurs in the cytosol where it contributes substantially to the cytoplasmic osmotic adjustment (Ketchum et al. 1991). It is osmotically very active and contributes to membrane stability and mitigates the effect of NaCl on cell membrane disruption (Mansour 1998). Even at supra-optimal levels, proline does not suppress enzyme activity. Maggio et al. (2002) are of the view that proline may act as a signaling/regulatory molecule able to activate multiple responses that are component of the adaptation process. Petrusa and Winicov (1997) demonstrated that salt tolerant alfalfa plants rapidly doubled their proline content in roots, whereas in salt sensitive plants the increase was slow. These results were corroborated by Fougere et al. (1991). However, Aziz et al. (1998) and Parida et al. (2004) report a negative correlation between proline accumulation and salt tolerance in tomato and *Aegiceras corniculatum* respectively.

There are two alternative routes in proline biosynthesis in higher plants: the L-ornithine and the L-glutamate pathways. It is also known that, as in plants, both ornithine and glutamate are precursors of proline biosynthesis in microorganisms and mammals (Figure 1). Delauney et al. (1993) showed that two enzymes: pyrroline-5-carboxylate synthetase (P5CS) and pyrroline-5-carboxylate reductase (P5CR), play major roles in proline biosynthetic pathway (Figure 1). Transgenic tobacco plants over-expressing P5CS have shown increased concentration of proline and resistance to both drought and salinity stresses (Kishor et al. 1995). However, whether proline accumulation in these transgenic plants resulted in increased stress tolerance through osmotic adjustment or other mechanisms is unknown (Sharp et al. 1996).

Transgenic approach to improve plant stress tolerance has appreciable results. Overproduction of proline by genetically manipulated tobacco plant showed tolerance to NaCl (Hong et al. 2000).

<table>
<thead>
<tr>
<th>Species</th>
<th>Response to salinity</th>
<th>References</th>
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<tbody>
<tr>
<td><em>Oryza sativa</em></td>
<td>decrease</td>
<td>Alamgir and Ali (1999)</td>
</tr>
<tr>
<td><em>Vicia faba</em></td>
<td>decrease</td>
<td>Gadallah (1999)</td>
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<tr>
<td><em>Amaranthus tricolor</em></td>
<td>decrease</td>
<td>Wang and Nil (2000)</td>
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<tr>
<td><em>Bruguiera parviflora</em></td>
<td>decrease</td>
<td>Parida et al. (2002)</td>
</tr>
<tr>
<td><em>Pancratium maritimum</em></td>
<td>increases at low salinity; decrease at high salinity</td>
<td>Khedr et al. (2003)</td>
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<tr>
<td><em>Arabidopsis thaliana</em></td>
<td>increase</td>
<td>Quintero et al. (1996)</td>
</tr>
<tr>
<td><em>Fragaria x ananassa cv. Camarosa</em></td>
<td>increase</td>
<td>El-Baz et al. (2003)</td>
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Nanjo et al. (2003) demonstrated that introduction of antisense proline dehydrogenase cDNA in Arabidopsis overexpresses proline and showed tolerance to freezing temperatures (−7°C) as well as salinity (600 mmol NaCl). The RD29A promoter-driven DREB1A transgenic plants exhibited tolerance to water deficit, salt stress, and freezing temperatures (Kasuga et al. 1999).

However, accumulation of proline in rice plants grown under salt stress was due to salt injury and not as an indication of salt tolerance (Lutts et al. 1999). Similarly, accumulation of proline in two sorghum genotypes contrasting in salt tolerance suggested that proline accumulation was a reaction to salt stress and not a plant response associated with tolerance (De Lacerda et al. 2003). In another experiment, Lutts et al. (1999) showed that under salt stress, higher concentration of proline was accumulated in sensitive rice cultivars than in tolerant genotypes. Currently, there is more evidence supporting the presence of a positive relationship.

Although amides generally accumulate in salt-stressed plants to a lesser extent than do other nitrogen containing compounds (Mansour et al. 2002), concentration of asparagine frequently increases in response to stress (Fougere et al. 1991). In fact, asparagine accumulated to a greater extent than proline in Agrostis stolonifera (Dubey 1997). In barley, NaCl stress enhanced the asparagine pool in roots, xylem sap, and leaf blades of seedlings, whereas glutamine increased only in roots and leaf blades (Yamaya and Matsumoto 1989).

**Quaternary ammonium compounds (QAC)**

In several plant species, a positive correlation between leaf osmotic potential and glycinebetaine, β-alaninebetaine and prolinebetaine has been observed. These organic compounds are now known to have osmoprotective effects in the cell as well. Of the QACs in plants subjected to salt stress, glycinebetaine (GB) occurs most abundantly (Mansour 2000). This organic compound is mainly localized in chloroplasts and plays a vital role in chloroplast adjustment and protection of thylakoid membranes, thereby maintaining photosynthetic efficiency and plasmamembrane integrity (Yokoi et al. 2002).

In higher plants, GB is synthesized in chloroplast from serine via ethanolamine, choline, and betaine aldehyde (Rhodes and Hanson 1993). Choline is converted to betaine aldehyde, by choline mo-
nooxygenase (CMO), which is then converted to GB by betaine aldehyde dehydrogenase (BADH) (Figure 2). Although other pathways such as direct \(N\)-methylation of glycine is also known, the pathway from choline to glycine betaine has been identified in all GB-accumulating plant species (Weretilnyk et al. 1989).

GB accumulates in response to stress in many crops, including spinach, barley, tomato, potato, rice, carrot and sorghum (Yang et al. 2003). According to Murata et al. (1992) GB protects the photosystem II (PS II) complex by stabilizing the association of the extrinsic PS II complex proteins under salt stress. The concentrations of GB in species that use it as a compatible osmoticum are variable, for example, in sorghum it is as much as ten-fold than those in maize. GB-deficient genotypes of both species have also been identified. Accumulation of glycinebetaine under saline conditions is also reported to be high in some salt tolerant plants of mulberry (Yang et al. 2003). According to Murata et al. (1992) GB protects the photosystem II (PS II) complex by stabilizing the association of the extrinsic PS II complex proteins under salt stress. The concentrations of GB in species that use it as a compatible osmoticum are variable, for example, in sorghum it is as much as ten-fold than those in maize. GB-deficient genotypes of both species have also been identified.

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Polyols

Polyols, the polyhydric alcohols, are among the compatible solutes involved in osmoregulation and are thought to play a role in plant salt tolerance (Bohnert and Shen 1999). They exist in both acyclic and cyclic forms and are widely distributed in the plant kingdom. The most common polyols in plants include acyclic forms, mannitol, glycerol, sorbitol, and cyclic (cyclitols) forms ononitol and pinitol. In general, they are thought to accumulate in the cytoplasm of some halophytes to overcome the osmotic disturbances caused by high concentrations of inorganic ions compartmentalized in vacuoles. Polyols make up a considerable percentage of all assimilated CO\(_2\) as scavengers of stress-induced oxygen radicals (Bohnert et al. 1995). Mannitol, a sugar alcohol that may serve as a compatible solute to cope with salt stress, is synthesized via the action of a mannose-6-phosphate reductase (M6PR) in celery (Zhifang and Loescher 2003) and its accumulation increases when plants are exposed to low water potential. The accumulation is regulated by inhibition of competing pathways and decreased mannitol consumption and catabolism (Stoop et al. 1996). Studies using transgenic tobacco and Arabidopsis have shown improved growth of man-
mannitol accumulating plants under stress (Thomas et al. 1995). Mannitol improves tolerance to stress through scavenging of hydroxyl radicals (OH•) and stabilization of macromolecular structures. In tobacco, mannitol protects the thiol-regulated enzymes phosphoribulokinase, thioredoxin, ferredoxin and glutathione from OH• (Shen et al. 1997). Abebe et al. (2003), however, state that the amount of mannitol accumulated in response to stress was small and its effect on osmotic adjustment was less than that of other carbohydrates. In contrast to previous approaches that used a bacterial gene to engineer mannitol biosynthesis in plants and other organisms, A. thaliana, a non-mannitol-producer, was transformed with the celery leaf M6PR gene under control of the CaMV 3SS promoter. In all independent Arabidopsis M6PR transformants, mannitol accumulated in plants in amounts ranging from 0.5 to 6 µmol/g fresh weight. A novel compound, not found in either celery or Arabidopsis, 1-O-β-D-glucopyranosyl-β-D-mannitol, also accumulates in vegetative tissues of mature plants in amounts up to 4 µmol/g fresh weight but not in flowers and seeds. In the absence of NaCl, all transformants are phenotypically the same as the wild type; however, in the presence of NaCl, mature transgenic plants show a high level of salt tolerance and complete normal development including production of seeds on soil irrigated with 300 mmol NaCl. These results demonstrate a major role of M6PR in developing salt-tolerant plants by means of introducing mannitol biosynthesis (Zhifang and Loescher 2003).

**ANTIOXIDANTS**

Defence mechanisms against free radical-induced oxidative stress involve: (i) preventative mechanisms, (ii) repair mechanisms, (iii) physical defences, and (iv) antioxidant defences. The plants defend against these reactive oxygen species by induction of activities of certain antioxidative enzymes such as catalase, peroxidase, glutathione reductase, and superoxide dismutase, which scavenge reactive oxygen species (Mittova et al. 2003).

Activities of antioxidative enzymes such as ascorbate peroxidase, glutathione reductase, monodehydroascorbate reductase (MDHAR), dehydroascorbate reductase (DHAR), and Mn-SOD increase under salt stress in wheat, while Cu/Zn-SOD remains constant and total ascorbate and glutathione content decrease (Hernandez et al. 2000). In soybean root nodules ascorbate peroxidase, catalase, and glutathione reductase activities decrease under salt stress, while superoxide dismutase and reduced glutathione increase and malondialdehyde and total protein remain unchanged (Comba et al. 1998). Overexpression of genes leading to increased amounts and activities of mitochondrial Mn-SOD, Fe-SOD, chloroplastic Cu/Zn-SOD, bacterial catalase, and glutathione-S-transferase (GST)/glutathione peroxidase (GPX) can increase the performance of plants under stress (Roxas et al. 2000). Gossett et al. (1994) reported that in cotton (Gossypium hirsutum L.) NaCl stress increases the activities of SOD, guaicol peroxidase, and glutathione reductase and decreases the activities of catalase and ascorbate peroxidase. Salt stress also causes a decrease in total ascorbate, total glutathione, and a-tocopherol levels in this case. In leaves of rice plant, salt stress preferentially enhances the content of H2O2 and the activities of SOD, APX, and GPX, whereas it decreases catalase activity (Lee et al. 2001). On the other hand, salt stress has a little effect on the activity levels of glutathione reductase (Lee et al. 2001). Lechno et al. (1997) reported that NaCl treatment increases the activities of the antioxidative enzymes catalase and glutathione reductase and the content of the antioxidants ascorbic acid and reduced glutathione but does not affect the activity of SOD in cucumber plants. In wheat, activities of APX, MDHAR, DHAR, and GR increase in the shoots and decrease in the roots (Meneguzzo and Navarilizzo 1999). The tomato under high salt concentration showed higher antioxidant enzyme activities such as SOD, catalase, ascorbate peroxidase, glutathione reductase, and GST (Rodriguez-Rosales et al. 1999). At higher concentration of NaCl Hernandez et al. (1999) showed that activity of cytosolic CuZn-SOD II, chloroplastic CuZn-SOD II, and mitochondrial and/or peroxisomal Mn-SOD increases with increasing concentration of NaCl (110–130 mmol) in pea.

The non-enzymatic antioxidants viz. vitamin C, vitamin E, carotenoids, lipoic acid and others in the protection against oxidative stress were also reported (Kojo 2004).

**FUTURE PERSPECTIVE**

Salt stress causes huge losses of agriculture productivity worldwide. Therefore, plant biologists aimed at overcoming severe environmental stresses needs to be quickly and fully implemented. Together with conventional plant physiology, ge-
netics and biochemical approaches to studying plant responses to abiotic stresses have begun to bear fruit recently. Relevant information on biochemical indicators at the cellular level may serve as selection criteria for tolerance of salts in agricultural crops.

Although there were many transgenic plants with high stress tolerance generated, plant abiotic stress tolerance is a complex trait that involves multiple physiological and biochemical mechanisms and numerous genes. Transgenic plants with commercial value should at the same time retain relatively high productivity and other traits important for agriculture. Moreover, genetic modification should be combined with marker-assisted breeding programs with stress-related genes and QTLs, and ultimately, the different strategies should be integrated, and genes representing distinctive approaches should be combined to substantially increase plant stress tolerance.

REFERENCES


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